



MAASTRICHT . THE NETHERLANDS

# IMSC 2022

ABSTRACT BOOK

27 AUGUST - 2 SEPTEMBER 2022



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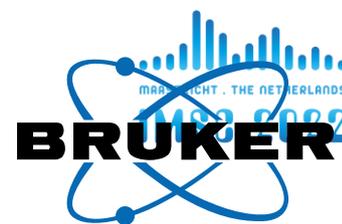
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## POSTER SESSION A

### Theme: Mass spectrometry across disciplines

### Session: Cultural heritage and conservation science

Poster number: **AD-PA-001**

#### **MINIMALLY-INVASIVE LIQUID MICROJUNCTION SAMPLING TO IDENTIFY DYE ON A 17TH CENTURY SPANISH ILLUMINATED MANUSCRIPT**

Abstract ID: **368**

**Presenting author: G. Asher Newsome, Smithsonian Museum Conservation Institute**

##### **Introduction**

Damage is usually defined for conservation purposes as the “degradation of a particular property of interest”. Artefacts must be preserved as much as possible to respect the culture of origin and protect the object for future study and so any visible sign of disruption can be undesirable. Mass spectrometry is the most molecularly specific technique, and ambient sampling is an attractive alternative to damaging methods like microsectioning. This work demonstrates artefact sampling by using microscale liquid handling to minimize disruption to an intact object. Red paint illuminations in the 1604 manuscript “Don Phelippe por la Gracia de Dios Rey” were targeted for identification.

##### **Methods**

Analysis of the manuscript was completed with a modified CombiPAL Autosampler (CTC Analytics) controlled using Cycle Composer software with Macro Editor (Autosampler Guys). The sample extraction was optimised to use as little as 0.1 mL solvent, 50:50 MeOH:H<sub>2</sub>O with 0.1% formic acid. The needle distance to the sample surface was measured with a Baumer distance sensor (OM70-11200061) and adjusted accordingly, whilst being carefully monitored with an endoscope. Samples were diluted to 50 mL for direct infusion using a heated ESI source on an Orbitrap Elite Mass Spectrometer (Thermo Fisher) in negative mode.

##### **Preliminary data (results)**

17<sup>th</sup> century organic red colourants were primarily cochineal from insects and madder root which contains alizarin and purpurin. The solvent system was optimized for water-soluble cochineal and alcohol-soluble madder; both were extractable using 50:50 MeOH:H<sub>2</sub>O +0.1% formic acid. Standards were produced from watercolour samples of cochineal made from ground beetle shells and direct extraction of madder roots were used to generate standards. Extraction volumes down to 0.1 mL showed intense signal, and disruptions to the colour field were practically invisible to the untrained, naked eye.

Selected illuminations on the manuscript parchment were analysed using a liquid microjunction surface sampling system. Further optimisations were done to minimise surface disruption, including careful selection of sample position. Any previously disrupted surface was used or the margins/edges of the colour field were selected to limit visibility of surface disruption. Twelve samples were taken from different pages which showed various degrees of water damage, these samples were chosen to be as indicative of the red colouration throughout the manuscript to ascertain the likelihood of cochineal being used throughout.

Of the eleven samples taken, nine showed the indicative cochineal [M-H]<sup>-</sup> peak at  $m/z$  491.070 +/- 0.015 above eight times more abundant than the background. This analysis was completed on all twelve samples, including blank parchment as a control. The average signal abundance was taken for each sample and showed that cochineal was detected in all coloured samples tested. Carryover signal when analysing the uncoloured section of parchment was within baseline levels.

### POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

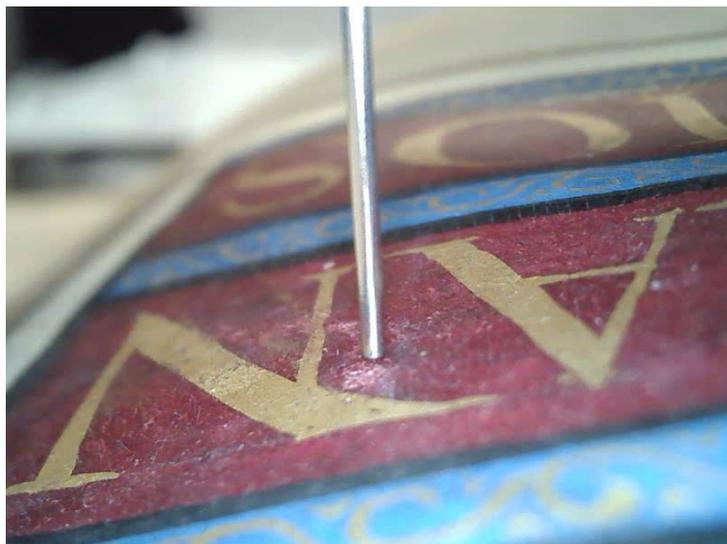
#### **Please explain why your abstract is innovative for mass spectrometry?**

A minimally-invasive liquid microjunction sampling method to detect dyes from an historical parchment manuscript using sub-microlitre volumes.

#### **Co-authors:**

*Hannah Lawther, Smithsonian Museum Conservation Institute, University of St Andrews*

*Vanessa Haight-Smith, Smithsonian Libraries and Archives*



A photograph showing the sampling process of a coloured section

Poster number: AD-PA-002

## GETTING UNDER THE SKIN: TOWARDS ON-TISSUE DIGESTION OF COLLAGEN IN PARCHMENT

Abstract ID: 452

**Presenting author: Antonia Malissa, TU Wien, Institute of Chemical Technologies and Analytics , Academy of Fine Arts Vienna, Institute of Natural Sciences and Technology in the Arts**

### Introduction

From its first introduction as alternative to papyrus within Late Antiquity, parchment functioned as primary writing support for scrolls and manuscripts until the introduction of paper production at the end of Medieval times. Being produced from the dermal layer of animal skins, it comprises and is to a great extent structured by different types of collagens. While the complex manufacturing process possibly alters the material during its preparation already, environmental stress, e. g. caused by UV light, moisture, or pollutants, induced changes over centuries and still is today without proper handling. Therefore, detailed knowledge of chemical modifications of collagen and their impact on the degradation behavior of parchment is essential for the conservation and preservation of these precious artifacts.

### Methods

In-solution digestion protocols with different buffer systems were developed for bovine collagen type I and sheep parchment. After reduction/alkylation, samples were enzymatically digested at 37 °C, cleaned up and peptides were co-eluted with  $\alpha$ -CHCA on a MALDI target. Tragacanth embedded samples were on-tissue digested: different enzymes were applied via spraying (HTX TM5) and after incubation at 37 °C at high humidity,  $\alpha$ -CHCA matrix was applied via spraying. MALDI TOF/TOF analysis was performed in positive reflectron mode and peptide fragmentation was performed with PSD in LIFT mode (Bruker, ultrafleXtreme). Resulting spectra were searched against a collagen database.

### Preliminary data (results)

Since parchment is processed dermis, different types of collagens are its main constituents next to non-collagenous components. The presented study focuses on developing an enzymatic digestion protocol, allowing the analysis of collagens in parchment, and additionally the assessment of degradation-induced alteration of collagen by studying peptide modifications. A digestion protocol employing trypsin/LysC was used as benchmark. The repetitive structural pattern of  $-(\text{Gly-X-Y})_n-$ , with Pro and Hyp highly present in X- and Y-position, makes glycine, proline, and hydroxyproline the most abundant amino acids in collagens. To enhance the digestion efficiency and increase collagen-specificity of the digestion, collagenase III and ProAlanase - a novel alanine-, proline-, and hydroxyproline-specific protease - were tested as alternatives to the commonly used arginine-/lysine-specific trypsin/LysC. Different digestion conditions were employed to define the most efficient workflow: Collagenase III and ProAlanase were used in single-enzyme digestions with different incubation times. Additionally, the enzymes were used consecutively on the same sample in varying orders for a potential multi-enzyme digestion. Performances were compared with each other for the single and the multi-enzyme approaches.

The most efficient in-solution digestion protocol was then translated into an on-tissue digestion methodology in combination with an adequate MALDI application for subsequent MALDI imaging MS of parchment. The established workflow was comparatively applied to the hair and flesh side of the same parchment to investigate possible differences due to the manufacturing process. In addition, the distribution of collagenous and non-collagenous regions in thin parchment sections was analyzed.

### Please explain why your abstract is innovative for mass spectrometry?

A collagen-specific digestion protocol allows for an in-depth analysis of collagenous and non-collagenous components in parchment to understand protein changes induced by environmental influences (e. g. oxidation, sulfation).

### Co-authors:

*Manfred Schreiner, TU Wien, Institute of Chemical Technologies and Analytics , Academy of Fine Arts Vienna, Institute of Natural Sciences and Technology in the Arts*

*Martina Marchetti-Deschmann, TU Wien, Institute of Chemical Technologies and Analytics*

Poster number: **AD-PA-003**

## **MINIMALLY INVASIVE PROTEOMICS WORKFLOW TO DETERMINE THE SPECIES OF ORIGIN OF IVORY OBJECTS**

Abstract ID: **587**

**Presenting author: Catherine Gilbert, CBMN, UMR CNRS 5248, University of Bordeaux, 33000 Bordeaux, France, Proteome Platform, University of Bordeaux, 33000 Bordeaux, France**

### **Introduction**

Ivory is a highly sought-after material in many cultures, both for its medicinal properties and its use as a structural medium. Ivory is essentially an elongated tooth, and like other mineralised tissues, the structural protein collagen composes 90% of the organic material in ivory. Structural proteins such as collagens are well preserved in mineralised tissues such as bones, teeth, and ivory, and can remain a source of taxonomic information long after the degradation of DNA. For this reason, the analysis of ancient proteins is often used to enable species identification of morphologically unidentifiable objects. In this study, we perform species identification on ivory objects from the collections of the Metropolitan Museum of Art, using optimised proteomics methods adapted to trace level detection.

### **Methods**

In order to perform successful minimally invasive analysis by rubbing with microabrasive films, it is necessary to miniaturise and simplify the sample preparation procedure. This can be done, for example, by integrating different chemical treatments into a single or few steps, or using miniaturized analytical workflows, both of which have already proven to improve recovery in the most challenging ancient samples. In addition to this, we have used data acquisition methods adapted to low sample amounts (Orbitrap Fusion Lumos, Thermo Fisher Scientific) and adapted our data processing steps to target for the high heterogeneity of the collagen proteins.

### **Preliminary data (results)**

In this presentation we will demonstrate the optimisation we have performed in order to robustly achieve proteomic analysis using minimal amounts of starting material, as well as introducing new methods that we are developing to further increase the sequence coverage of collagens. These new methods are essential in order to establish minimally invasive sampling with microabrasive films in the cultural heritage analysis workflow and to offer a method of species identification where visual assessment is not possible.

In many cases we were able to identify several hundred peptides attributable to both collagen alpha1-(I) and alpha2-(I), from objects as old as 3200 years old; e.g. 957 AAs COL1A1 fragment identified by 92% sequence coverage and 343 peptides.

This means that we were also able to perform confident species identification based on several unique peptides that were confirmed with y and b fragment ions covering the sequences.

In addition to this, since many of the objects that we are analysing in this study originate from Egypt, the objects are likely to originate from either elephant or hippopotamus ivory. A challenge here is that the proteomes of these species are not available on public databases, and so we must rely on protein sequences that are either incomplete, sequences that have been calculated from data sequences, and on de novo sequencing. While de novo sequencing is commonly required in the field of palaeoproteomics, it has, as of yet, rarely been applied to cultural heritage objects to identify the species of origin.

### **Please explain why your abstract is innovative for mass spectrometry?**

Here we report the use of miniaturised protocols, specifically adapted mass spectrometry data acquisition methods and targeted data analysis strategies to analyse immeasurably small samples taken from cultural heritage objects.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

**Co-authors:**

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*Caroline Tokarski, CBMN, UMR CNRS 5248, University of Bordeaux, 33000 Bordeaux, France, Proteome Platform, University of Bordeaux, 33000 Bordeaux, France*

Poster number: **AD-PA-004**

## **IDENTIFYING PAINT BINDERS IN PAINTINGS BY MARK TOBEY USING MASS SPECTRAL TECHNIQUES**

Abstract ID: **595**

**Presenting author: Vanessa Johnson, Portland State University**

### **Introduction**

Mark Tobey was an influential painter working in the mid-twentieth century in the Pacific Northwest of the United States. He created expressionistic works inspired by his spirituality, applying paints he mixed himself to paper and cardboard supports. While his media are described as "gouache" or "tempera", these terms can refer to a range of binder mixtures. There is no evidence in the literature that his paint binders have undergone compositional analysis. This study sought to understand the composition of Tobey's paints by utilizing Pyrolysis coupled to Gas Chromatography Mass Spectrometry (Py-GC/MS) to identify key components of Tobey's paint binders.

### **Methods**

Paint microsamples were collected for Py-GC/MS analysis from twelve paintings by Mark Tobey at the Seattle Art Museum and the Jordan Schnitzer Museum of Art in Eugene, Oregon. Reference MS libraries were created from a range of binding media such as plant gums, egg yolk, animal glue, linseed oil, resins and waxes. Binder types were determined by matching MS data from museum samples to mass spectra in the usercreated binder libraries. In addition to Py-GC/MS, proteomics via Liquid Chromatography/Mass Spectrometry (LC/MS) was utilized to detect and quantify a range of amino acids in proteinaceous binding media.

### **Preliminary data (results)**

Analysis revealed that Tobey painting microsamples associated with tempera paints contained abundant dicarboxylic acids along with indene- and indole-related molecules. Both egg and linseed oil reference binders were nearly identical and did not facilitate identification of egg in tempera paints. Samples labelled watercolors matched well with unique mass spectra from gum arabic and gum tragacanth reference binders. Some watercolors contained an abundance of mono-carboxylic acids as well as anticipated trimethoxy benzenes. One painting assigned a watercolor designation was found to instead be comprised of wax-based encaustic paints. Complications arose from the presence of paper substrate in some analyzed samples, although unique paper molecules such as furan-containing compounds were identified while many molecules related to gum binding media were not found in paper references, allowing the assignment of gum binding media despite the presence of paper. Proline-related molecules were found in samples with attached paper substrate as well as those which may have contained a gesso-layer. Triterpenes appear in samples which were painted on cardboard, indicating these may be substrate related rather than deriving from the binder. Work is ongoing to verify the presence and type of proteins in Tobey's tempera paints using proteomics. Additionally, characterization of the abundance of mono- and dicarboxylic acids in paint samples and reference binders is ongoing and will provide another point of reference for differentiating between binder types.

### **Please explain why your abstract is innovative for mass spectrometry?**

The two MS techniques in this study show potential as methods of identifying both polysaccharide and proteinaceous binding media, particularly when collected from paper substrates which contain very similar molecules.

### **Co-authors:**

*Nicholas Dorman, Seattle Art Museum*

*Chris White, Jordan Schnitzer Museum of Art*

*Tami Lasseter Clare, Portland State University*

Poster number: **AD-PA-005**

## **IDENTIFICATION AND DISCRIMINATION OF METAL-CONTAINING PIGMENTS AND ADDITIVES IN OIL PAINTS EXPLORED BY ULTRA-HIGH RESOLUTION (FT-ICR) MASS SPECTROMETRY.**

Abstract ID: **623**

**Presenting author: Elena Giaretta, Université de Lille, CNRS, USR 3290 - MSAP laboratory - Miniaturization for Synthesis, Analysis & Proteomics**

### **Introduction**

Metals complexes catalyze the oxidative drying of oil-based paint as they are present as additives or as pigments in paint formulations. Hence, the catalytically active species present a fundamental role in the process of dioxygen uptake, followed by hydroperoxides decomposition to free radicals resulting in the binding of the pigment to the painting support, giving rise to hardened cross-linked polymer networks. The key step in order to identify metal-containing pigments consists in the chelation of the metal with a chelating agent bearing a dimethylamino group for efficient ionization. We hereby propose a procedure implying metal chelation followed by concentration on an ion-exchange strong cation column and a method to analyze metal-containing pigments and additives by means of ultra-high resolution mass spectrometry.

### **Methods**

Pigments extracted from oil paint tubes were reacted in amber glass vials with the chelator, 2-(5-Bromo-2-Pyridylazo)-5-dimethylaminophenol (5-Br-DMPAP), in acidic environment by adding an acetate buffer solution at pH 5.5 for at least 30 minutes. Sample concentration was performed on a 3 mL Phenomenex® STRATA™-X-C 33 µm column washed with 3,5 mL of ultrapure water, 6 mL of acetonitrile and eluted with 14 mL of a solvent solution acidified by formic acid (pH = 5). After solvent evaporation, analyses were performed by nano-ESI and MALDI ionization on a 9.4 Tesla Bruker™ Solarix™ FT-ICR.

### **Preliminary data (results)**

Pigment samples were prepared by extracting 1 g of oil or alkyd paint in a 15 mL Falcon tube with 5 mL of ethyl acetate. The Falcon tube undergoes centrifugation for 10 minutes at 5000 rpm. The pigment precipitates in the tube, while the ethyl acetate now contains the binder. The latter is analyzed distinctly by NMR while metal chelation essays with the 5-Br-DMPAP were tested in different samples amount ranging from 100 mg to 10 mg for optimizing a method complying with the small samples available in cultural heritage. Sodium acetate buffer (pH 5.5) is added to the reaction mixture, as working under acidic conditions is crucial for having metallic ions in solution and thus for the chelation reaction to take place. After sample concentration and purification on SPE columns the samples were, analyzed using an FT-ICR mass spectrometer. The advantage of FT-ICR MS is that the typical isotopic patterns of the different metals, as copper (II), lead (II), manganese (II), cobalt (II), iron (II) and iron (III), can be observed in mass spectra, allowing for metal discrimination followed by pigment identification. In the analyzed samples the formation of mono- or doubly-chelated metals was observed. In a further stage, this protocol will be optimized for museum-size painting samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

We present an innovative procedure, extensible to forensic analyses, allowing for the identification of metals and consequently the recognition of pigments and materials included in paint formulations.

### **Co-authors:**

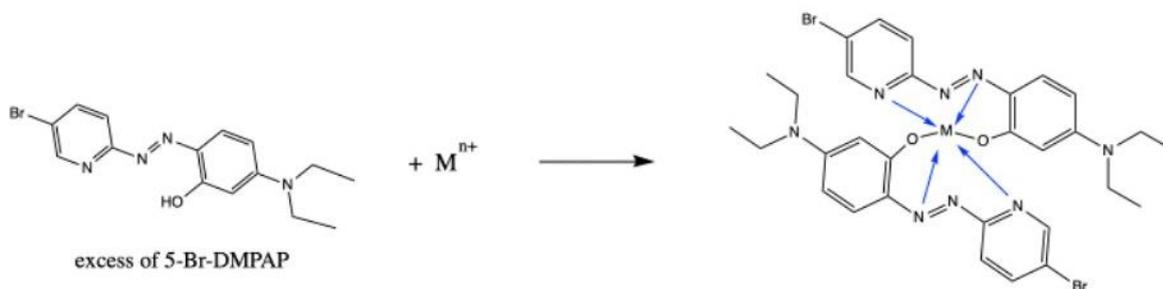
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*Fabrice Bray, Université de Lille, CNRS, USR 3290 - MSAP laboratory - Miniaturization for Synthesis, Analysis & Proteomics*

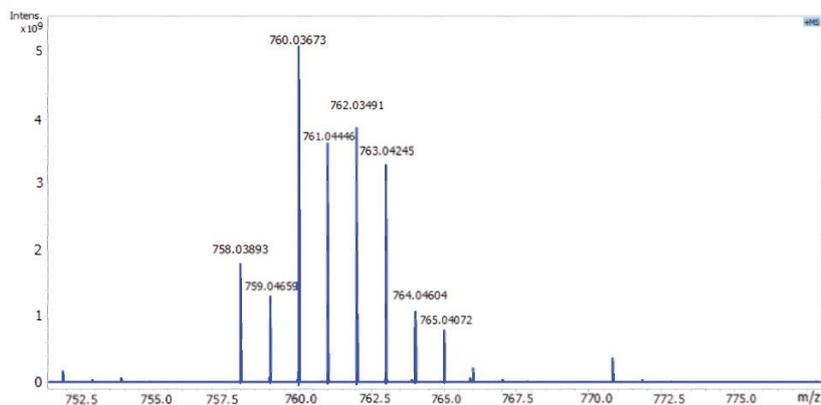
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## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Formation of the metal complex.



Isotopic distribution of the complex signal with Cu (II).

Poster number: AD-PA-006

## DYE ANALYSIS OF HISTORICAL TEXTILES USING DESI-MS

Abstract ID: 757

**Presenting author: Edith Sandström, University of Edinburgh, National Museums Scotland**

### Introduction

One key issue in the heritage science field is the necessity of sampling culturally significant objects. Non-invasive spectroscopic techniques have been developed but offer less information than many invasive analyses. Ambient mass spectrometry techniques, such as desorption electrospray ionisation mass spectrometry (DESI-MS), bypass the need for sampling without reducing the information gained from the analysis. The use of DESI-MS in heritage science thus enables the analysis of hitherto inaccessible objects.

Sampling opportunities of historical textiles for dye analysis are limited due to structural integrity concerns and conservation practises. Therefore, a DESI source has been built and optimised for historical dye analysis to aid the understanding of the socioeconomic context in which previously unattainable objects were made.

### Methods

A Bruker 7T Solarix FT-ICR-MS was equipped with an in-house developed DESI source (Fig. 1). The source has the sprayer holder mounted to positioners for manual control of the x-, y- and z-axes and a rotation mount for manual adjustment of the sprayer angle. A camera was added for sample spot monitoring. LaserGRBL CNC control was used to control the motorised XY stage for DESI-MS imaging.

Substrates tested include silk, wool, viscose, polyester and barkcloth dyed with early synthetic and natural dyes as well as historical samples from *Tabular overview of artificial organic colourants and their application* (Adolf Lehne, 1893).

### Preliminary data (results)

Initial studies used wool samples dyed with rhodamine B or turmeric as standards for synthetic and natural dyes respectively (Fig. 2). The investigation found that the most important parameters to control for dye analysis using DESI-MS are sprayer angle, sprayer-to-sample distance and solvent system. However, the variety of chemical structures and dyeing processes used across natural and early synthetic dyes means that the parameters used for DESI-MS depend substantially on the nature of the suspected dye as well as the substrate analysed.

Reproducible detection of nitro, azo, triphenylmethane and xanthene dyes as well as anthraquinone, flavonoid and indigoid chromophores with good signal-to-noise ratios have been achieved on all tested substrates. In both negative and positive mode, greater ion abundance of the ion of interest is achieved using shallower angles and smaller sprayer-to-sample distances in comparison to their analysis using steeper angles and larger distances.

A higher flowrate and capillary voltage were needed for desorption from wool in comparison to silk and barkcloth. Preliminary studies also suggest that a 3:1 v/v MeOH:H<sub>2</sub>O solvent system gives better signal-to-noise ratios for analysis in positive mode while 3:1 v/v ACN:H<sub>2</sub>O gives better signal-to-noise ratios in negative mode.

After damage assessment, this use of DESI-MS will be applied to historical samples from *Tabular overview of artificial organic colourants and their application* (Adolf Lehne, 1893). Future potential investigations also include the analysis of Renaissance embroideries and a collection of 19<sup>th</sup> and early 20<sup>th</sup> centuries barkcloths both housed at National Museums Scotland.

### Please explain why your abstract is innovative for mass spectrometry?

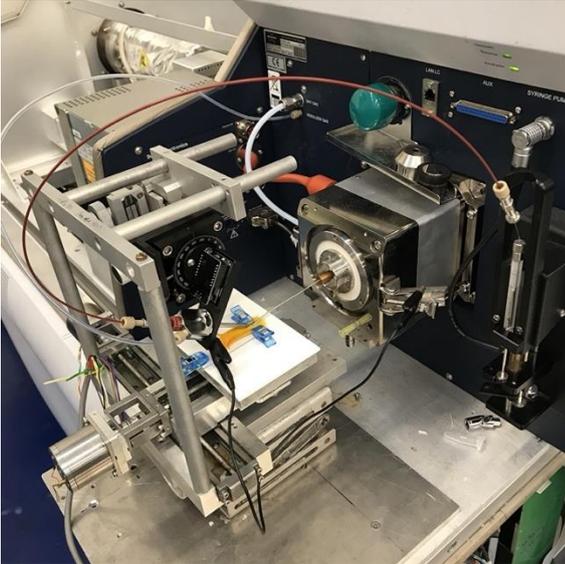
Development and application of a desorption electrospray ionisation (DESI) source for the analysis of both early synthetic and natural dyes in historical textiles.

## POSTER SESSION A

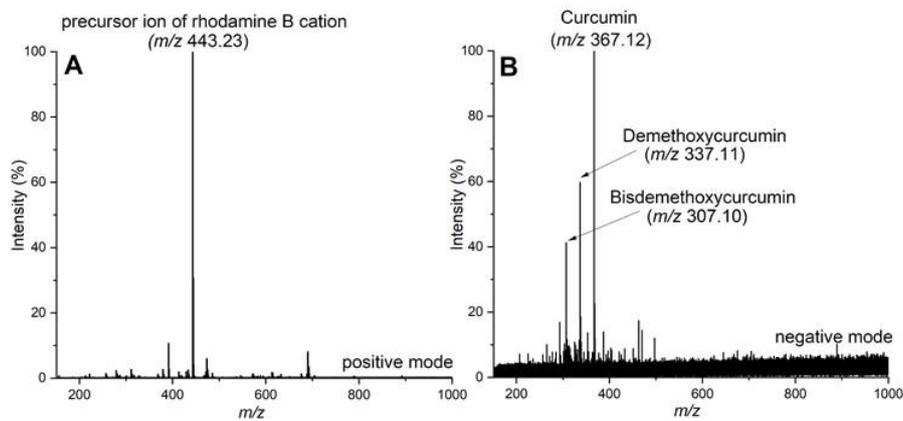
Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Co-authors:

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C. Logan Mackay, University of Edinburgh  
Lore G. Troalen, National Museums Scotland  
Alison N. Hulme, University of Edinburgh



DESI source developed in-house.



DESI-MS spectra of rhodamineB (A) and turmeric (B) on wool.

Poster number: **AD-PA-007**

## **CHARACTERIZATION OF ARCHEOLOGICAL RESIDUES BY PYROLYSIS-GCMS**

Abstract ID: **994**

**Presenting author: Christelle Absalon, Bordeaux University-ISM-UMR5255**

### **Introduction**

The chemical analysis of residues collected on ceramic permits to connect the object to its function. However, the difficulty comes from the strategy to be used since the chemical nature of the compounds constitutive of the residue is not known. Pyrolysis-GCMS is one of the technique which permits to obtain structural information without intensive chemical extraction or derivatization. This study concerns archeological vessels found during excavation sites in Villeneuve sur Lot (France).

### **Methods**

Samples were analyzed by TMAH pyrolysis-GCMS on a frontier lab instrument, 1 to 2 mg of organic residue were sampled on vessels.

### **Preliminary data (results)**

Twenty one samples were analyzed by pyrolysis-gas chromatography mass spectrometry. We were able to detect a significant quantity of organic compounds on 17 samples and a small quantity of organic compounds was detected on 4 samples.

The profiles obtained for those samples are quite similar and the presence of a hundred of organic compounds could be established. The different compounds detected were grouped by chemical category, 7 families were identified: sugar, terpenic compounds, resinic acid, abietane derivatives, fatty acid, HAP, di and trimethoxylated compounds.

In all samples, we find mostly dehydroabietic acid as well as a series of resin acids: isopimaric acid, dextropimaric, oxodehydroabietic and abietic acids; these resinic acids are markers of a pine resin. These compounds are also associated with the presence of retene and norabietane derivatives which are markers of an anthropogenic thermal degradation of dehydroabietic acid (1). The combined presence of resin acids associated with abietane derivatives allows us to conclude that vessels were waterproofed by pine pitch.

Among the other detected compounds, we could detect mono or dimethoxylated aromatic compounds. These compounds are characteristic of the presence of tannin and therefore of wine ( 2 and 3).

1. Colombini, M.P., Modugno, F., Ribechini, E. (2005) *Journal of Mass Spectrometry*, 40 (5), pp. 675-687
2. Garnier, N., Richardin P., Cheynier V., Regert M. (2003) *Analytica Chimica Acta*, 493, pp. 137-157
3. Saint-Raymond C., Frugier C., Cantin N., Verdin F., Lemaître S., Absalon C., Pianet I. (2021) *Bioeng Biosci.* 4(4), pp. 377-384

### **Please explain why your abstract is innovative for mass spectrometry?**

Identification of wine marker in vessels without chemical extraction

### **Co-authors:**

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*Claire Mouche, Bordeaux University-ISM-UMR5255*

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*Alexandra Hanry, INRAP - Ausonius - UMR5607*

Poster number: **AD-PA-008**

## **IDENTIFICATION OF ORGANIC PIGMENTS AND DYES IN HISTORIC COLOUR CHARTS OF ARTISTS' PAINTS - THE "DEUTSCHES FARBENBUCH" (1925)**

Abstract ID: 1031

**Presenting author: Alina Astefanei, Van 't Hoff Institute for Molecular Sciences, Faculty of Science, University of Amsterdam, Center for Analytical Sciences Amsterdam (CASA)**

### **Introduction**

The "*Deutsches Farbenbuch*" was the result of a long discussion about the quality of commercial paints that began at the "*1<sup>st</sup> Conference for Painting Technique*" held in Munich in 1893.

The book aimed at establishing quality assurance and correct denotation standards for commercial paints and to fight against "*Farbenschwindel*" (paint deception). It was published by the chemist and paint producer Heinrich Trillich in 3 parts (1923-1926); the second part (1925) was dedicated to artists' paints (TRILLICH 1925). The book contains several commercial colour charts of different German paint producers such as Schoenfeld, Schmincke, Pelikan, Bössenroth and others.

### **Methods**

In this work, we present the use of a combination of spectroscopic (Raman and SERS) and ultra-high pressure liquid chromatography - mass spectrometry (UPLC-MS) techniques for the accurate identification of organic pigments and dyes in artists' colour charts of the "*Deutsches Farbenbuch*".

### **Preliminary data (results)**

We have identified a significant number of different synthetic organic pigments, lakes and dyes belonging to different chemical classes and often present in mixtures. The identified colorants (30 different colorants up to now) have mainly poor to fair, and only few have good fastness to light. The labels of the commercial paints do not give a clue of the colorants present in the tube colors. These findings indicate that the conservation and exhibition of artworks from this period must be performed with special care. Furthermore, the results demonstrate the need of combining different complementary analytical tools such as Raman, SERS and UPLC-PDA-MS for a comprehensive characterization such complex samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

Raman and SERS alone cannot deal with complex mixtures of colorants present in paint samples. Mass spectrometry tools are crucial to accurately identify the dyes and pigments present.

### **Co-authors:**

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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Das Deutsche Farbenbuch* (Heinrich Trillich (1923-1926) part II (1925) color charts (1925)



## **Session: Forensic Sciences**

Poster number: **AD-PA-009**

### **FIRST STEPS TOWARD UNCOVERING GENE DOPING WITH CRISPR/CAS BY IDENTIFYING SPCAS9 IN PLASMA VIA HPLC–HRMS/MS**

Abstract ID: 17

**Presenting author: Alina Paßreiter, Center for Preventive Doping Research/ Institute of Biochemistry, German Sport University Cologne**

#### **Introduction**

The discovery of the CRISPR/Cas system as a programmable, RNA-guided endonuclease has revolutionized the utilization of gene technology. Because it enables the precise modification of any desired DNA sequence and surpasses all hitherto existing alternatives for gene editing in many ways, it is one of the most frequently used tools for genome editing. However, these advantages also potentially facilitate the illicit use of the CRISPR/Cas system in order to achieve performance enhancing effects in sporting competitions. This abuse is classified as gene doping, which is banned in sports according to the Prohibited List of the World Anti-Doping Agency (WADA). Therefore, there is a pressing need for an adequate analytical method to detect the misuse of the CRISPR/Cas system by athletes.

#### **Methods**

Identification of the exogenous protein Cas9 from the bacterium *Streptococcus pyogenes* (SpCas9) in plasma samples was accomplished by means of a bottom-up analytical approach via immunoaffinity purification, tryptic digestion, and subsequent detection by HPLC–HRMS/MS.

#### **Preliminary data (results)**

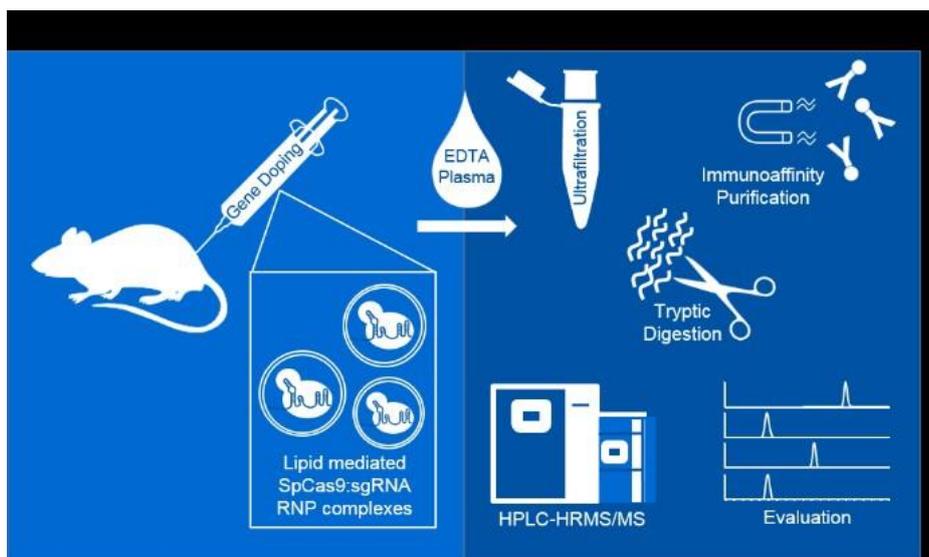
A qualitative method validation was conducted with three specific peptides allowing for a limit of detection of 25 ng/mL. Additionally, it was shown that the developed method is also applicable to the detection of (illicit) gene regulation through the identification of catalytically inactive Cas9. A proof-of-concept administration study employing an in vivo mouse model revealed a detection window of SpCas9 for up to 8 h post administration, confirming the suitability of the test strategy for the analysis of authentic doping control samples.

#### **Please explain why your abstract is innovative for mass spectrometry?**

With this study, first steps were undertaken to directly uncover gene doping with CRISPR/Cas by means of HPLC–HRMS/MS.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Graphical abstract

Poster number: **AD-PA-010**

## **FORENSIC INVESTIGATION OF BLOODSTAINS AND BLOOD FINGERMARKS BY MALDI MSI**

Abstract ID: **25**

**Presenting author: Simona Francese, Sheffield Hallam University**

### **Introduction**

Blood is the most frequent type of evidence encountered at the scene of major crimes. In addition to developing methods for the operational and reliable detection of this biofluid, efforts have been directed towards providing intelligence to narrow down the pool of suspects. In this context, the detection of Haemoglobin variants (HbV) from bloodstains of bloodmarks yields suspect profiling intelligence relating to their physiological or pathological state. Given the relationship between these variants and areas of incidence, their detection may also give insights into bio-geographical provenance. In the work presented here, a MALDI MS Imaging -proteomics combined approach has been developed to detect, confirm and visualise the distribution of six Haemoglobin variants in bloodmarks in blind patients samples.

### **Methods**

Blood stains were enzymatically digested with trypsin at 20 µg/mL (containing 0.1% RapiGest) and incubated at 37 °C for 1 h. Blood marks were digested with trypsin at 25 ng/µL containing 0.1% RapiGest, using the TM3 Sprayer (HTX Technologies LLC) and incubated for 2 h at 50 °C. Matrix coating with α-cyano-4-hydroxycinnamic acid was performed at 10 mg/mL in 70:30 ACN:TFAaq 0.2% using the TM M3 Sprayer. MALDI profiling data were acquired from three high end MALDI mass spectrometers, whereas MALDI imaging data were primarily acquired from the ttleX (Bruker Daltonik, Germany).

### **Preliminary data (results)**

At least one of the proteotypic peptides predicted for each of the six variants (HbC, HbS, HbD Iran, HbD Punjab, HbJ Baltimore, HbE) was detected in in solution digests of the blood and in high mass accuracy on the Synapt G2 HDMS (Waters Corp. Manchester) the rapifleX and the ttleX (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). The highest mass accuracy was exhibited by the ttleX which also correctly identified most patients samples, in terms of variants, in a blind study. Tandem MS was also possible with this instrument so that all the variants putatively identified could be confirmed. The six variants were all imaged in blood fingermarks with varying degree of ridge flow and ridge detail considered the original blood fingermark produced (example shown in Fig 1). A certain level of separation of two overlapping blood fingermarks was achieved by imaging two proteotypic peptides belonging to the HbC and HbJ-Baltimore respectively (Fig 2).

### **Please explain why your abstract is innovative for mass spectrometry?**

- Haemoglobin variants have been correctly reported for patients in a blind study
- First time that peptides proteotypic to six Haemoglobin variants have been visualised in blood fingermarks through MALDI MSI

### **Co-authors:**

*Cameron Heaton, foster +freeman*

*Matthias Witt, Bruker Daltonik*

*Laura Cole, Sheffield Hallam University*

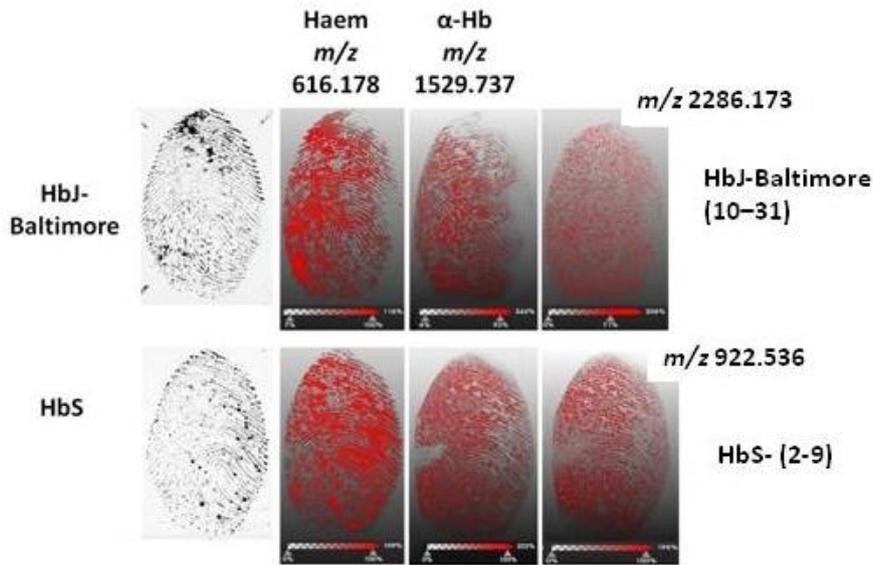
*Richard McColm, Defense Science and Technology Laboratory*

*Jason Eyre, Sheffield Teaching Hospitals*

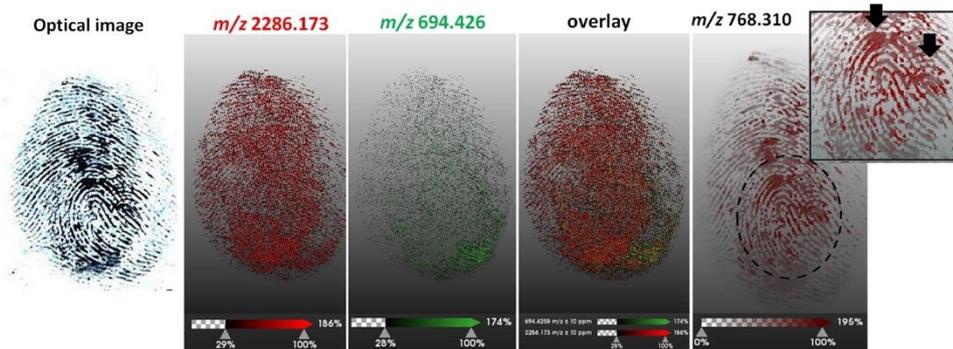
*Simon Tazzyman, Sheffield Teaching Hospitals*

**POSTER SESSION A**

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MALDI MSI of bloodmarks contaminated with HbJ and HbS respectively



MALDI MSI of overlapping HbVs-containing bloodmarks

Poster number: **AD-PA-011**

## **INVESTIGATIONS INTO THE ELIMINATION PROFILES AND METABOLITE RATIOS OF MICRO-DOSED SELECTIVE ANDROGEN RECEPTOR MODULATOR LGD-4033 FOR DOPING-CONTROL PURPOSES**

Abstract ID: **56**

**Presenting author: Felicitas Wagener, German Sport University Cologne**

### **Introduction**

LGD-4033 (ligandrol) is a selective androgen receptor modulator (SARM), which is prohibited in sports by the World Anti-Doping Agency (WADA) and led to 62 adverse analytical findings (AAFs) in 2019. But not only deliberate doping with LGD-4033 constitutes a problem. In the past years, some AAFs that concerned SARMs can be attributed to contaminated dietary supplements (DS). Thus, the urgency to develop methods to differentiate between inadvertent doping and abuse of SARMs to benefit from the performance-enhancing effect of the compound in sports is growing.

### **Methods**

To gain a better understanding of the metabolism and excretion patterns of LGD-4033, human micro-dose excretion studies at 1, 10 and 50 µg LGD-4033 were conducted. Collected urine samples were prepared for analysis using enzymatic hydrolysis followed by solid-phase extraction and analyzed via LC-HRMS/MS. The method was validated for qualitative detection of LGD-4033, allowing for a limit of detection (LOD) of 8 pg/mL.

### **Preliminary data (results)**

Including isomers, a total of 15 phase-I metabolites of LGD-4033 were detected in the urine samples after ingestion of LGD-4033. The metabolite M1, representing the epimer of LGD-4033 was synthesized and the structure elucidated by NMR spectroscopy. As the M1/LGD-4033 ratio changes over time, the ratio and the approximate LGD-4033 concentration can contribute to estimating the time point of drug intake and dose of LGD-4033 in doping control urine samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

The approach of time and dose estimation will aid in improving anti-doping result management.

### **Co-authors:**

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*Christian Görgens, German Sport University Cologne*

*Yiannis S. Angelis, National Center for Scientific Research "Demokritos"*

*Michael Petrou, Cyprus Anti-Doping Authority*

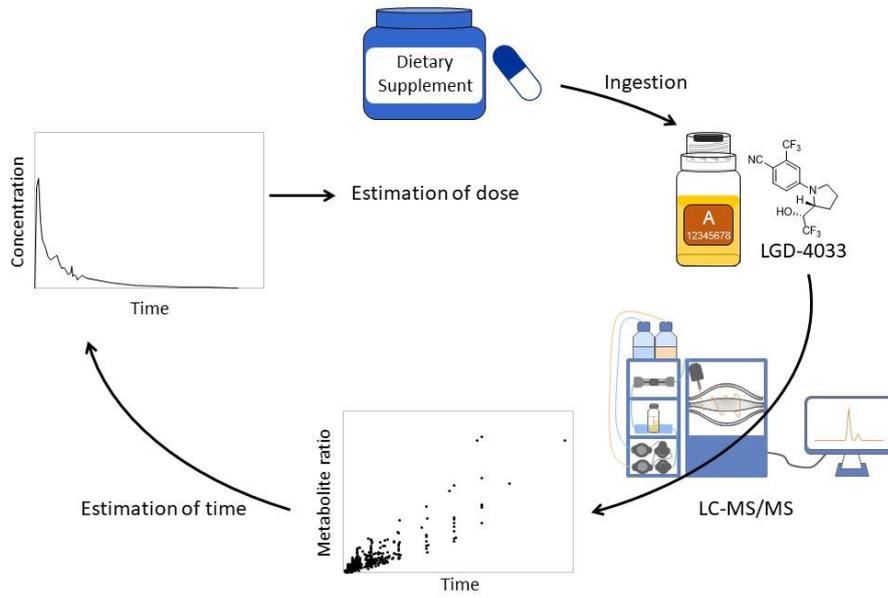
*Andreas Lagojda, BayerCropScience AG*

*Dirk Kühne, BayerCropScience AG*

*Mario Thevis, German Sport University Cologne, European Monitoring Center for Emerging Doping Agents*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Poster number: **AD-PA-012**

## **PROBING FOR FACTORS INFLUENCING EXHALED BREATH DRUG TESTING IN SPORTS – PILOT STUDIES FOCUSING ON THE TESTED INDIVIDUAL'S TOBACCO SMOKING HABIT AND SEX**

Abstract ID: **180**

**Presenting author: Ann-Marie Garzinsky, Center for Preventive Doping Research/Institute of Biochemistry, German Sport University Cologne**

### **Introduction**

Exhaled Breath (EB) was found to be a promising matrix in the field of sports drug testing due to the non-invasive and non-intrusive sampling procedure, but significant interindividual variations regarding detected drug concentrations have been observed in previous studies. In order to investigate whether the detectability of doping agents in EB is affected by the sex or tobacco smoking, two administration studies were conducted with male and female smokers and non-smokers concerning the elimination of the beta blocker propranolol and the stimulant pseudoephedrine into EB.

### **Methods**

Following the administration of 40 mg propranolol or 30 mg pseudoephedrine, a total of 19 participants, including female and male non-smokers as well as female and male smokers, collected EB and Dried Blood Spot (DBS) samples over a period of 24 h. Respective analyte concentrations were determined using liquid chromatography and high-resolution tandem mass spectrometry and semi-quantitative assays were characterized with regard to selectivity, limit of detection and identification, precision, linearity, and carry-over.

### **Preliminary data (results)**

Both propranolol and pseudoephedrine were identified in post-administration EB samples from female and male non-smokers as well as female and male smokers, and maximum detected drug levels ranged from 9 to 2847 pg/cartridge for propranolol and from 26 to 4805 pg/cartridge for pseudoephedrine. Corresponding DBS levels were in a range of 4-30 ng/mL for propranolol and 55-186 ng/mL for pseudoephedrine. Neither the consumption of cigarettes nor the sex appear to represent decisive criteria as to the detectability of propranolol or pseudoephedrine in EB, but interindividual variations regarding the detected drug levels were observed among all studied population groups. A cross-comparison with additionally collected DBS samples indicated that the varying concentrations detected in EB are not due to the varying resorption of the substances into the blood. Overall, EB is a promising matrix in the context of sports drug testing, but further research is required to characterize its benefits and limitations, as well as its application as a complementary matrix.

### **Please explain why your abstract is innovative for mass spectrometry?**

Exploring complementary biological matrices in the field of sports drug testing, e.g. Exhaled Breath, involves overcoming mass spectrometric challenges such as high sensitivity and unidentified matrix effects.

### **Co-authors:**

*Andreas Thomas, Center for Preventive Doping Research/Institute of Biochemistry, German Sport University Cologne*  
*Mario Thevis, Center for Preventive Doping Research/Institute of Biochemistry, German Sport University Cologne,*  
*European Monitoring Center for Emerging Doping Agents*

Poster number: AD-PA-013

## SPATIAL CHEMOMETRICS AND CORRELATIVE CHEMICAL IMAGING BASED MOLECULAR HISTOPATHOLOGY DELINEATES ANATOMICAL HETEROGENEITY AT CELLULAR LENGTH SCALES

Abstract ID: 376

**Presenting author: Patrick Wehrli, Department of Psychiatry and Neurochemistry, Sahlgrenska Academy at the University of Gothenburg**

### Introduction

Imaging mass spectrometry (IMS) is used to visualize the spatial distribution of molecules on a sample surface. In particular, matrix-assisted laser desorption/ionization (MALDI) IMS is a popular approach for studying biochemical processes related to disease pathology. Biochemical distribution maps obtained from the analysis of biological tissues can be matched to histological features, the technique has thus also been referred to as molecular histology.

We have been developing multimodal imaging strategies to investigate pathology associated distribution patterns of lipids and proteins in transgenic mouse models of Alzheimer's disease. In here, we present a continuation of these efforts by means of improved sample preparation routines, data processing workflows and advanced spatial chemometrics analysis for direct and unsupervised multivariate image analysis of multimodal IMS data.

### Methods

We applied the trimodal MALDI imaging workflow as previously described with improvements. Brain tissue sections of transgenic AD mice (tgSwe) were analyzed using four imaging modalities: matrix-assisted laser desorption/ionization (MALDI) IMS of lipids in (1) negative and (2) positive ion mode, (3) peptides in positive ion mode, and (4) hyperspectral fluorescence imaging of amyloid plaques. All four datasets were acquired on the same tissue section permitted by sample preparation workflows and analysis parameters. Data processing including image registration and spatial chemometrics were performed using MATLAB routines and SIMCA software.

### Preliminary data (results)

Improvements in sample preparation and imaging analysis workflows allowed quadrumodal dataset acquisition on a single biological tissue. Data processing workflows including accurate image registration allowed the application of novel cross-modality spatial chemometrics modeling at the measured image resolution. Comprehensive multimodal MSI data were evaluated using a novel approach based on multiblock orthogonal component analysis to identify joint and unique covariations between and within modalities revealing lipid-peptide relationships within AD pathology at MSI resolution. Finally, we predicted high-resolution ion distributions by image fusion<sup>6</sup> of MSI and pathological staining data to enhance histological interpretation.

### Please explain why your abstract is innovative for mass spectrometry?

We present novel correlative chemical imaging workflows enabling multimodal MSI data acquisition on a single tissue sample for interrogating lipid-peptide relationships within complex biological systems at cellular length scales.

### Co-authors:

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*Henrik Zetterberg, Department of Psychiatry and Neurochemistry, Sahlgrenska Academy at the University of Gothenburg, Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, Department of Neurodegenerative Disease, Queen Square Institute of Neurology, University College London, London, United Kingdom, UK Dementia Research Institute at UCL, London, United Kingdom*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



*Jörg Hanrieder , Department of Psychiatry and Neurochemistry, Sahlgrenska Academy at the University of Gothenburg,  
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United Kingdom*

Poster number: AD-PA-014

## CREATININE TAUTOMERISM AS PREDICTOR OF SOLUTION PHASE PRESERVATION IN THE GAS PHASE

Abstract ID: 388

**Presenting author: Maria Helena Florencio, FTICR and Structural Mass Spectrometry Lab of FCUL, MARE-Marine and Environmental Sciences Centre of FCUL**

### Introduction

The small creatinine compound ( $MW = 113 \text{ gmol}^{-1}$ ), a byproduct of muscle metabolism, is removed from the blood mostly by the kidneys. The serum creatinine level is the most widely used and commonly accepted measure of renal function in clinical medicine

### Methods

It is therefore expected that techniques such as liquid chromatography coupled to mass spectrometry (LC-MS), can be developed for the accurate quantification of creatinine in serum

### Preliminary data (results)

We are not presenting a more suitable strategy to deal with the complex serum samples where creatinine levels can be determined, but instead the behavior of creatinine weakly basic compound ( $pK_a = 4.83$  at  $25^\circ \text{ C}$ ) under electrospray ionization mass spectrometry (ESI-MS) conditions.

The behavior of creatinine in the gas phase has not been completely elucidated and remains almost unexplored experimentally at least. By direct injection of creatinine aqueous solutions in the electrospray ionization source we observed that the creatinine molecule softly ionizes, as expected, and the most abundant ions in the ESI-MS spectra are those corresponding to the dimerization (and trimerization) of creatinine forms .

The dimer cation identified, such as  $m/z$  307 ions (Fig. 1), includes in its structure the interaction of one zwitterionic creatinine form (enolate) and one non-zwitterionic creatinine form (keto). In the dimer cation composition the formation of a salt bridge in deprotonated creatinine dimer cations structures is possible. This behavior has been observed in the gas phase for deprotonated glycine dimer anions. Moreover, in the creatinine dimer cations composition two water molecules can be seen (Fig. 1). Solution theory reveals that interconversion of the two creatinine tautomers occurs by a relay mechanism in which two water molecules play the role of "bridge" for proton transfer. It appears that the predicted solution behavior for creatinine tautomers interconversion is preserved in the gas phase. Moreover creatinine tautomers interconversion can give us information on how much from the solution behavior is preserved in the gas phase.

### Please explain why your abstract is innovative for mass spectrometry?

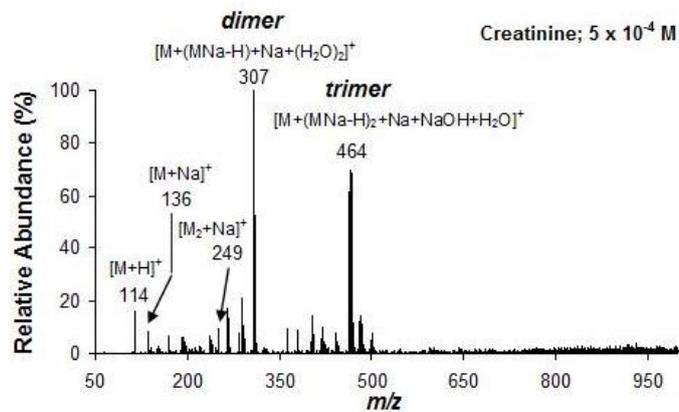
The presented system is important due to the creatinine biological relevance besides that it can also be used to better understand the mechanism of electrospray ionization. This work is financed by national funds through FCT \_ Fundação IP, under the project UIDB/04292/2020.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

**Co-authors:**

Marco Antonio Saraiva, Technical Institute, IST, Technical Institute, IST, Structural Chemistry Centre



Can be used to better understand the electrospray ionization mechanism

Poster number: AD-PA-015

## UNIDIRECTIONAL DOUBLE AND TRIPLE HYDROGEN REARRANGEMENT REACTIONS PROBED BY IR ION SPECTROSCOPY

Abstract ID: 482

Presenting author: Mathias Schäfer, University of Cologne, Department of Chemistry

### Introduction

Unidirectional double- (2H) and triple-hydrogen (3H) rearrangement reactions, *i.e.*, unimolecular gas-phase redox processes had been found to be predominant fragmentation pathways in electron ionization (EI-) mass spectrometry of electron-rich 2-benzylindanols by Kuck and Filges.[1,2] In the present work, we investigated the structure of the product ions of the 2H and 3H rearrangement reactions by an independent experimental approach. We used ESI-MS to produce the molecular radical cations and MS<sup>2</sup> experiments combined with IR ion spectroscopy and theory to identify the reaction products and thereby propose modified mechanisms for these complex fragmentation reactions.

### Methods

The radical cations of *trans*-2-(4-*N,N*-dimethylaminobenzyl)-1-indanol and 4-(4-*N,N*-dimethyl-aminophenyl)-2-butanol as well as that of 1-(4-*N,N*-dimethylaminophenyl)-5-(4-methoxyphenyl)-3-pentanol were generated by electrospray ionization (ESI) from anhydrous acetonitrile solutions and served as precursor ions for collision-induced dissociation (CID) experiments. The 2H and 3H fragment ions were obtained by ESI-MS<sup>2</sup> experiments in a quadrupole ion trap. The respective product ions, C<sub>9</sub>H<sub>14</sub>N<sup>+</sup> and C<sub>8</sub>H<sub>13</sub>N<sup>+</sup>, were characterized by IR ion spectroscopy and DFT calculations.[3]

### Preliminary data (results)

The radical cationic molecular ions of several precursor compounds were formed by ESI from anhydrous solutions.[4] The product ions of the specific 2H and 3H rearrangement reactions were generated upon CID in an ion trap, which underlines that the 2H and 3H rearrangement reactions occur not only under EI but also under ESI-MS<sup>2</sup> CID conditions. Comparison of the experimental and calculated IR ion spectra allowed us to identify the 2H rearrangement product ion, C<sub>9</sub>H<sub>14</sub>N<sup>+</sup> (*m/z* 136), as the *N,N*-dimethyl-*para*-toluidinium ion bearing the extra proton *ortho* to the amino group. The open-shell 3H rearrangement product ion, C<sub>8</sub>H<sub>13</sub>N<sup>+</sup> (*m/z* 123), was initially assumed to be a distonic ion, Me<sub>2</sub>HN<sup>+</sup>-C<sub>6</sub>H<sub>6</sub>·.[1] Our results indicate that it is actually a conventional radical cation, *viz.*, ionized *N,N*-dimethyl-2,3-dihydro-*para*-toluidine, Me<sub>2</sub>N-C<sub>6</sub>H<sub>7</sub><sup>+</sup>. Thus, the 3H rearrangement reaction represents an intramolecular redox process that involves non-catalyzed transfer hydrogenation between a secondary alcohol and an ionized aromatic ring. More detailed mechanisms for the unidirectional 2H and 3H rearrangement reactions are proposed.[3]

[1] Kuck, D.; Filges, U., *Org. Mass Spectrom.* **1988**, 23, 643–653.

[2] Kuck, D.; Salameh, L. C.; Onwuka, K. I.; Letzel, M. C., *Eur. J. Mass. Spectrom.* **2014**, 20, 51–61.

[3] D. Zeh, M. Bast, J. Martens, G. Berden, J. Oomens, S. Brünken, S. Schlemmer, M. Schäfer, D. Kuck, *J. Am. Soc. Mass Spectrom.*, **2022**, submitted.

[4] M. Schäfer, M. Drayß, A. Springer, P. Zacharias, K. Meerholz, *Eur. J. Org. Chem.* **2007**, 5162-5174.

### Please explain why your abstract is innovative for mass spectrometry?

Mechanisms of unidirectional 2H and 3H rearrangement reactions are investigated. Characteristic fragment ions were structurally characterized by IR ion spectroscopy and theory.

### Co-authors:

Dennis Zeh, University of Cologne, Department of Chemistry  
 Marcel Bast, University of Cologne, I. Physikalisches Institut

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

Dietmar Kuck, University Bielefeld, Department of Chemistry

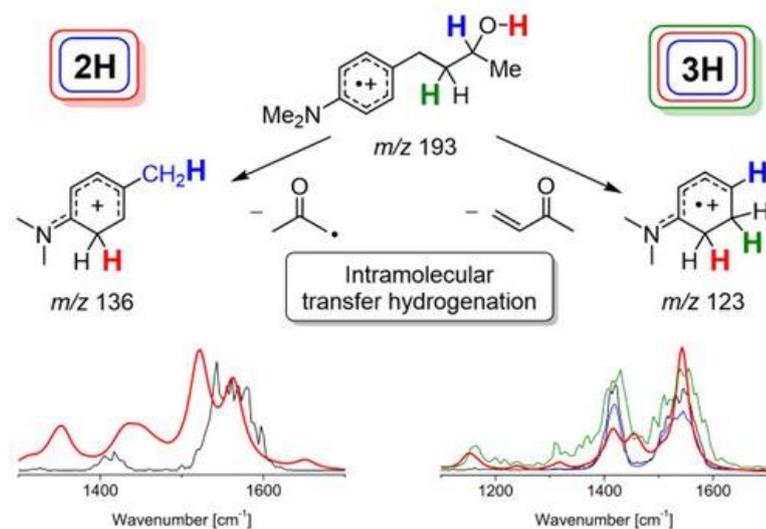
Sandra Brünken, Radboud University, Institute for Molecules and Materials, FELIX Laboratory

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Giel Berden, Radboud University, Institute for Molecules and Materials, FELIX Laboratory

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2H and 3H rearrangement products investigated by IR ion spectroscopy.

Poster number: AD-PA-016

## OXYGEN <sup>16</sup>O/<sup>18</sup>O ISOTOPE EXCHANGE FOR SMALL MOLECULE LC-MS BASED IDENTIFICATION

Abstract ID: 542

Presenting author: Sergey Osipenko, Skoltech

### Introduction

LC-MS based workflows to identify chemical compounds operate with retention time, accurate mass and fragmentation spectra, which are compared to reference values of pure chemicals standards. This information is often insufficient or unavailable and new features for identification are desired. One of such features is the number of certain functional groups of a molecule that can be counted via derivatization, particularly with isotope exchange techniques. Hydrogen/deuterium exchange is used widely for mass spectrometry, while oxygen <sup>16</sup>O/<sup>18</sup>O exchange is less investigated. Nevertheless, it is known that some functional groups may be easily exchanged in <sup>18</sup>O enriched media. Here we study the capabilities of <sup>16</sup>O/<sup>18</sup>O isotope exchange to enhance small molecule LC-MS based identification.

### Methods

For isotope exchange the sample containing the analytes of interest was dried, redissolved in H<sub>2</sub><sup>18</sup>O:acetonitrile (7:3) mixture with 1% TFA, and incubated at 37°C and 95°C for 24 h, and then analyzed using Waters ACQUITY UPLC system coupled with Thermo Q Exactive mass spectrometer in DDA mode with stepped collision energy. Separation was done on a ACQUITY UPLC BEH C18 column in gradient mode at 0.4 mL min<sup>-1</sup> flow rate. MS analysis was performed in ESI positive mode with ESI voltage 4.5 kV. All scripts were prepared using Python and rdkit library.

### Preliminary data (results)

Enumeration of “exchangeable” groups was performed for a set of 100 compounds. Incorporation of <sup>18</sup>O was observed for carbonyl groups, some carboxyl groups, and for hydroxyl groups in benzyl and allyl positions at 37°C making it possible to operate with biological samples. Increasing the temperature to 95°C resulted in deeper exchange of some groups. For other oxygen containing groups (phenols, esters, amides, alcohols) exchange was not observed. Although for most “exchangeable” groups we have found several exceptions, the experimentally observed number of exchanges validated the maximum number calculated from the structure of a candidate thus being a filter to eliminate isomeric species. For warfarin we observed one mass shift in the spectrum, corresponding to the introduction of <sup>18</sup>O atom. Calculating maximum possible exchanges for warfarin isomers (1611 molecules in PubChem) allowed filtering out 553 molecules (34%) without exchangeable groups. Such search space reduction was calculated for 45 molecules, and it was 9-92% (median 62%).

As <sup>18</sup>O label is relatively stable, MS/MS data can be used to filter candidates. We implemented an approach of mapping fragment formulas on the candidate structure. If a fragment can not be mapped on the parent structure, such candidate should be considered false-positive. As such method does not account for rearrangements, heavy fragments are preferred for this procedure. Labeled data allows eliminating false identities. For warfarin with non labeled MS/MS data we could eliminate 31 more candidates, while with labeled MS/MS data 236 additional molecules were filtered out.

### Please explain why your abstract is innovative for mass spectrometry?

The <sup>16</sup>O/<sup>18</sup>O exchange in mild conditions with computational approach to use tandem MS data in identification process is proposed.

### Co-authors:

Alexander Zhrebker, Skoltech

Lidia Rumiantseva, Skoltech

Oxana Kovaleva, Skoltech

Eugene Nikolaev, Skoltech

Yury Kostyukevich, Skoltech

Poster number: **AD-PA-017**

## **VALIDATION OF THE METABOLITE ERGOTHIONEINE AS A FORENSIC MARKER IN BLOODSTAINS**

Abstract ID: **767**

**Presenting author: Jiyeong Lee, Department of Biomedical Laboratory Science, College of Health Science, Eulji University - Uijeongbu Campus, Uijeongbu, Republic of Korea**

### **Introduction**

Metabolites are commonly targeted substances for drug analysis, mainly in the forensic science field. However, there are studies that have attempted to obtain various information through metabolites in bloodstains to reconstruct the crime scene. Ergothioneine in bloodstains is a potential forensic marker for estimating the age of bloodstain and the bloodstain donor's age. In this study, a quantitative analysis of the ergothioneine in the bloodstain is conducted to confirm the usability of ergothioneine as a forensic marker for estimation of the elapsed time of bloodstain and age marker of bloodstain donor based on the quantitative results of the ergothioneine.

### **Methods**

Blood samples were prepared by obtaining venous blood from 35 participants. Bloodstains were formed by fresh blood directly dripped onto filter paper. The bloodstain samples were stored away from light in a room, under temperature  $22.9 \pm 1.1$  °C and relative humidity  $64.9 \pm 10.9\%$ , for up to 7 days. Metabolites were extracted from the bloodstains on days 0, 1, 2, 3, 5, and 7. The metabolites in the sample were quantified through multiple reaction monitoring (MRM) analysis. After obtaining the quantitative results, statistical analysis was performed for comparison between groups. MetaCyc open databases were used for pathway analysis.

### **Preliminary data (results)**

#### 1. Concentration of ergothioneine in fresh blood

The concentration of ergothioneine in bloodstains was compared by categorizing participants into the youth group and the elderly group. Ergothioneine was present at a higher concentration in the bloodstain of the elderly group than in the youth group. Additionally, when participants were grouped by sex, the ergothioneine concentrations in day 0 bloodstains from both male and female elderly groups tended to be higher than the youth groups.

#### 2. Changes in ergothioneine concentration in aged bloodstain and estimation of age of bloodstain donors

As a result of statistical analysis to compare whether the concentration of ergothioneine between each time point was significantly different, there was a significant difference on days 2 and day 3 when compared with day 0. In addition, The difference in ergothioneine concentration between the youth and elderly groups at days 0, 5 and 7 was statistically significant.

#### 3. Time-dependent concentration changes in histidine and mercynine concentrations in bloodstains

In the changes in the concentrations of histidine, mercynine, and ergothioneine present in the ergothioneine biosynthesis pathway in bloodstains left in an in vitro environment. The concentration of mercynine that can be produced from histidine in ergothioneine-producing organisms gradually decreased until day 2 and maintained until day 7. Ergothioneine, which can be generated from mercynine, decreased until day 2 and showed a tendency to increase after day 3, and it was confirmed that the trend from day 3 to day 7 was reversed with histidine.

### **Please explain why your abstract is innovative for mass spectrometry?**

Based on metabolite quantitative information obtained through mass spectrometry, useful information can be provided for crime scene reconstruction in the field of forensic science.

## POSTER SESSION A

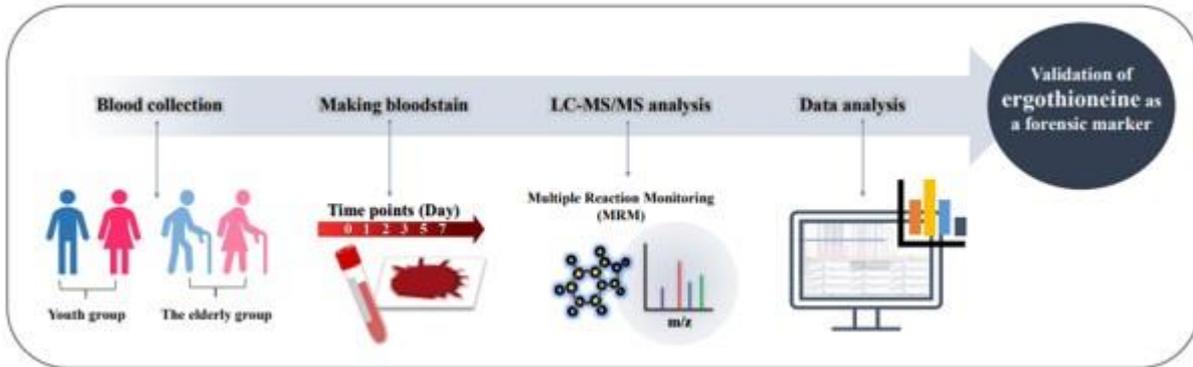
Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Co-authors:

Seungyeon Lee, Department of Senior Healthcare, Graduate School, Eulji University - Uijeongbu Campus, Uijeongbu, Republic of Korea

You-Rim Lee, Department of Senior Healthcare, Graduate School, Eulji University - Uijeongbu Campus, Uijeongbu, Republic of Korea

Hee-Gyoo kang, Department of Biomedical Laboratory Science, College of Health Sciences, Eulji University, Seongnam, Republic of Korea



Experimental workflow

Poster number: **AD-PA-018**

## **DISCOVERY OF THE FORENSIC MARKER FOR ESTIMATION OF THE AGE OF BLOODSTAIN USING METABOLOMIC APPROACH**

Abstract ID: **768**

**Presenting author: Hee-Gyoo Kang, Department of Biomedical Laboratory Science, College of Health Sciences, Eulji University, Seongnam, Republic of Korea**

### **Introduction**

The bloodstains generated by blood leakage are commonly found at the scene of an incident and are valuable evidence that provides information related to the incident. Data on changes in the components of the bloodstain according to the time elapsed after bloodstain deposition can be used to estimate bloodstain age, which serves as useful information for reconstruction of the event site, such as determining the time when the blood leaked or the event occurred. The purpose of this study is discovery of metabolite markers that can estimate the age of bloodstain in a week unit from the bloodstains aged up to 28 days.

### **Methods**

The discovery set consisted of 10 males and 10 females, and the validation set consisted of 16 males and 24 females. Fifty microliters of the blood dropped onto filter paper to generation bloodstains. All bloodstain samples kept at room temperature ( $23.5 \pm 0.8^\circ\text{C}$ ) and relative humidity ( $61.0 \pm 6.1\%$ ) for 28 days. The metabolites in bloodstains were extracted at a predetermined time point. Then, the extracted metabolites were analyzed by HPLC-MS/MS. After that, statistical analysis was performed for comparison between days based on the obtained mass spectrometry data. Candidate metabolites selected through statistical analysis were validated using multiple reaction monitoring (MRM).

### **Preliminary data (results)**

To observe the change of metabolites in bloodstains over time and compare the metabolite concentrations between days, a total of 10 combinations were created by grouping 2 of day conditions each through volcano plot analysis and sparse partial least squares discriminant analysis (sPLS-DA). In the volcano plot analysis, molecular features (MFs) that passed fold change  $> 2.0$  and  $p\text{-value} < 0.05$  conditions were listed then, in the sPLS-DA, MFs constituting the first component that contributed the most to the distinction between day conditions were listed. As a result, a total of 57 metabolites showed statistically significant changes over time in the integrated MF list of the volcano plot analysis and the sPLS-DA. After identifying these metabolites in an open database, three metabolites were finally selected as bloodstain age estimation markers and validated using multiple reaction monitoring (MRM). In the case of 5'-AMP, a significant difference was confirmed in comparison of day 0 versus day 7, 14, and 21 and day 7 versus day 14, 21, and 28. For choline, there was a significant difference between day 0 versus all other days, and day 7 versus days 14 and 28. In the case of pyroglutamic acid, a significant difference was confirmed in comparison of day 0 vs. day 7, 21 and day 7 vs. day 14, 21, and 28. In comparison combinations after day 14, the concentration of the three metabolites did not differ significantly between days, but in common, day 0 versus day 7 and day 7 versus day 28 showed significant differences.

### **Please explain why your abstract is innovative for mass spectrometry?**

Mass spectrometer with high throughput, high accuracy and precision can analyze metabolites in bloodstains simultaneously. Therefore, crime-related information contained in various bloodstain metabolites can be obtained through one analysis.

### **Co-authors:**

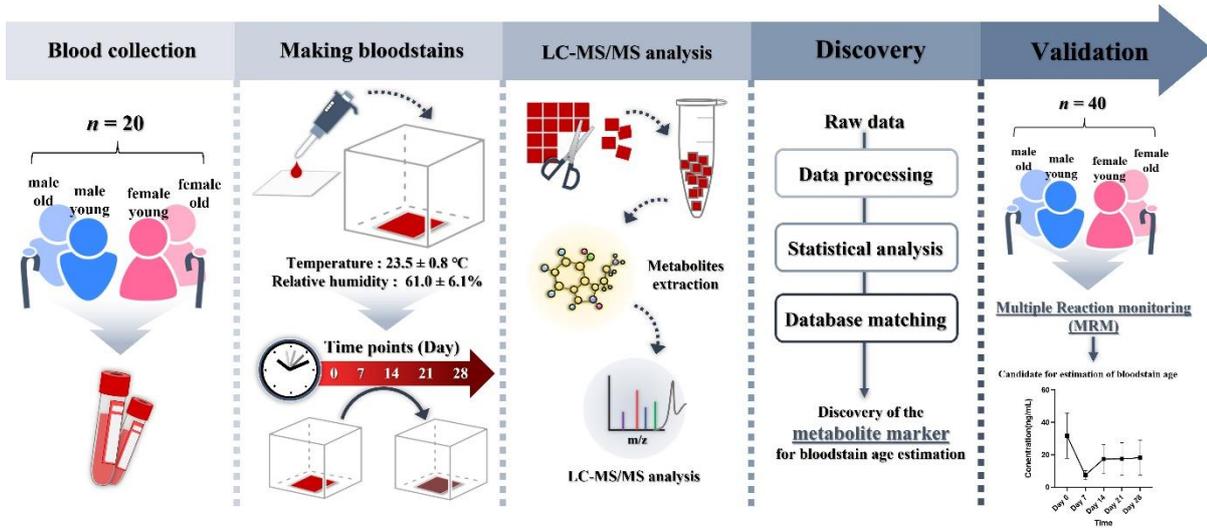
*Seungyeon Lee, Department of Senior Healthcare, Graduate School, Eulji University - Uijeongbu Campus, Uijeongbu, Republic of Korea*

*You-Rim Lee, Department of Senior Healthcare, Graduate School, Eulji University - Uijeongbu Campus, Uijeongbu, Republic of Korea*

*Jiyeong Lee, Department of Biomedical Laboratory Science, College of Health Science, Eulji University - Uijeongbu Campus, Uijeongbu, Republic of Korea*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Experimental workflow

Poster number: AD-PA-019

## ISOTOPE ANALYSIS OF U AND PU FOR 6TH COLLABORATIVE MATERIALS EXERCISE (CMX-6) SAMPLES BY MC-ICP-MS IN KAERI

Abstract ID: 909

**Presenting author: Ranhee Park, Environment and Disaster Assessment Research Division, Korea Atomic Energy Research Institute**

### Introduction

Nuclear forensics is the investigating activity of nuclear materials to find evidences for their sources, illegal trafficking possibility, nuclear materials (NM) enrichment, etc. In the nuclear forensic R&D, the mass spectrometry can be a powerful technique to obtain inherent NM information. Every two years, the Nuclear Forensics ITWG (International Technical Working Group) organizes a scenario based training program named Collaborative Materials eXercise (CMX) in order to enhance international nuclear forensic capability. Typically, each required analysis result derived from CMX samples shall be reported within the determined timeframes of 24 hours, a week or two months since the seizure of unknown NM. KAERI has participated in the sixth CMX program. In this paper, we present mainly isotope ratios of CMX-6 samples measured by MC-ICP-MS.

### Methods

KAERI has received two samples in plastic bags. According to our analysis plan, leaching of samples was good enough to perform isotope analysis. For leaching, the ingot taken out of the pipe was immersed in 8M nitric acid. 0.01M HCl and 0.02M  $\text{NH}_2\text{OH} \cdot \text{HCl}$ /Ascorbic acid in 2M  $\text{HNO}_3$  were used for effective U and Pu chemical separation through UTEVA resin. The U and Pu isotope ratios were measured using a highly-sensitivity MC-ICP-MS equipped with a desolvation system. Since MC-ICP-MS can measure simultaneously target isotopes using multi-detectors, U and Pu isotopes has been measured at the same time.

### Preliminary data (results)

Within 24 hours, KAERI has reported the physical characterization such as dimension, mass, and photography of CMX-6 samples including their preliminary elemental analysis from XRF. The 1-week report included elemental analysis result obtained by ICP-OES using the leached solution. In the ICP-OES results, the ingot in the ES-1 is composed of large amount of Ce, Y, and Fe and that in the ES-2 is mainly composed of U, Y and Fe. From these results, the main elements and concentration could be identified to determine an appropriate dilution factor. For the 2-month report, U and Pu were chemically separated from samples after acid digestion. Then the isotope ratios with a high precision were analyzed by MC-ICP-MS. For measurement by MC-ICP-MS, U and Pu eluents were diluted to 100 ppt and 1-2 ppt, respectively. In the ES-1, the atom ratio of U were measured at  $^{235}\text{U}/^{238}\text{U} = 2.075[0.007] \times 10^{-3}$ . The atom ratio of Pu in ES-1 were measured at  $^{240}\text{Pu}/^{239}\text{Pu} = 6.14[0.04] \times 10^{-2}$ . In the ES-2, the atom ratio of U were measured at  $^{235}\text{U}/^{238}\text{U} = 2.051[0.007] \times 10^{-3}$ , and the atom ratio of Pu were measured at  $^{240}\text{Pu}/^{239}\text{Pu} = 6.15[0.03] \times 10^{-2}$ . Based on these results, the CMX-6 samples were identified to contain the depleted U and the weapon grade Pu. This conclusion agreed with the consensus data from 20 participating laboratories worldwide.

### Please explain why your abstract is innovative for mass spectrometry?

Mass spectrometric techniques have played important roles in the safeguards and nuclear forensics fields. Here, we demonstrated highly accurate isotopic ratio measurement of uranium and plutonium at sub-ppt levels.

### Co-authors:

*Sang Ho Lim, Nuclear Chemistry Research Team, Korea Atomic Energy Research Institute*  
*Jungwoon Choi, Environment and Disaster Assessment Research Division, Korea Atomic Energy Research Institute*  
*Kyuseok Song, Environment and Disaster Assessment Research Division, Korea Atomic Energy Research Institute*  
*Kun Ho Chung, Environment and Disaster Assessment Research Division, Korea Atomic Energy Research Institute*

Poster number: AD-PA-020

## SYNTHESIS AND MASS SPECTROMETRIC CHARACTERIZATION OF THE SELECTIVE ANDROGEN RECEPTOR MODULATOR SARM 2f FOR DOPING CONTROL PURPOSES

Abstract ID: 936

Presenting author: Tristan Möller, Institute of Biochemistry/ Center for Preventive Doping Research, German Sports University Cologne

### Introduction

In recent years, selective androgen receptor modulators (SARMs) have become an emerging class of drugs investigated for the treatment of several diseases such as sarcopenia, cachexia or muscular dystrophy. Due to their anabolic effects on muscle and bone, SARMs pose a potential for misuse in sports and are prohibited by the World Anti-Doping Agency (WADA) at all times. The novel SARM 2f (4-((2*R*,3*R*)-2-ethyl-3-hydroxy-5-oxopyrrolidin-1-yl)-2-(trifluoromethyl)benzonitrile) shows beneficial effects on muscle growth in numerous animal models, such as rodent models or cynomolgus monkeys, which warrants consideration in preventive doping research.

### Methods

In order to synthesize SARM 2f for doping control purposes, a multi-step synthesis was conducted starting from commercially available *trans*-hexenoic acid **1**. Additionally, an appropriate internal standard (4-((2*R*,3*R*)-3-hydroxy-2-methyl-5-oxopyrrolidin-1-yl)-2-(trifluoromethyl)benzonitrile) **7b** was synthesized, using an analog mythology starting from methyl *trans*-pent-3-enoate **2b**. All reaction steps were monitored, using either RP-LC/HRMS or GC/HRMS.

### Preliminary data (results)

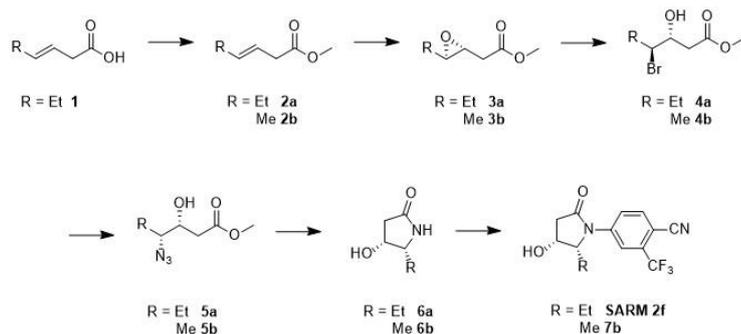
SARM 2f as well as the internal standard **7b** were synthesized using six, respectively five reaction steps. Mass spectrometric data was obtained for each intermediate and all steps resulted in good to excellent yields. The Buchwald-Hartwig-Amination reaction step 6 shows formation of undesired by-products, which cannot be separated using preparative LC-UV. The usage of suitable protection groups may increase the selectivity of this reaction in order to obtain pure products, which is necessary for doping control analysis.

### Please explain why your abstract is innovative for mass spectrometry?

The synthesised compounds SARM 2f and **7b** can be implemented into doping control analysis and will therefore improve anti-doping testing results.

### Co-authors:

Mario Thevis, Institute of Biochemistry/ Center for Preventive Doping Research, German Sports University Cologne, European Monitoring Center for Emerging Doping Agents (EuMoCEDA) Cologne/Bonn



Multi-step synthesis of SARM 2f and internal standard **7b**.

## Theme: Biomaterials

### Session: Polymers and synthetic macromolecules

Poster number: **BM-PA-001**

## MASS SPECTROMETRY STUDY OF THE PHOTOISOMERIZATION AND THERMAL BACK-ISOMERIZATION OF AZOBENZENE-FUNCTIONALIZED PEPTOIDS FOR THE CHEMICAL STORAGE OF SOLAR ENERGY

Abstract ID: 77

Presenting author: Benjamin Tassignon, University of Mons

### Introduction

Storing solar energy represents a major challenge in modern science. Chemical storage with **MO**lecular **SOL**ar **THER**mal systems (**MOST**) appears promising though challenging. The working principle of those systems is based on iterative closed cycles of photoisomerization and back-isomerization between a parent compound and its metastable isomer (**Figure 1**). Energy is stored within the metastable isomer which possesses a certain half-life time and thermal energy is released during the thermal back-isomerization process. Among the MOST systems, azobenzene with its E to Z photoisomerization has been studied but characteristics such as storage enthalpy and half-life time must be improved. To do so, anchoring chromophores on a macromolecular backbone appears to be an elegant strategy since cooperative effects between chromophores may help enhancing these properties.

### Methods

We are nowadays exploring the possibility of preparing MOST systems based on a peptoid-type backbone supporting different azobenzene chromophores incorporated at key positions. We will first describe the synthesis and MS/(MS)-based characterization of original peptoids realized on a Waters QToF Premier. We will further present the LC-MS methodology developed for measuring first-order kinetics of photoisomerization and thermal back-isomerization at different temperatures in solution, allowing for the photoisomer half-life time determination as well as the back-isomerization activation barrier. These values in solution will be compared to values obtained for gas-phase back-isomerization kinetics by tandem IMS on an original instrument.

### Preliminary data (results)

We synthesized different peptoids containing one or more azobenzene(s) at key positions with solid support protocol. Primary structures of the molecules were confirmed with MS and MS/MS analyses based on B/Y and A/Y fragmentation patterns. With irradiation, different isomers are produced and their relative proportions against irradiation time are monitored using LC-MS analyses. The same method was applied for thermal back-isomerization. Determination of half-life times shows that peptoids containing only one azobenzene behave similarly as pristine azobenzene ( $t_{1/2}$  between 40 and 50 hours at 20 °C). However, when two or more azobenzenes are incorporated on the peptoid chain, the half-life times significantly increase. We were able to achieve half-life time up to 290 hours in solution when 5 azobenzenes are incorporated, showing cooperativity effects between chromophores.

Using tandem IMS on an original instrument (at Université Lyon) where the drift tubes are separated by a heated trap, we were able to determine rate constants at different temperatures in gas phase for a peptoid containing one azobenzene (**Figure 2**) and obtained an activation barrier ( $111 \text{ kJ}\cdot\text{mol}^{-1}$ ) close to that of pristine azobenzene in solution (between 95 and  $110 \text{ kJ}\cdot\text{mol}^{-1}$ ).

LC-MS analyses at different temperatures are currently performed to obtain activation barriers in solution and to compare them to gas-phase data.

### Please explain why your abstract is innovative for mass spectrometry?

Use of tandem IMS to determine photoisomer half-life times, which is crucial in the MOST field.

Co-authors:

Julien De Winter, University of Mons  
 Fabien Chirot, University of Lyon  
 Philippe Dugourd, University of Lyon  
 Jérôme Cornil, University of Mons  
 Pascal Gerbaux, University of Mons

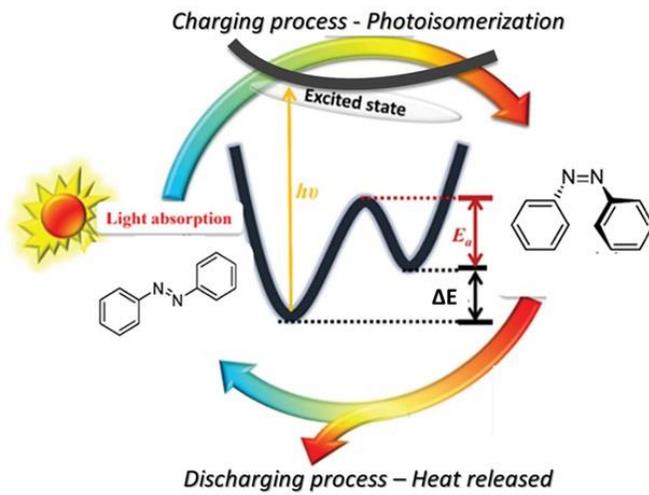
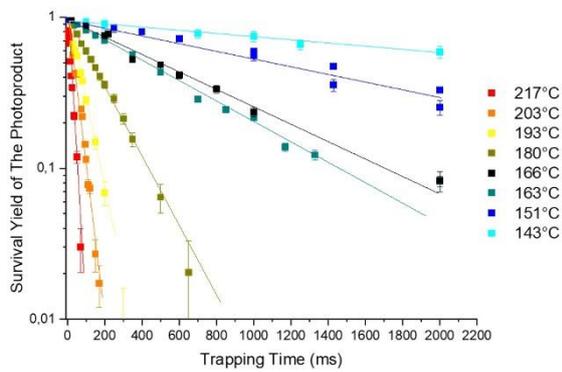


Figure 1. Working principle of azobenzene as a MOST system.

a) Kinetics at different temperatures



b) Eyring's plot

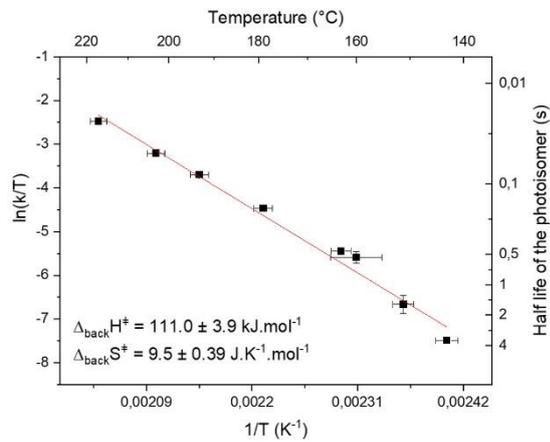


Figure 2. Kinetics study at different temperatures using tandem IMS.

Poster number: **BM-PA-002**

## **IMITATION IS THE HIGHEST FORM OF FLATTERING SWAMP-MS: A NEW SYSTEMATIC WORKFLOW OF ANALYZING MULTIPLE- FRAGMENTED POLYMERS WITH MASS SPECTROMETRIC**

Abstract ID: 78

**Presenting author: Ynze Mengerink, DSM - ACC**

### **Introduction**

Sequence distributions (SD) of monomers in synthetic polymers are not easy to determine and often so-called triads (three consecutive monomeric units) are measured with NMR to evaluate randomness [1]. MSMS on intact molecules can be used for SD evaluation but all examples in open literature are based on selection of well-defined m/z values. These are typically below 5kDa as higher masses are difficult to isolate for fragmentation purposes

[1] J. Devaux, P. Godard, J. P. Mercier, *Journal of Polymer Science: Polymer Physics Edition*, Vol. 20, 1875-1880 (1982)

### **Methods**

A limited number of references reported a broad MS signal after ESI of supercharged synthetic polymers (Mw=20-40kDa) in the m/z 200-2000Da range [2,3]. The broad molecular weight distribution, possible different monomers, isotope distributions, and the charge distribution over the before mentioned molecules swamp the ESI-spectrum into an unreadable Mass Spectrum.

[2] Y. Mengerink, R. Peters, C.G. de Koster, Sj. van der Wal, H.A. Claessens, C.A. Cramers, *J. Chromatography A*, 914(2001)131-145

[3] A. Ghaffar, G.J.J. Draaisma, G. Mihov, A.A. Dias, P.J. Schoenmakers, Sj. van der Wal, *Biomacromolecules*12 (2011) 3243-3251

### **Preliminary data (results)**

By applying the SWAMP-MS workflow (MSMS related) on the supercharged synthetic polymers, highly detailed chemical information of the complete polymer can be obtained, including never revealed sequence distributions up till 20 consecutive monomeric units.

Analogousness and differences towards the SWATH-MS [4] workflow for complex protein samples will be discussed

[4] L.C. Gillet, P. Navarro, S. Tate, S., H. Roest, N. Selevsek, L. Reiter, R. Bonner, and R. Aebersold, *Mol. Cell Proteomics* 2012, 11(6):O111.016717

### **Please explain why your abstract is innovative for mass spectrometry?**

Highly detailed chemical information of synthetic polymers can be obtained, including never revealed sequence distributions up till 20 consecutive monomeric units .

### **Co-authors:**

*Jan Jordens, Vito*

*Harry Philipsen, DSM - ACC*

*Rob van der Hoeven, DSM - DFS*

*Ron Peters, Covestro Innovation Group, University of Amsterdam*

Poster number: **BM-PA-003**

## MASS SPECTROMETRY AND PHOTOCHEMICAL STUDY OF PHOTOISOMERIZATION AND THERMAL BACK-ISOMERIZATION OF SUBSTITUTED AZOBENZENE ANCHORED ON PEPTOIDS FOR THE CHEMICAL STORAGE OF SOLAR ENERGY

Abstract ID: 157

Presenting author: Gwendal Henrard, University of Mons

### Introduction

Molecules that undergo light-induced isomerization to a metastable isomer can be used to store solar energy. Such systems are known as **MO**lecular **SOL**ar **THER**mal systems. Exposing compound to sunlight generates a high energy photoisomer whose lifetime is considered as a key criterion for storage purpose. When energy is needed, the photoisomer is converted back to the stable compound, releasing the excess energy in the form of heat. **Azobenzenes (ABs)** with their E  $\leftrightarrow$  Z photoisomerization are among the most widely studied molecular photoswitches. Properties such as storage enthalpy and half-life time need to be improved. To do so, grafting azobenzenes at selected positions all along a polymer backbone appears to be an elegant strategy to enhance these properties thanks to cooperative effects between the chromophores.

### Methods

Anchoring AB photoswitches on a peptoid chain is performed using an on-resin step-by-step synthetic procedure allowing to incorporate different side chains at selected positions (**Figure 1**). To strengthen the inter side-chain interaction, we intend to incorporate H-bond donors and acceptors on the pending chains to create an H-bond network. The original peptoids presented in Figure 1 are subjected to UV photoillumination experiments. The photoisomers are analyzed using LC-MS experiments that allow to separate/identify the photo-products. Kinetics of E-to-Z and Z-to-E isomerization, composition of the photostationary states and half-time ( $t_{1/2}$ ) of the metastable isomers are here originally determined using LC-MS.

### Preliminary data (results)

Several peptoids ranging from 3 to 6 units and bearing 1 or 2 azobenzene side chains were prepared using a solid-phase protocol. Deprotection of the carboxylic acid groups, without affecting the peptoid integrity, failed so that we focused on peptoids bearing esterified azobenzene residues. All prepared azobenzene monomers and peptoids were subjected to UV-vis illumination and the evolution of the photoisomer distribution, including the photostationary state distribution (PSD) determination, has been conducted using LC-MS analysis. The thermal retro isomerization process was also monitored over time using LC-MS (**Figure 2**). The chromatogram presented in **Figure 2** presents 4 peaks that can respectively be associated to the ZZ, ZE (and/or EZ) and EE isomers. The experimental data demonstrated that the substitution of the azobenzene ring with an ester function slightly increases the proportion of the metastable isomers in the PSD. The determined thermal retro-isomerization kinetics revealed that the 6-unit peptoid carrying 2 chromophores possesses a longer metastable isomer lifetime than the 3-unit peptoid with a unique chromophore. Globally, our investigation attests that our strategy, based on the incorporation of multiple interacting chromophores all along a peptoid backbone, is promising for the development of efficient MOST systems.

### Please explain why your abstract is innovative for mass spectrometry?

Use of LC-MS for the on-line and off-line study of photoisomerization processes

### Co-authors:

*Benjamin Tassignion, University of Mons*

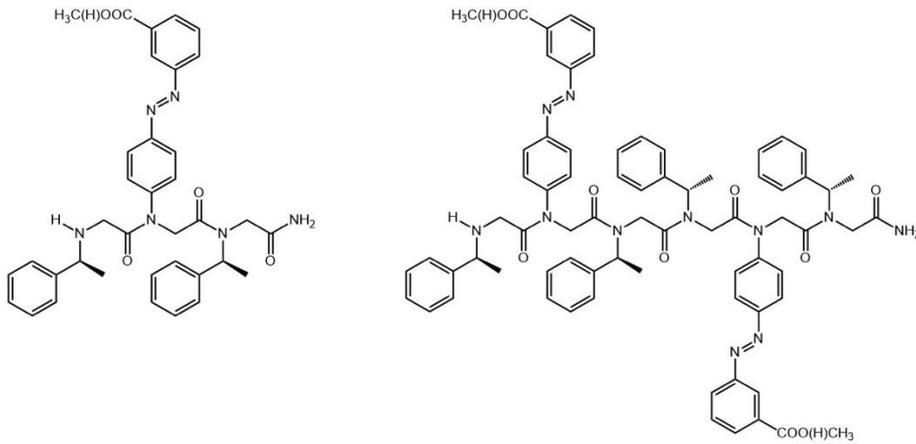
*Julien De Winter, University of Mons*

*Jérôme Cornil, University of Mons*

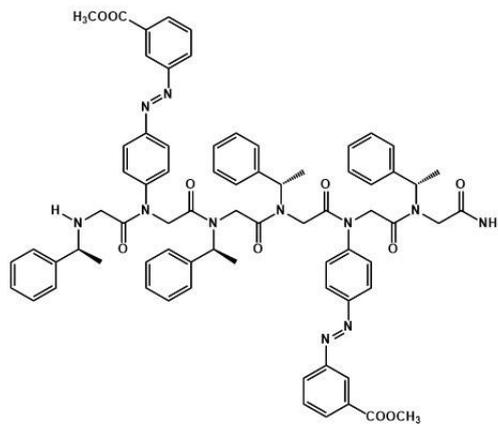
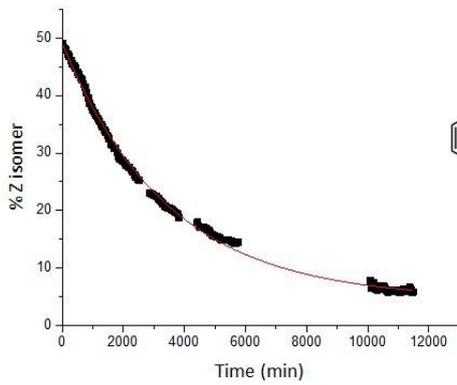
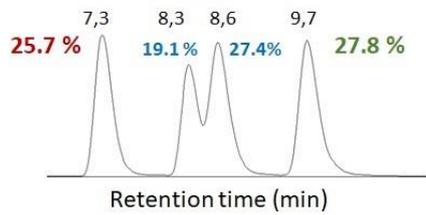
*Pascal Gerbaux, University of Mons*

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Targeted azobenzene-decorated peptoids for MOST applications



Monitoring of the photoisomerization by LC-MS

Poster number: **BM-PA-004**

## APPLICATION OF MALDI-TOF MS FOR THE STRUCTURAL IDENTIFICATION OF NEW POLYOL BUILDING BLOCKS FOR POLYURETHANES

Abstract ID: 265

**Presenting author: Katalin Czifrák, University of Debrecen, Department of Applied Chemistry, 4032, Egyetem tér 1**

### Introduction

The synthesis of poly(*w*-pentadecalactone) (PPDL) and poly(*d*-valerolactone) (PDVL) (co)polymers, or amine-diglycidyl ether bisphenol-A oligomer (A-DGEBA) were carried out by different polymerization technics. The new polyols were incorporated into segmented polyurethanes chains. The structural identification of new polyols was performed by MALDI-TOF MS. The use of new types of polyols in the synthesis of crosslinked polyurethanes provides new switching elements in shape memory programming. These novel crosslinked polyurethanes systems may find their applications among the well-tuned  $T_m$ -based shape memory and self-healing polymers.

### Methods

The polymerization of PPDL and PDVL was carried out without solvent by the reaction of *w*-pentadecalactone (1 eq.), diol (1 eq.) and catalysator (2 mol %) at 100 °C for 2 days. The polymerization of DGEBA resin was performed by the reaction of mono- or diamine (2 eq.) and DGEBA (1 eq.) in dry toluene at 100 °C for 24 h. The obtained oligomers were analyzed by MALDI-TOF MS. For the MALDI-TOF MS measurements a Bruker BIFLEX III<sup>TM</sup> mass spectrometer equipped with a time-of-flight (TOF) mass analyzer was used. The ions were detected in the reflectron mode.

### Preliminary data (results)

For the determination of the structure of poly(*w*-pentadecalactone) (PPDL) and poly(*d*-valerolactone) (PDVL) copolymers and Amine-DGEBA oligomers, MALDI-TOF MS was applied. In the synthesis of PPDL and PDVL (co)polymers PEG(200), PCD(2000) and 1,4-butanediol served as initiators. In order to confirm that successful initiation from the targeted initiators took place, MALDI-TOF MS measurements were performed. The MALDI-TOF MS spectra of these copolymers show the presence of various series as illustrated in Figure 1. In addition of the block copolymers, the formation of homopolymers can be detected and MALDI-TOF MS were also used to confirm the presence of A-DGEBA oligomers with different number of repeat units and amine end-groups. The  $M_n$  and the number average functionality of the A-DGEBA oligomer ( $f_n$ ) were determined by MALDI-TOF MS. The values of  $M_n$  and  $f_n$  of the A-DGEBA oligomer series were determined to be 1150 g/mol and 3.3, respectively.

Acknowledgment: The work was supported by the grant No. FK-132385, K-132685 and FK-128783 from National Research, Development and Innovation Office (NKFI)

### Please explain why your abstract is innovative for mass spectrometry?

Our results demonstrated that these types of copolymers and oligomers can unambiguously be detected by MALDI-TOF MS and the results can be used for further molecular designing.

### Co-authors:

*Csilla Lakatos, University of Debrecen, Department of Applied Chemistry, 4032, Egyetem tér 1*  
*Marcell Kordován, University of Debrecen, Department of Applied Chemistry, 4032, Egyetem tér 1*  
*Lajos Nagy, University of Debrecen, Department of Applied Chemistry, 4032, Egyetem tér 1*  
*Lajos Daróczy, University of Debrecen, Department of Solid State Physics, 4026, Bem tér 18/B*  
*Miklós Zsuga, University of Debrecen, Department of Applied Chemistry, 4032, Egyetem tér 1*  
*Sándor Kéki, University of Debrecen, Department of Applied Chemistry, 4032, Egyetem tér 1*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

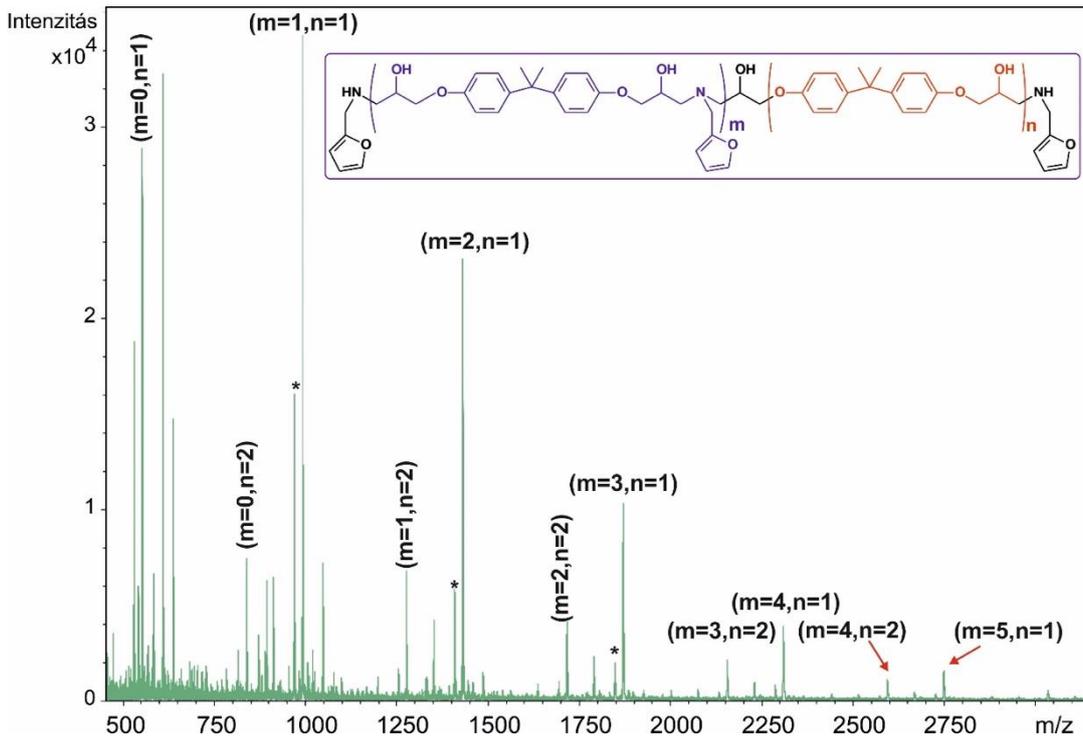


Figure 1. MALDI-TOF MS spectrum of FA-DGEBA oligomer.

Poster number: **BM-PA-005**

## **PATTERN TARGETING SOFTWARE FOR THE DETECTION AND ASSIGNMENT OF POLYMERIC COMPOUNDS IN DATA ACQUIRED USING LIQUID CHROMATOGRAPHY-ION MOBILITY-MASS SPECTROMETRY**

Abstract ID: **277**

**Presenting author: Jeff Goshawk, Waters Corporation**

### **Introduction**

Liquid chromatography-ion mobility-mass spectrometry (LC-IM-MS) is used for the non-targeted acquisition of samples containing polymeric compounds. The analysis of the resulting data is challenging due to the presence of a range of charge-state distributions, multiple adducts and isomeric oligomers. It is common for such samples to contain many individual ions related to each polymeric species and this can make the assignment of the individual polymers difficult.

### **Methods**

We present a dedicated software application that can target user specified patterns within complex data and enables the assignment of polymers, determines the individual oligomeric contributions, and measures the overall abundance of each polymer in a sample. The application processes data that has been previously peak detected and reduced to a list of ions. The ions are clustered into isotope distributions over a range of charge states thereby yielding a collection of components each of which is uniquely defined by a combination of  $m/z$ , retention-time, and drift-time.

### **Preliminary data (results)**

The application presents the clustered data as a single mass spectrum from which it is straightforward to identify repeating mass differences (Figure 1). Linear homopolymers can then be defined as targets in which repeat units and end groups are represented by either elemental formulae or mass values. The polymers are subsequently targeted in the measured data and, following processing, the application presents the assignments of the polymeric targets together with the residual, unassigned data (Figure 2). The unassigned data may reveal the presence of additional repeating mass differences which can then be targeted by defining additional polymeric distributions. This process can be repeated until all distinct repeating mass differences in the acquired data have been successfully assigned.

For each of the assigned polymers, the pattern targeting software presents spectra displaying the assignments for each of the target polymeric distributions specified. Additionally, standard polymeric calculations are performed for each of the targets to provide a measure of the number average molecular weight, weight average molecular weight, polydispersity index, and total abundance of each polymer.

### **Please explain why your abstract is innovative for mass spectrometry?**

Detection and identification of polymers using ion-mobility mass spectrometry and pattern targeting software.

### **Co-authors:**

*Andrew Tudor, Waters Corporation*

*Rachel Sanig, Waters Corporation*

*Isabel Riba, Waters Corporation*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

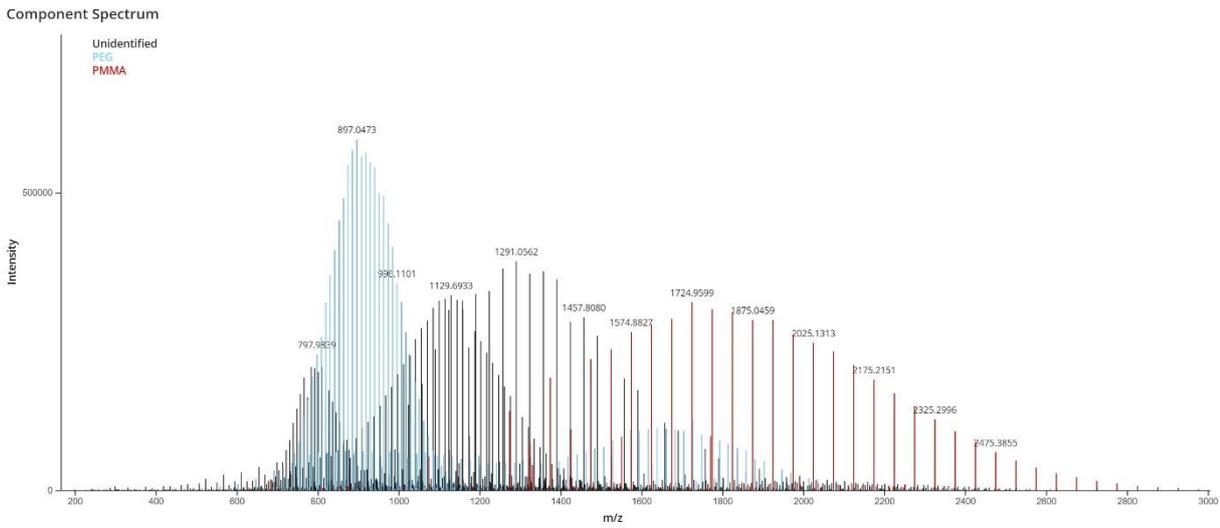


Figure 1: Component Spectrum

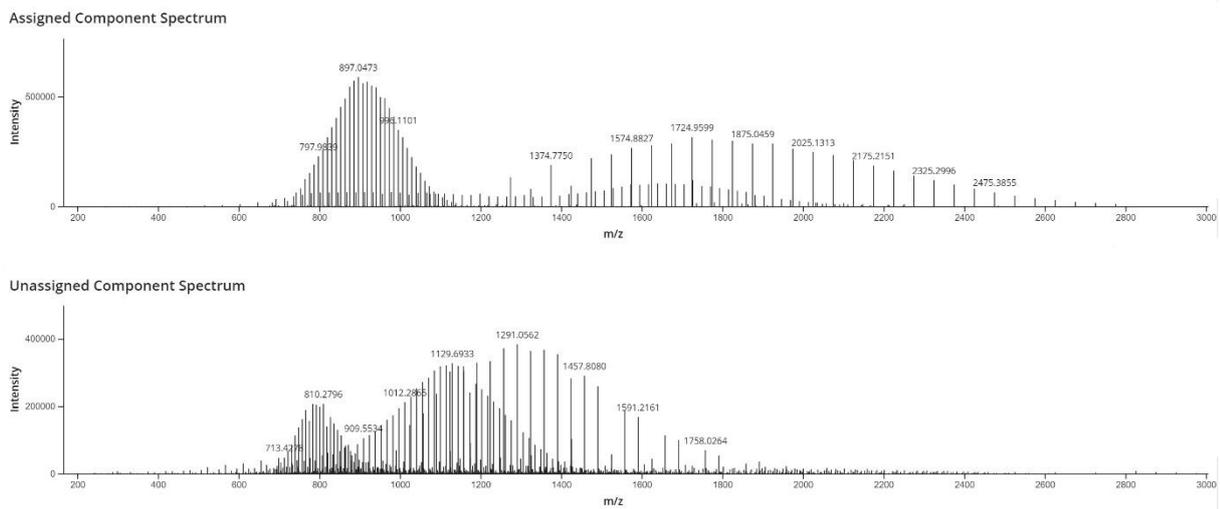


Figure 2: Assigned and Unassigned Spectra

Poster number: **BM-PA-006**

## THE POLYDISPERSITY RATIO AND ITS IMPLEMENTATION FOR THE INTERPRETATION OF PLURONICS

Abstract ID: **282**

**Presenting author: Gergő Róth, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary, Doctoral School of Chemistry, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary**

### Introduction

Nowadays, molecular engineers have the need for precise analysis to understand the properties of copolymers. Our method is a powerful tool to identify every polymer component in the mass spectrum, moreover, we introduce a novel parameter the Polydispersity Ratio (PDR), that helps comprehend the shape of the composition drift.

### Methods

Chemicals: EO/PO block copolymers were obtained from BASF (Ludwigshafen, Germany) and Sigma Aldrich (Taufkirchen, Germany). Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). MALDI-TOF MS measurements were made by a Bruker Autoflex Speed mass spectrometer (Bruker Daltonik, Bremen, Germany). Reflectron mode was used for all of the measurements, where 19 kV (ion source voltage 1), 16.65 kV (ion source voltage 2), 21 kV (reflector voltage 1), and 9.55 kV (reflector voltage 2) were utilized. The solid phase laser (355 nm) was used at 200 Hz, and 10 000 shots were summed. The spectra were internally calibrated.

### Preliminary data (results)

The characterization of copolymer structures is essential to design the physical properties of the copolymers. A robust algorithm was developed for the evaluation of spectra of copolymers. The efficiency of the algorithm was proved by the measurement of ethylene-oxid (EO)-propylene-oxid(PO) block and random copolymers with different chemical compositions and molecular weight. We have determined the composition drift and composition distribution, which are relevant polymer quantities. In a lot of case, the measured and calculated molecular weight was different than the value provided by the manufacturer. For instance, the L64 (Sigma Aldrich, BASF product) and the Pluronic PE6400 (BASF) copolymer have a similar molecular weight (3000 g/mol) and composition (40 m/m% EO), based on their datasheets. Our analysis finds a significant difference between the two poloxamers. Figure 1. shows the composition drift of these polymers and the dissimilarity is obvious. The PE6400 has higher EO content with broader distribution. Furthermore, we studied the aggregation behavior of these two polymers and the size of the micelles is different, which indicates, little difference in the composition fine determine physical properties.

Acknowledgment: The work was supported by grant No. FK-132385, K-132685 and FK-128783 from National Research, Development and Innovation Office (NKFI).

### Please explain why your abstract is innovative for mass spectrometry?

A robust algorithm was developed for the evaluation of mass spectra of copolymers, furthermore, a new copolymer quantity was introduced, which can be determined from mass spectra.

### Co-authors:

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## POSTER SESSION A

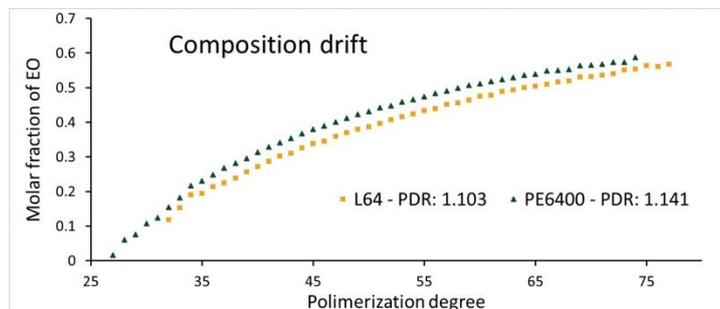
Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

Miklós Zsuga, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary

Sándor Kéki, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary



Composition drift-plot of L64 and PE6400 Mn:2900g/mol-EO%:40

Poster number: **BM-PA-007**

## **MONITORING AND CHARACTERIZATION OF SUCROSE-HDI COOLIGOMER FORMATION BY MALDI-TOF MASS SPECTROMETRY**

Abstract ID: **292**

**Presenting author: Csilla Lakatos, Department of Applied Chemistry, University of Debrecen**

### **Introduction**

Nowadays, carbohydrates are often used in polymer syntheses as monomers or even as crosslinking agents. This is because they are easy to access, biocompatible, and cheap. The polymers are thus prepared to have a wide range of uses in medicine, as they are non-toxic and biodegradable. Sucrose also belongs to this family of compounds, is present in a renewable form in nature, easy to obtain in high purity forms. However, it appears to be a compound used only as a crosslinker. However, this research focuses on synthesis and characterization of longer chain sucrose-1,6-hexamethylene diisocyanate (HDI) and sucrose-4,4'-diphenyl diisocyanate (MDI) cooligomers, that are appropriate polyols for polyurethanes. The chain formation from sucrose and HDI was monitored by MALDI-TOF mass spectrometry.

### **Methods**

To prepare the sucrose oligomer, dried sucrose was dissolved in anhydrous DMSO. The resulting solution was heated to 80 °C under anhydrous conditions and then 1 equivalent of HDI or MDI was added. The mixture was stirred for different hours and samples were taken for MALDI-TOF mass spectrometric analysis at predetermined intervals. Samples (10 ml) were quenched in THF-methanol 1:1 (990 ml). A solution of DHB (20 mg/ml) and NaTFA (10 mg/ml) was also added to the samples for MALDI-TOF MS measurements. Samples were analyzed in both reflectron and linear modes.

### **Preliminary data (results)**

The results show that a molecular weight of 6300 Da was obtained for the sucrose-HDI oligomers. In addition, it was observed that the obtained polyol has a relatively wide molecular weight distribution. The resulting polyol was further reacted with a poly( $\epsilon$ -caprolactone)-based, diisocyanate-ended prepolymer. It has also been shown that sucrose-HDI / sucrose-MDI units appear as hard segments in the polymer and the resulting polyurethanes revealed excellent mechanical properties.

Acknowledgment: The work was supported by the grant No. FK-132385, K-132685 and FK-128783 from National Research, Development and Innovation Office (NKFI).

### **Please explain why your abstract is innovative for mass spectrometry?**

Our measurements demonstrated that these types of sucrose oligomers can be detected by MALDI-TOF mass spectrometry and the results can be used for designing polyurethane networks.

### **Co-authors:**

*Marcell Árpád Kordován, Department of Applied Chemistry, University of Debrecen, Doctoral School of Chemistry, University of Debrecen*

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*Bence Vadkerti, Department of Applied Chemistry, University of Debrecen, Doctoral School of Chemistry, University of Debrecen*

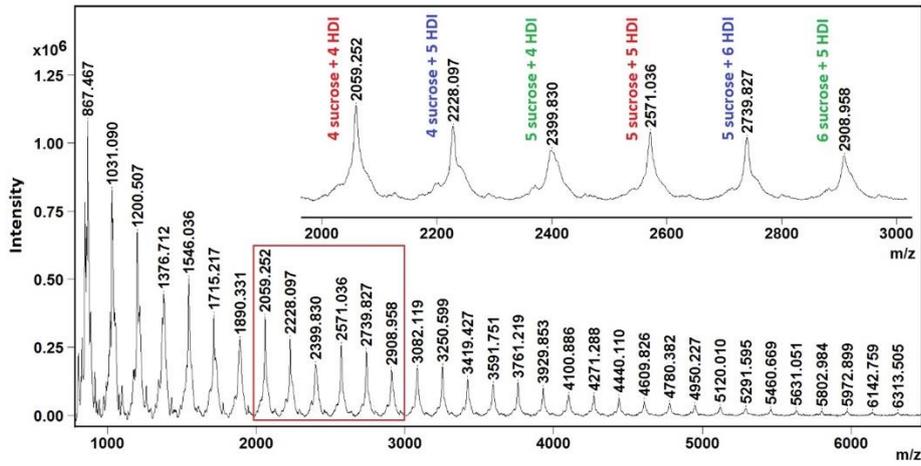
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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



MALDI-TOF MS spectrum of the products of sucrose-HDI reaction.

Poster number: **BM-PA-008**

## **INFORMATION CODING BY PEG – ALIPHATIC ISOCYANATE REACTION AND DECODING BY MALDI-TOF MS**

Abstract ID: **300**

**Presenting author: Bence Vadkerti, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen, Doctoral School of Chemistry, University of Debrecen**

### **Introduction**

In this research the possibility and capability of encoding information applying alcohol-isocyanate reactions were studied. Monoethoxy polyethylene glycols (PEG) (ethylene oxide units from 5 to 8) were reacted with linear aliphatic isocyanates (CH<sub>2</sub> units from 3 to 7) yielding 20 different products. This combination allowed us to encode picture, text and audio file. Since the products had similar chemical structures and ionization efficiencies, MALDI-TOF-MS signals were excellent for the complete decoding.

### **Methods**

The MALDI-TOF-MS measurements were performed by a Bruker Autoflex Speed mass spectrometer operating in the reflectron mode. Each measurement was carried out with 19 and 21 kV (ion source voltage 1 and 2), as well as 21 and 9.55 kV (reflector voltage 1 and 2) voltages. The solid phase laser (355 nm) was operated at 500 Hz with 60% laser attenuation. The spectra were externally calibrated with PEG400. The information spots were prepared with sodium-trifluoroacetate and 2,5-dihydroxybenzoic acid.

### **Preliminary data (results)**

The reactions between mPEGs and isocyanates were carried out in toluene in the presence of tin(II) 2-ethylhexanoate without any side reaction. Quenching and dilution of the reaction mixtures occurred with methanol. The reading was performed by MALDI-TOF-MS in the reflectron mode. If a given m/z value was found, it was marked by “1”, or if not by “0” bit. Due to the high intensity of the peaks, reading was applied with high accuracy and efficiency. The data storage capability was presented for picture, text, and audio file, decoding of which was 100% in each case. It was found that the theoretical limit of data storage density is approximately 5 Mbit/cm<sup>2</sup>. This value is higher than that of a conventional floppy disc (0.36 Mbit/cm<sup>2</sup>), however, it is significantly lower than that of a modern hard-disk drive (207,700 Mbit/cm<sup>2</sup>).

### **Acknowledgement**

This work was supported by the grants No. FK-128783, FK-132385 and K-132685 from National Research, Development and Innovation Office (NKFI)

### **Please explain why your abstract is innovative for mass spectrometry?**

This research provides a new opportunity to store information by means of chemical substances and to read it by a mass spectrometer.

### **Co-authors:**

*Lajos Nagy, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen*  
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*Tibor Nagy, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen*  
*Zoltán Erdélyi, Department of Solid State Physics, Faculty of Science and Technology, University of Debrecen*  
*Levente Kárpáti, Department of Organic Chemistry, Faculty of Pharmacy, Semmelweis University*  
*Miklós Zsuga, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen*  
*Sándor Kéki, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen*

Poster number: **BM-PA-009**

## **DETAILED KINETIC STUDY ON THE REACTIVITIES OF THE EIGHT HYDROXYL GROUPS OF SUCROSE IN URETHANE FORMING REACTION**

Abstract ID: **304**

**Presenting author: Lajos Nagy, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen**

### **Introduction**

The crosslinking agents play a significant role in the polyurethane chemistry since these can affect the physical/mechanical properties of the produced polyurethanes. In the last decades for many applications the sucrose was chosen as a primary feedstock for production of hydrocarbon based biopolymers. The addition reaction of the glucose OH groups with isocyanates provides an invaluable synthetic route to prepare urethane (carbamate) functionalized glucose derivatives and/or crosslinked polymers. Thus, the knowledge of the reactivities of the sucrose's OH groups is important in order to rationalize the synthesis of various sucrose containing materials. In this research the relative reactivities of sucrose's OH groups were determined with phenyl isocyanate applying pseudo-first order reactions.

### **Methods**

For the High-Performance Liquid Chromatographic (HPLC) investigation 10 mL sample was injected into the chromatographic system consisting of a Waters 2695 Separations Module equipped with a thermostable autosampler (5 °C), a column module (45 °C), and a Waters 2996 diode-array detector (DAD). The separation of the reaction products was achieved by reverse phase C18 column. Monitoring of the reactions was performed by UV (233 nm) areas obtained in the chromatograms. For the assignments of the OH groups tandem mass spectrometry (Bruker MicroTOF-Q mass spectrometer equipped with an ESI source), NMR and DFT (Density Functional Theory) calculations were performed.

### **Preliminary data (results)**

The reactions were performed in high molar excess of sucrose to phenyl isocyanate and the eight possible products were successfully separated by HPLC. Based on the peak areas detected in the UV chromatogram the relative peak area values were calculated. The obtained values were plotted against the reaction time and the rate constants were determined by fitting them to the experimental data. It was found that there were two OH groups whose reactivities were significantly higher than those of the others. Furthermore, one magnitude order difference was obtained in the reactivities between the most and less reactive OH groups. The next step was the assignation of the OH groups that was achieved using HPLC-MS/MS and 2D-NMR measurements. Based on the HPLC-MS/MS chromatogram of the products obtained in the reaction of phenyl isocyanates with a sucrose derivative, which was labeled with a <sup>13</sup>C-atom at C-1 position in the glucose moiety. The obtained peaks were separated to two four-membered groups as the fragmentation of sucrose takes place at the glycosidic bond. As the primary OH groups have higher reactivity than that of the secondary ones and using the NMR results that showed the highest reactivities for the three primary OH groups in OH(6')>OH(6)>OH(1') order the most reactive OH groups could be assigned.

### **Acknowledgement**

This work was supported by the grants No. FK-128783, FK-132385 and K-132685 from National Research, Development and Innovation Office (NKFI)

### **Please explain why your abstract is innovative for mass spectrometry?**

Tandem mass spectrometry was used to assign the rate constants to the corresponding OH group of sucrose.

### **Co-authors:**

*Bence Vadkerti, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen  
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Miklós Zsuga, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen  
Sándor Kéki, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen*

Poster number: **BM-PA-010**

## ANALYSIS OF MULTIBLOCK COPOLYMERS BY MASS SPECTROMETRY

Abstract ID: **324**

**Presenting author: Tibor Nagy, Department of Applied Chemistry, University of Debrecen**

### Introduction

Multiblock copolymers are a widely studied class of functional materials, however, to understand their properties characterization of these copolymers is required. Mass spectrometry-based methods can provide details about the chemical composition, however, the evaluation of spectra is challenging due to the hundreds of peaks detected in a single spectrum. The identification of compounds is not enough to calculate the polymer quantities, correction of overlapping peaks is essential. Recently, we have developed the Mass-Remainder Analysis (MARA) and related methods, to achieve effective sorting and determine the intensity fraction of copolymer compounds detected in a spectrum. The MARA and related methods were applied for the characterization of multiblock copolymers and studying polymerization reactions.

### Methods

Copolymers were characterized by an Autoflex Speed MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen, Germany). Reflector mode was used with the voltages ionsource1, ionsource2, reflector1 and reflector2: 19kV, 16.65kV, 21kV and 9.55kV, respectively. DCTB matrix and NaTFA ionizing agent was applied in the concentration of 15mg/mL and 5mg/mL. The polyacrylamide copolymers were synthesized by RAFT polymerization. The chain transfer agent was 2-(((butylthio)-carbonothioyl)thio)propanoic acid, the initiator was VA-044. The Pluronic copolymers were generous gifts from BASF or bought from Merck. A Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) was applied for the DLS measurements.

### Preliminary data (results)

Herein the Mass-Remainder Analysis (MARA) and the Multistep Mass-Remainder Analysis (M-MARA) were further developed and applied for the characterization of different multiblock copolymers. The Mass-Remainder Analysis is simply based on the calculation of remainders after dividing by a base unit. The proper selection of the base results in the ordering of the homolog series. For samples with higher complexity, like copolymer blends or multiblock copolymers with more than two types of monomer units, additional ordering criteria must be used. The intensity correction step of the MARA method was improved which is based on the fitting of the simulated isotope pattern of all possible compounds, allowing the accurate determination of the intensity fraction of the copolymer chains even at higher molecular weight regions. The method was applied for the investigation of the composition-selfassembly connection of Pluronic copolymers. Furthermore, the M-MARA method was applied for the characterization of multiblock copolymers of acrylamide derivatives with four different monomer units. The suitable base units for the M-MAA method were determined to identify the composition of the polymer chains and determine the possible overlaps. The prepared block copolymers were analyzed before the synthesis of new blocks on them. The obtained copolymer quantities were used to interpret the reversible addition-fragmentation chain-transfer (RAFT) polymerization of the investigated monomers.

Acknowledgment: The work was supported by grant No. FK-132385, K-132685 and FK-128783 from National Research, Development and Innovation Office (NKFI), János Bolyai Research Scholarship (BO/00212/20/7), and New National Excellence Program (ÚNKP-21-05-DE-476).

### Please explain why your abstract is innovative for mass spectrometry?

The Mass-Remainder Analysis and related methods were improved for the analysis of spectra of copolymers with higher molecular weight and copolymers with four different monomer units.

### Co-authors:

*Ákos Kuki, Department of Applied Chemistry, University of Debrecen*

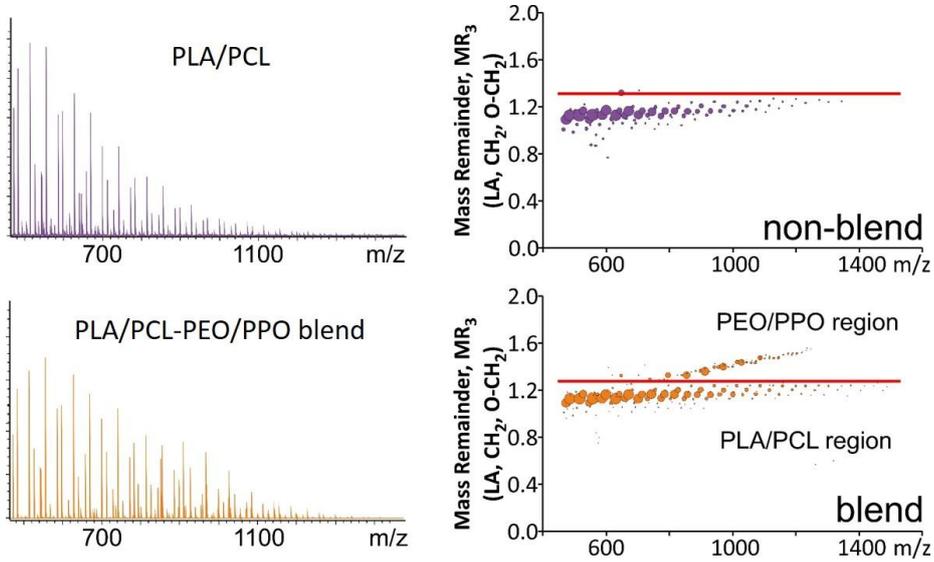
*Gergő Róth, Department of Applied Chemistry, University of Debrecen*

*Mahir Hashimov, Department of Applied Chemistry, University of Debrecen*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Miklós Zsuga, Department of Applied Chemistry, University of Debrecen  
Sándor Kéki, Department of Applied Chemistry, University of Debrecen



Spectra and MR<sub>3</sub> plot of a copolymer and copolymer blend

Poster number: **BM-PA-011**

## DEVELOPMENT OF CHEMICAL BARCODES AND THEIR READING BY DART-MS

Abstract ID: **335**

**Presenting author: Veronika Csilla Pardi-Tóth, Department of Applied Chemistry, University of Debrecen,**

### Introduction

Nowadays, following the evolution of technology, there is an increasing field of research in storing digital information in chemicals and making this data secure, easy and repeatable to read back. Single molecules and a mixture of molecules can be used to store digital data, while mass spectrometry is a suitable method to decode the stored information. As a reading method, the Direct Analysis in Real Time mass spectrometry is a promising technique due to the lack of sample preparation and high speed of measurements. Our aim was to find a class of low molecular weight thermally stable compounds with high ionization efficiency. The nicotinic acid esters are promising compounds where nicotinic acid and alcohols can be varied to increase the number of compounds for storage.

### Methods

For our studies, Nicotinic acid, nicotinic acid N-oxide, 4-aminonicotinic acid, 2- (3-pyridinyloxy) nicotinic acid, and 5- (3-formyl-4-methoxyphenyl) nicotinic, 2-chloronicotinic acid, 5-bromo-6-chloronicotinic acid, methyl-6-chloronicotinic acid, 2-amino-6-chloronicotinic acid were used. DART-MS measurements were performed with a MicroTOF-Q type Qq-TOF MS instrument from Bruker (Bruker Daltonics, Bremen, Germany) equipped with aDART SVP source from IonSense (IonSense, Inc., Saugus, MA, USA). He gas was used as ionizing gas, the temperature was varied in the range of 250-450 °C degree.

### Preliminary data (results)

Nicotinic acid and nicotinic acid derivatives were reacted with different alcohols to produce a series of compounds with different lengths of the ester groups. Esterification reactions were carried out at 65°C and the resulting compounds were purified by flash chromatography and tested by DART-MS method, relative ionization efficiencies were determined. These compounds were applied as a molecular bit, i.e., the presence or absence of a particular compound in the spectrum corresponds to a value of 0 or 1. Furthermore, we successfully converted the mass spectra of the synthesized nicotinic acid ester derivatives into chemical barcodes. The writing and the reading of the method were tested with the production of different barcodes and multiple readings of stored data. The mixtures were placed on different surfaces to determine the efficiency of the quality of materials on the reading. It was obtained that the compounds were detectable in most cases, however, their concentrations must be selected properly. Furthermore, the temperature dependence of the ionization of compounds was also investigated in order to determine the applicability of our compounds as anti-counterfeiting agents in thermally sensitive materials.

**Acknowledgment:** The work was supported by grant No. FK-132385, K-132685 and FK-128783 from National Research, Development and Innovation Office (NKFI).

### Please explain why your abstract is innovative for mass spectrometry?

The DART-MS method was successfully applied to read stored data in the mixture of nicotinic acid ester derivatives.

### Co-authors:

Ákos Kuki, Department of Applied Chemistry, University of Debrecen,  
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Sándor Kéki, Department of Applied Chemistry, University of Debrecen,

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
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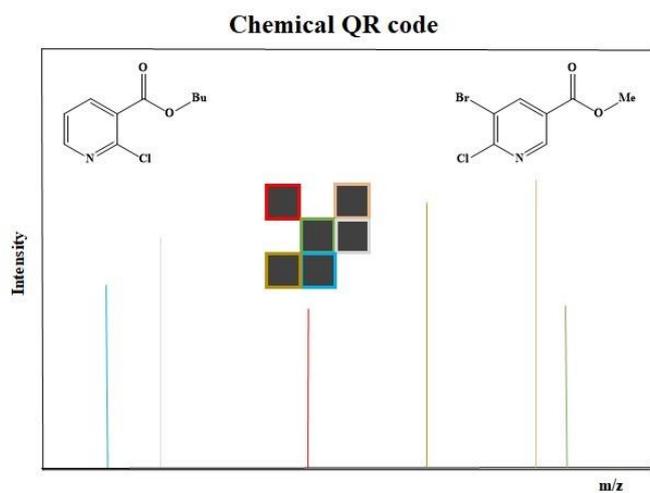


Figure 1. A simulated spectrum and the created QR code.

Poster number: **BM-PA-012**

## **MONITORING THE REACTIONS OF 1,5-ISOCYANAMINAPHTHALENE IN THE PRESENCE OF WATER/PROTIC SOLVENT MIXTURES AND Hg (II) IONS BY FLUORIMETRIC AND MASS SPECTROMETRIC METHODS**

Abstract ID: **340**

**Presenting author: Anita Dékány-Adamoczky, University of Debrecen**

### **Introduction**

Mercury is one of the most toxic heavy metals found in several forms in our environment. Different methods have been developed to detect Hg (II) concentration in the water including titrimetry, electrochemical and other instrumental methods. However, fluorometry proved to be one of the most efficient. We developed a new class of isocyanonaphthalene derivatives, which are effective fluorescence sensors for the quantification of Hg (II) ions in water utilizing the results of mass spectrometry.

Our aim was to investigate the mechanism the reaction of 1-isocyano-5-aminonaphthalene (1,5-ICAN) with water in the presence of as a Hg(II) ions. To understand the mechanism, the kinetics of the reactions was investigated using mass spectrometry and fluorescence spectroscopy.

### **Methods**

The reaction of ICAN with water and protic solvents was investigated at a constant Hg (II) concentration. HgCl<sub>2</sub> was dissolved in the mixture of water and co-solvent (e.g., iPrOH) to obtain a concentration of  $1.3 \times 10^{-3}$  mol/L. The water co-solvent ratios were varied during the experiments.

The MS and MS/MS measurements were carried out by a MicroTOF-Q instrument (Bruker Daltonik, Bremen, Germany) equipped with an ESI source. The mass spectra were calibrated using cluster ions of sodium trifluoroacetate [(NaTFA)<sub>n</sub>+Na].

### **Preliminary data (results)**

The transformations of 1-isocyano-5-amino naphthalene in various aqueous / co-solvent solutions including protic and aprotic solvents were investigated in the presence of Hg(II) ions.

To determine the reaction product(s) formed in the water/iPrOH mixture, ESI-MS measurements were performed. The reaction mixtures for ESI-MS were prepared similarly to those for the fluorescence experiments, but in the ESI-MS experiments, 1,5-ICAN was applied at a higher concentration by one order of magnitude.

Applying water/isopropanol co-solvent the ESI-MS spectrum showed the presence of protonated 1,5-DAN, (5-aminonaphthalen-1-yl) carbamic acid and isopropyl (5-aminonaphthalen-1-yl) carbamate (urethane), *m/z* 159, 203 and 245. Further tandem mass spectrometric measurements were carried out to confirm the structure of the formed products. The MS / MS spectra showed the loss of H<sub>2</sub>O and CO<sub>2</sub> molecules, supporting the presence of carbamic acid in the reaction mixture. We proved that the intermediates of carbamic acid or carbamates were produced and the decomposition of these resulted in the formation of the amino group.

Acknowledgment: The work was supported by the grant No. FK-132385, K-132685 and FK-128783 from National Research, Development and Innovation Office (NKFI)

### **Please explain why your abstract is innovative for mass spectrometry?**

The structure of the product and intermediates were successfully confirmed by MS and MSMS methods.

### **Co-authors:**

Lajos Nagy, University of Debrecen  
Miklós Zsuga, University of Debrecen  
Sándor Kéki, University of Debrecen

Poster number: **BM-PA-013**

## MASS SPECTRAL FILTERING BY MASS-REMAINDER ANALYSIS (MARA) AND ITS APPLICATION TO FLAVONOID SCREENING

Abstract ID: 353

**Presenting author:** Ákos Kuki, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen

### Introduction

Flavonoids represent an important class of secondary metabolites because of their potential health benefits and functions in plants. Based on the degree of oxidation and saturation the flavonoids can be divided into several groups, e.g., flavones, flavonols, flavanones, flavanonols, flavanols. The mass accuracy and resolving power of high resolution mass spectrometry has increased the relevance of non-hyphenated MS methods. Direct infusion mass spectrometry (DIMS) can significantly increase the analytical throughput compared to LC-MS. However, the rapid identification of thousands of mass peaks requires advanced data mining techniques. Recently, we have developed a data mining algorithm, called mass-remainder analysis (MARA) for the processing of complex mass spectra. Here, we propose a novel method for the filtering of flavonoids in the DIMS spectra of natural extracts.

### Methods

15 commercially available brands of three herbs, such as yarrow (*Achillea millefolium* L.), elder (*Sambucus nigra* L.), and birch (*Betula* L.) were investigated. Plant samples were ground, the powders were swollen in ethanol: water-7:3 (v/v) for 16 h. After the swelling process, the diluted samples were sonicated for an hour and then filtered. The filtrates were dried under vacuum to constant weights.

MS measurements were performed using a Maxis II type Qq-TOF MS instrument (Bruker Daltonics, Bremen, Germany) equipped with an electrospray ion-source. Mass accuracy: <600 ppb, resolution: 40,000 at m/z 400 (FWHM).

### Preliminary data (results)

Multi-step Mass-remainder analysis (M-MARA) is able to produce the simplified graphical representations of the peak-rich ESI-DIMS mass spectra of various herb extracts. M-MARA eliminates most of the differences in the elemental composition of the flavonoids, and a single characteristic difference – for example the double bond equivalent (DBE) value – is highlighted and used for filtering (Figure 1, green: DBE = 11; orange: DBE = 10; blue: DBE = 9). Moreover, our flavonoid-filtering algorithm performs the mass remainder calculation twice (M-MARA(DBE) and M-MARA(O)), using different divisors, decreasing thereby the number of false hits of the mass filter, and facilitating the determination of the elemental composition. Each point in Figure 2a corresponds to one or more peaks of the mass spectrum. By applying our M-MARA(DBE)-M-MARA(O) filter algorithm, the interference mass spectral peaks can be removed, and therefore, Figure 2b reveals only the points, which correspond to possible flavonoid constituents.

The suggested application area of our M-MARA filtering method is the comprehensive metabolite profiling of flavonoids in herb extract, foods and beverages of plant origin. As a pilot study to test our approach, the flavonoid analysis of altogether 15 commercially available brands of three herbs, such as yarrow, elder, and birch were performed. M-MARA flavonoid-filtering uses simple mathematical and logical operations and thus, it can easily be implemented in a regular spreadsheet software.

**Acknowledgement:** The work was supported by the grant no. FK-132385, K-132685 and FK-128783.

### Please explain why your abstract is innovative for mass spectrometry?

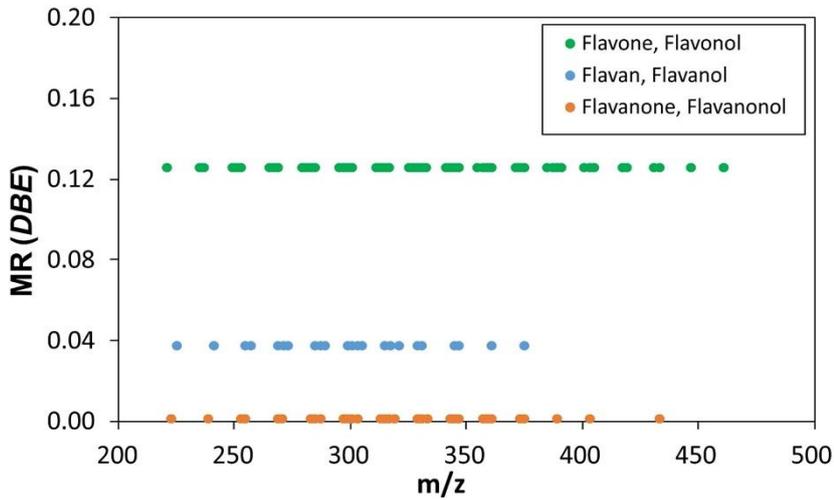
Processing of complex direct infusion mass spectra with high speed and low demand for computing power and memory that enables the real time application.

### Co-authors:

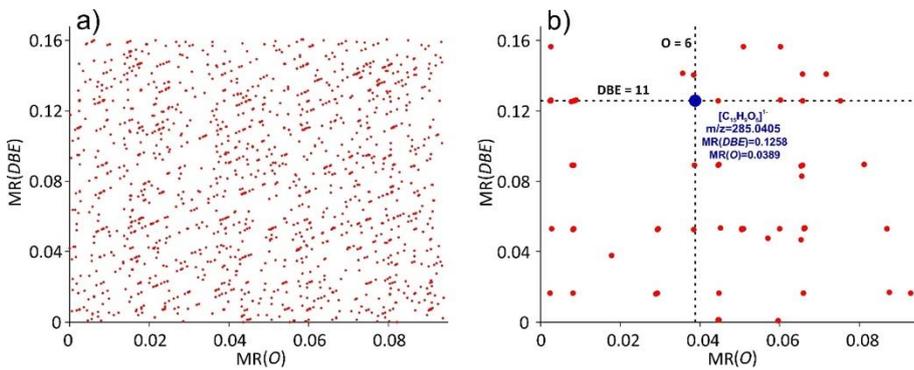
POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Tibor Nagy, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen  
Gergő Róth, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen, Doctoral School of Chemistry, University of Debrecen  
Miklós Zsuga, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen  
Sándor Kéki, Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen



Mass-remainder versus  $m/z$  plot of about one hundred flavonoids.



Raw and filtered MR(O) – MR(DBE) plots

Poster number: **BM-PA-014**

## **ANALYSIS OF POLYBUTADIENE POLYDISPERSITY BY ION MOBILITY COUPLED WITH A TIME OF FLIGHT MASS SPECTROMETRY**

Abstract ID: **435**

**Presenting author: Caroline Damseaux, Mass Spectrometry Laboratory-ULiège**

### **Introduction**

Polydispersity and chain end identification are key parameters used to characterize polymeric compounds. A well-known technique to retrieve this information is mass spectrometry classically coupled to matrix assisted laser desorption ionization (MALDI). Other ionization sources such as electrospray ionization (ESI) is also being considered for the transfer of polymers from solution to the MS instrument. Nevertheless, the analysis of polybutadiene by MS is challenging because this polymer has a low ionization capacity related to its electroneutrality and is poorly soluble in polar solvent, such as methanol or acetonitrile, commonly used for ESI and APCI. Here, we propose a method to characterize the chain length and the chain ends of polybutadiene by direct infusion into an ion mobility mass spectrometer.

### **Methods**

The study is conducted on a timsToF Pro. It is a trapped ion mobility spectrometer (tims) coupled to high resolution time-of-flight (ToF) mass analyser. The tims cell is able to separate and accumulate ions according to their mobility in gas-phase. The mobility ( $k_0$ ) is related to the projection of the conformation (mass, size and shape) of the polymer in gas phase and therefore to the three-dimensional structural information of the polymers. The analysis of the high-resolution mass spectra for each separated conformation gives access to additional structural information such as the polymer length and the nature of its chain ends.

### **Preliminary data (results)**

Polybutadiene is injected by direct infusion onto the electrospray source of a timsToF Pro. However, as polybutadiene is poorly ionized, a doping agent has been added to the solution. To promote ionization, lithium and silver have good properties to bind to polybutadiene. Two lithium salts have been tested to cationize the polymer: lithium chloride (LiCl) and lithium lactate (CH<sub>3</sub>CH-OH-COOLi). Two other salts have been tried to cationize the polybutadiene: silver nitrate (Ag-NO<sub>3</sub>) and sodium acetate (CH<sub>3</sub>COONa). The ratio between the amount of polybutadiene and salts has been optimized to increase the polymer signal while avoiding the bonding of the doping agent which would complicate the data interpretation. Stronger signals have been observed with lithium lactate and silver nitrate used at a ratio of 1000/30 and 1000/20 respectively (Polybutadiene/Salt, concentration ratio). The ionization parameters are optimized for low polar molecules on an ESI source set to positive mode and the mass spectrometer operate between 50 to 2000m/z in full scan mode. Trapped ion mobility scanning parameters have been optimized to improve separation in high purity dry nitrogen in the interest scan range between 0.65 and 1.75 V.s/cm<sup>2</sup> expressed in 1/ $k_0$ . Kendrick mass defect filtering has been used to process the data and extract the polybutadiene signal. Families can thus be distinguished by the unit of repetition of the monomer (Kendrick mass reference), by the state of oxidation and by the polymer length to polybutadiene. Each mobility signal provides a specific determination of a single mass-related polymer conformation.

### **Please explain why your abstract is innovative for mass spectrometry?**

The interplay of ion mobility mass spectrometry and Kendrick analysis allows rapid characterization of polybutadiene in solution by a direct infusion method.

### **Co-authors:**

*Christopher Kune, Mass Spectrometry Laboratory-ULiège*

*Georges Scholl, Mass Spectrometry Laboratory-ULiège*

*Gauthier Eppe, Mass Spectrometry Laboratory-ULiège*

Poster number: **BM-PA-015**

## **HIGH RESOLUTION MS FOR SINGLE-USE SYSTEMS EXTRACTABLE AND LEACHABLE ANALYSIS**

Abstract ID: **857**

**Presenting author: Weifeng Lin, Thermo Fisher Scientific**

### **Introduction**

Single Use Systems (SUS) are widely used in biopharmaceutical industry for biological drug and vaccine production. To ensure drug quality and patient safety, extractable & leachable analysis of SUS is required by end-user and regulatory approval for new products.

High-resolution mass spectrometry (HRMS) coupled with UHPLC are routinely used for nonvolatile extractable and leachable analysis. HRMS full scan, MS/MS and MS<sup>n</sup> data ensure confident elemental composition determination and structure identification.

This poster presents an effective workflow for extractable analysis of single-use system for bioproduction using Thermo Scientific™ Orbitrap Exploris™ 120 MS and Vanquish UHPLC system. The data was processed with Thermo Scientific Compound Discoverer 3.3 software.

### **Methods**

Films for single-use bags were extracted using WFI (water-for-injection), 50% EtOH, 0.1M H<sub>3</sub>PO<sub>4</sub>, and 0.5N NaOH at 40°C for 21 days following Biophorum operations group (BPOG) protocol. The extracts solutions were analyzed directly by Orbitrap Exploris 120 MS and Vanquish UHPLC system for nonvolatile compounds. DAD total scan was collected from 200 to 400 nm.

HRAM data was acquired using electrospray ionization with full MS followed by top 4 dd-MS<sub>2</sub> with positive/negative polarity switching. An EASY-IC Internal calibration was employed to ensure high mass accuracy throughout.

The HRAM data were processed with software Compound Discoverer 3.3.

### **Preliminary data (results)**

For routine SUS untargeted E&L analysis, HRAM full scan and MS/MS data acquisition with polarity switching are critical for the detection of structurally diverse compounds and to provide complete extractable profiles.

In this study, the high scan speed of the Orbitrap Exploris 120 enabled a duty cycle of ~1 second for 10 scan events data acquisition: full MS followed by 4 DDA MS<sub>2</sub> with polarity switching at resolution 60,000 (full MS) and 15,000 (MS/MS), respectively. The confirmative positive and negative full scan data and MS/MS fragments facilitated confident extractables identification.

Compound Discoverer 3.3 software was used for data processing. It used multiple database search in parallel for known compounds identification. The databases used in this study were: mzCloud high resolution spectral database, mzVault E&L database, Thermo Scientific extractables and leachables compound database, NIST high resolution ESI database, and ChemSpider.

The results show that Orbitrap Exploris 120 and Vanquish UHPLC, coupled with Compound Discoverer 3.3 software data processing, provide an effective workflow for SUS extractables and leachables analysis.

### **Please explain why your abstract is innovative for mass spectrometry?**

Orbitrap Exploris 120 MS high-resolution, high scan speed, high sensitivity, and fast polarity switching with EASY IC ensure high quality full scan and MS/MS data for E&L analysis.

## POSTER SESSION A

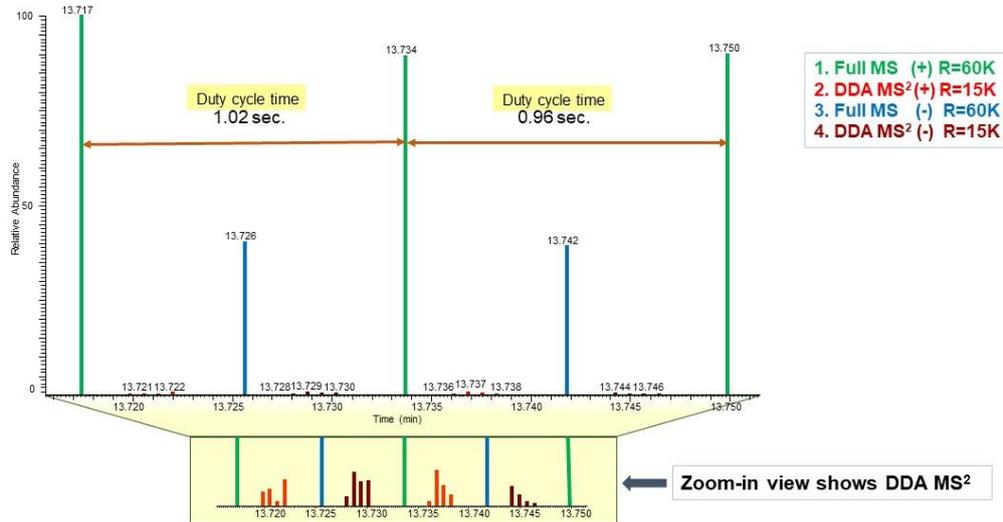
Monday 29 August 2022 from 11:30 to 13:00 hours  
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### Co-authors:

Kate Comstock, Thermo Fisher Scientific

## Fast Scan Speed for Short Duty Cycle

Full MS + Top 4 DDA MS<sup>2</sup> with Pos/Neg switching: 10 scan events in ~1.0 sec duty cycle



Poster number: **BM-PA-016**

## **ELECTROCHEMISTRY MEETS IMAGING – ELECTRODE SURFACE ANALYSIS BY MEANS OF MALDI-TOF-MS**

Abstract ID: **917**

**Presenting author: Arne Behrens, Bruker Daltonics GmbH & Co. KG, Institute of Inorganic and Analytical Chemistry, University of Münster**

### **Introduction**

Minimization of side reactions is a crucial step during the development of a typical organic synthesis route to maximize product yield and reduce educt consumption. In the last decades, electrochemistry has emerged to be a powerful tool in organic chemistry, since it enables easier access to several moieties via single-electron transfer. The efficacy of electrochemical reactions strongly depends on parameters such as electrolyte composition and electrode condition. Electrochemical side reactions, e.g., polymerization, can cause deposition of substrate material, thus limiting the activity of the electrode's surface. This process is predominantly known as "electrode fouling" and is a crucial factor not only in electroorganic synthesis, but also in the design and functionality of modern battery developments.

### **Methods**

In this work, matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) is presented as valuable tool for the surface analysis of commonly used boron-doped diamond (BDD) electrodes. Three different organic compounds with common functional groups, including aniline and phenol, have been investigated. A thin-layer flow-through cell was used to perform oxidative polymerization experiments. The treated BDD electrodes were subsequently analyzed by MALDI-ToF-MS to visualize the resulting deposition of oligomers on the electrode's surface.

### **Preliminary data (results)**

All three compounds show significant adsorption when polymerized via electrochemical oxidation with different localization on the surface depending on the respective oligomer size. Additionally, MALDI imaging reveals a dependency of oligomer size and the flow direction and therefore the mean contact time of the compounds.

### **Please explain why your abstract is innovative for mass spectrometry?**

MALDI-TOF-MS provides deep molecular and lateral insight into surface reactions to optimize electrochemical processes.

### **Co-authors:**

*Jens Fangmeyer, Institute of Inorganic and Analytical Chemistry, University of Münster*  
*Uwe Karst, Institute of Inorganic and Analytical Chemistry, University of Münster*

Poster number: **BM-PA-017**

## **CONFIRMATION OF SYNTHESIS OF SPARINGLY SOLUBLE COMPOUNDS BY ACCURATE MALDI-TOF MASS SPECTROMETRY**

Abstract ID: **924**

**Presenting author: Ei-Ichi Matsuo, Shimadzu Corporation**

### **Introduction**

The MALDI method is tolerant to various characteristics of samples, and thus, for example, can be used to perform mass spectrometry of poorly soluble compounds that are difficult to measure with LC-MS. For the analysis of less polar compounds, an ionizing agent is sometimes added to the sample and matrix to promote ionization. They must be mixed and dissolved, and then spotted and dried on a sample plate to form cocrystals. Therefore, it is necessary to dissolve all components in the same solvent. Meanwhile, for highly accurate mass measurement in MALDI-TOF MS analysis, it is desirable to perform mass calibration using internal standards. However, calibrants with similar physical properties and molecular weight to the sample compound may not be easily found.

### **Methods**

DHB (50 mg/mL) was dissolved in 70% acetonitrile aqueous solution containing 0.1% TFA and used as the external standard. Mass calibration was performed using DHB cluster ions (3M to 7M) as the standard peak.

A trace amount of each sample was dissolved in chloroform and used directly for analysis. DCTB (10 mg/mL) was used as a matrix and was dissolved in chloroform. Sodium trifluoroacetate (2 mg/mL) dissolved in tetrahydrofuran was added as a cation donor for SIE3. These were mixed and a small amount was spotted on the SUS target, dried, and used for measurement.

### **Preliminary data (results)**

Each poorly soluble compound (SIE1, SIE3, SIE4; Fig. 1) was measured with the iMScope QT - LCMS-9030 under appropriate conditions, with or without the sample compound, and peaks derived from the sample were confirmed. As a result, SIE1 and SIE4 were observed as  $[M+H]^+$  ions, and SIE3 was observed as  $[M]^+$  ions. Then, the accurate mass was measured three times, and the average value was calculated. It was found that the accurate mass of each compound could be measured with an accuracy within 1 ppm of the theoretical value. In this case, the exact mass was confirmed by accurate mass measurement, but this approach is also useful for formula prediction and structure analysis of unknown compounds.

### **Please explain why your abstract is innovative for mass spectrometry?**

Accurate mass measurement is achievable even with external calibration methods. Thus, analysis of poorly soluble compounds can be performed easily without the need to find suitable internal calibrants.

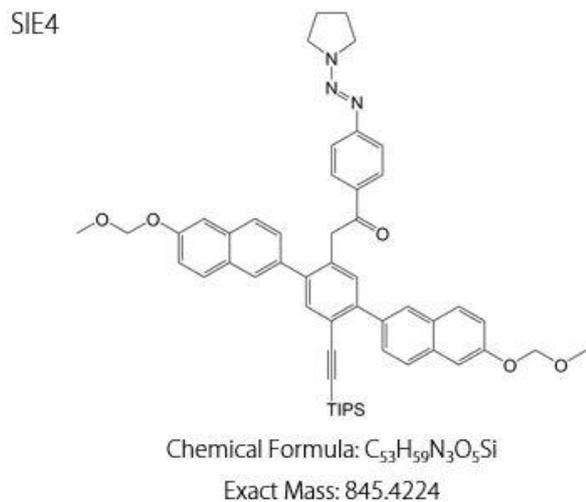
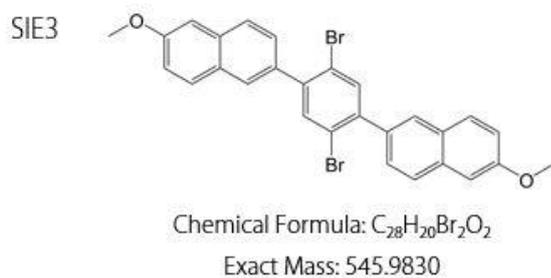
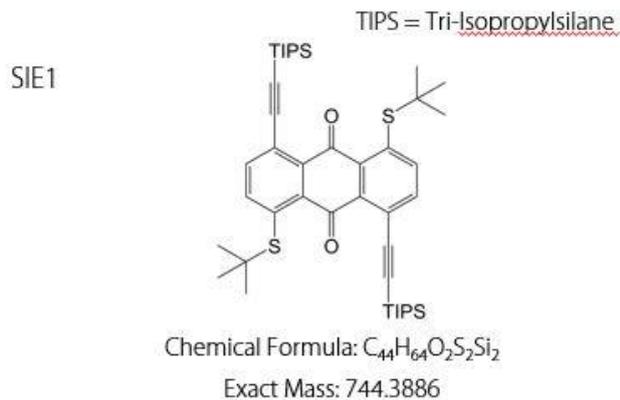
### **Co-authors:**

*Andreas Baumeister, Shimadzu Europa GmbH*

*Ann-Christin Niehoff, Shimadzu Europa GmbH*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
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Structures of poorly soluble compounds

Poster number: **BM-PA-018**

## **ION MOBILITY MASS SPECTROMETRY AS A TOOL TO STUDY STRUCTURAL CHEMISTRY OF METAL COORDINATION CAGES AND GOLD CLUSTERS**

Abstract ID: **948**

**Presenting author: Elina Kalenius, University of Jyväskylä, Department of Chemistry, Nanoscience center, Jyväskylä, Finland**

### **Introduction**

Mass spectrometric methods has been solidifying its position in analysis of different metal coordination complexes since soft ionization techniques became more frequent. In its simplest form, MS analysis provides a way to characterize samples by determination of molecular weight and molecular formulae. Ion mobility mass spectrometry (IM-MS) provides another dimension to structural analytics. Structural analytics of metal coordination cages and coinage metal clusters is extremely difficult with other structural chemistry experimental methods, especially when multiple structures or isomers are formed. IM-MS can effortlessly separate different structures and comparison between experimental and theoretical CCS values provides new insight to molecular structures otherwise impossible to obtain. We show here two examples highlighting the importance of IM-MS methods for structural analytics of gold nanoclusters and metal coordination cages.

### **Methods**

Ion mobility mass spectrometry experiments were performed with Agilent 6560 ESI-IM-QTOF mass spectrometer equipped with dual AJS ion source, Drift Gas Upgrade Kit (Agilent Technologies, USA) and in-source activation fragmentor lens. High purity N<sub>2</sub> or He was used as drift gas directly from the gas cylinder (purity grade 6.0). Experimental <sup>DT</sup>CCS values were determined using multifield method and theoretical values were calculated with IMoS Suite 1.10.

### **Preliminary data (results)**

Very recently, drift tube ion mobility mass spectrometry was used to study gas-phase isomer of the ubiquitous, extremely well-studied Au<sub>25</sub>(SR)<sub>18</sub> cluster both in anionic and cationic form. The IM-MS arrival time distribution (ATD) showed a new isomeric structure, which was the first experimental evidence on recently theoretically predicted isomer. The relative abundance of the isomeric structures was controlled by in-source activation prior the drift-tube and showed the structural dynamics which implies that it may play an important role in their gas-phase reactions, and that isomerization could be controlled by external stimuli.

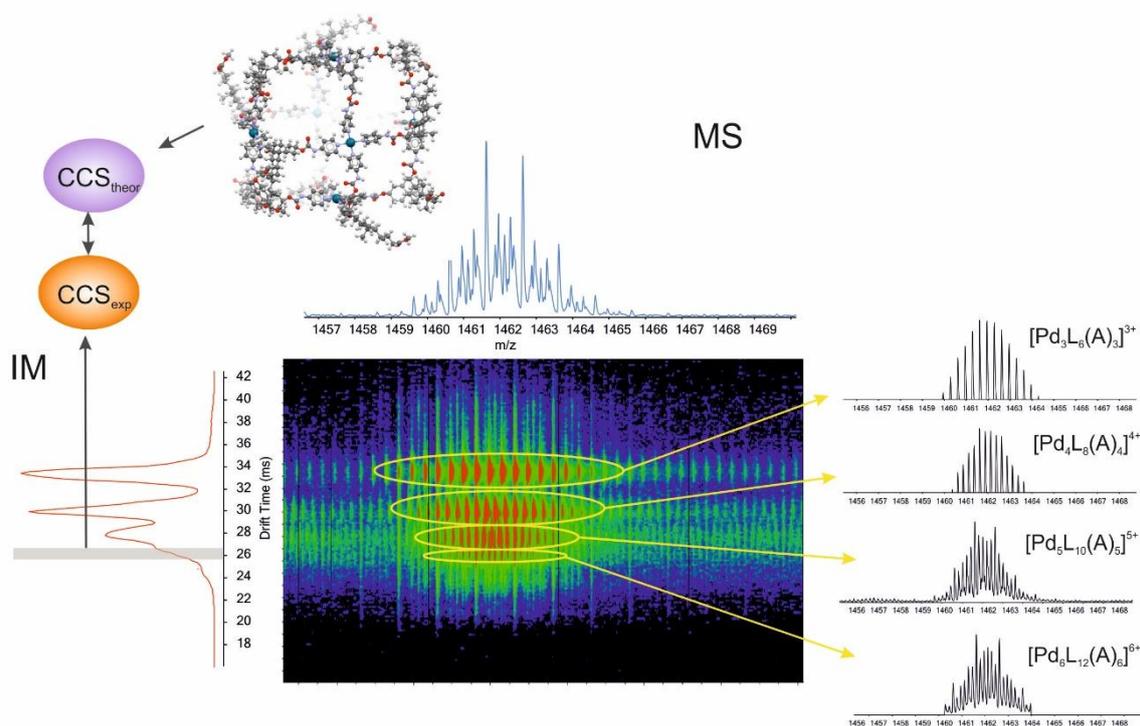
An ion mobility analyzer separates ions in drift tube according to their mobility, which is linked to physical dimensions and charge state of the ion. Therefore IM-MS can be utilized to separate metal coordination cages with overlapping *m/z* values, but different molecular weight and charge state. We have studied various metallosupramolecular cage compounds formed by coordination between organic ligands (*e.g.* cholic acid derivatives) and polyisotopic metals like Pd, Cu, Ni, Ag etc. These often form multiple structures with different charge states and overlapping *m/z*, which can though easily be separated by IM-MS methods (see Figure 1).

### **Please explain why your abstract is innovative for mass spectrometry?**

IM-MS provides means to separate, analyze and visualize different structures from their mixture, which using other methods would be often impossible.

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IM-MS of metallosupramolecular coordination cage.

Poster number: **BM-PA-019**

## **STRUCTURAL CHARACTERIZATION OF HARDWOOD XYLAN WITH DIRECT-INFUSION ESI FT-ICR MASS SPECTROMETRY**

Abstract ID: **961**

**Presenting author: Mikko Nikunen, University of Eastern Finland**

### **Introduction**

Profiling the structure and composition of plant-based biopolymer feedstocks has become an interest during the past decade. Xylan is a complex polysaccharide, consisting of 1,4-b-linked xylose residues with various substituents, mainly arabinose and glucuronic acid as well as acetyl groups. Hardwood and softwood xylans differ considerably by the degree of polymerization and substituents. Xylan is one of the major hemicelluloses in nature. A challenge in the characterization of these polysaccharides is mainly caused by heterogeneity in the structure of polymer as well as poor solubility in most of the common solvents.

### **Methods**

In this study we screened different solvent systems in order to enhance the measurability of native hardwood xylan with ESI FT-ICR MS. The hardwood xylan samples were measured in different solvent systems (i.e., H<sub>2</sub>O/NH<sub>4</sub>OH, H<sub>2</sub>O/HCOOH, MeOH, DCM, DMSO, and CHCl<sub>3</sub>) by high-field FT-ICR mass spectrometry (12-T Bruker Solarix XR), using direct-infusion positive- and negative-ion electrospray ionization (ESI).

### **Preliminary data (results)**

While (+)ESI targeted mainly neutral mono- and oligosaccharides in the sample, (–)ESI mainly ionized acidic glucuronic acid-substituted ones. The best solvent system for negative-ion ESI was H<sub>2</sub>O/NH<sub>4</sub>OH, while H<sub>2</sub>O/HCOOH was the most efficient for positive-ion ESI. The weight-average molecular weights of the xylan samples were considerably lower than that measured with size exclusion chromatography. It is not yet clear if this is due to the sample solubility or the ionization efficiency. For further studies, a chemical modification, e.g. permethylation, will be tested in order to overcome challenges in solubility of the sample matrix.

### **Please explain why your abstract is innovative for mass spectrometry?**

New methods for characterization of native xylan polysaccharide by FT-ICR

### **Co-authors:**

*Eemeli Eronen, University of Eastern Finland*

*Janne Jänis, University of Eastern Finland*

Poster number: **BM-PA-020**

## **MALDI ANALYSIS OF NON-IONIC SURFACTANTS : STRUCTURAL CHARACTERIZATION AND MIXTURE ANALYSIS**

Abstract ID: **973**

**Presenting author: Christelle Absalon, Bordeaux University**

### **Introduction**

Surfactants are surface-active agents widely used in manufacture of housecleaning products, personal care products, industrial detergents, medicine and plastics industry.

The determination and structural characterization of surfactants in complex mixture (1,2) is a real analytical challenge. In this study, we developed method by mass spectrometry to build a library of various non ionic surfactants, several classes of surfactants were chosen: fatty alkanolamide, sorbitan ester, alkyl polyglucoside, fatty acid ethoxylates, alkylphenoethoxylates, alcoholethoxylates.

### **Methods**

Standards and reference samples were analyzed by MALDI with different matrices to determine the best conditions to characterize the chosen surfactants. Surfactants and mixture of surfactants were analyzed on a autoflex instrument in reflector mode.

### **Preliminary data (results)**

Optimization of matrix conditions permits do determine DHB as the more convenient matrix, in particular because of the presence of only one main adduct for each peak.

The different surfactants were fully characterized, we obtain for each compound : Mp, mass range, repeat unit and endgroups. The obtained information permits to build a specific fingerprint for each surfactant. Non-ethoxylated compounds are less easily ionizable, we succeed to find conditions in the sample preparation to improve their ionization (for example addition of co-solvent helps to improve solubilization and then ionization).

The method was then used on several mixtures in order to deconvolute composition of those mixtures.

1. Pascale, R, Acquavia, MA, Onzo, A, Cataldi, TRI, Calvano, CD, Bianco, G. Analysis of surfactants by mass spectrometry: Coming to grips with their diversity. *Mass Spectrometry Reviews*, (2021); 1– 32.
2. Klaus Raith K., Christian E.H. Schmelzer C.E.H., Reinhard H.H. NeubertR.H.H. Towards a molecular characterization of pharmaceutical excipients: Mass spectrometric studies of ethoxylated surfactants, *International Journal of Pharmaceutics*, (2006), Vol 319, Issues 1–2, 1-1

### **Please explain why your abstract is innovative for mass spectrometry?**

Direct analysis by maldi mass spectrometry to analyze complex mixtures of surfactants

### **Co-authors:**

*Claire Mouche, Institut des Sciences Moléculaires, Université de Bordeaux -Talence, France*  
*Yann Rayssac, Institut des Sciences Moléculaires, Université de Bordeaux - Talence, France*  
*Geoffroy Germain, SNF SA - Andrézieux, France*

Poster number: **BM-PA-021**

## **STRUCTURAL FEATURES AND COMPLEXATION PROPERTIES OF A SOLOMON LINK STUDIED BY ION MOBILITY MASS SPECTROMETRY**

Abstract ID: **996**

**Presenting author: Anniina Kiesilä, University of Jyväskylä**

### **Introduction**

Molecular knots are important research field in supramolecular chemistry, for their fascinating applications as molecular machines. Catenane is a molecule consisting of two interlocked rings. Solomon link is doubly interlocked catenane with more rigid structure.

Within recent years, mass spectrometry has become a standard tool to study various supramolecular complexes. With electrospray ionization (ESI) even molecular machines can be transformed to gas phase as intact. Ion mobility mass spectrometry (IM-MS) adds another dimension to analytics and enables us to study structural features of supramolecular complexes and molecular machines, which would be time-consuming or impossible to observe with other structural chemistry methods. Here, we present results how IM-MS can be utilized to study structures of one molecular machine and its structural dynamics upon host-guest chemistry.

### **Methods**

Ion mobility mass spectrometry experiments were performed with Agilent 6560 ESI-IM-QTOF mass spectrometer equipped with dual AJS ion source, Drift Gas Upgrade Kit (Agilent Technologies, USA) and in-source activation fragmentor lens. High purity N<sub>2</sub> or He was used as drift gas directly from the gas cylinder (purity grade 6.0). Experimental <sup>DT</sup>CCS values were determined using multifield method and theoretical values were calculated with IMoS Suite 1.10.

### **Preliminary data (results)**

Solomon link (in Figure 1) is constructed in an elegant way using hydrophobic, aromatic building blocks. Catenated (knotted) structure was verified by ESI-MS and IM-MS experiments. Drift tube ion mobility mass spectrometry resulted in structure related collision cross section values when measured using both N<sub>2</sub> and He. Moreover, this Solomon link was observed to operate as an efficient anion receptor enabling simultaneous complexation of two SO<sub>4</sub><sup>-</sup> ions, which is connected to conformational change of the Solomon link. Here we present a detailed study on structural features of Solomon link including conformational dynamics and receptor properties with different anions.

### **Please explain why your abstract is innovative for mass spectrometry?**

Ion mobility mass spectrometry was innovatively used to study structural features of supramolecular complexes and molecular machines.

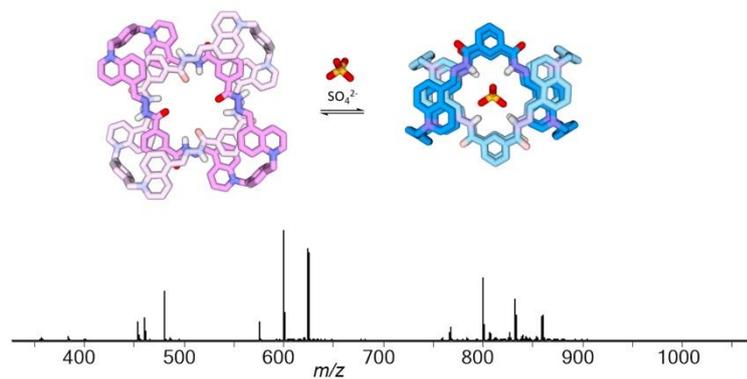
### **Co-authors:**

*Fabien Cougnon, University of Jyväskylä*

*Elina Kalenius, University of Jyväskylä*

**POSTER SESSION A**

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Solomon link goes to gas phase

## Theme: Food and (bio)pharma

### Session: Biosimilars, Biobetters and Glycoengineering

Poster number: FP-PA-001

## USE OF PASEF FOR ACCELERATED PROTEIN SEQUENCE CONFIRMATION AND DE NOVO SEQUENCING WITH HIGH DATA QUALITY

Abstract ID: 126

Presenting author: Detlev Suckau, Bruker Daltonics GmbH & Co. KG

### Introduction

Biopharmaceutical sequences can be well confirmed by multiple protease digests - e.g., trypsin, elastase and chymotrypsin – followed by LC-MS/MS data analysis. High quality data can be used for *de novo* sequencing as well. PASEF (Parallel Accumulation and Serial Fragmentation) on the timsTOF instrument has been used to accelerate proteome studies and increase sequence coverage concomitantly.

Here we applied PASEF to generate exhaustive protein sequence coverage maps by combination of results from 2 or 3 enzyme digests using a short LC gradient. The data quality obtained was high and adequate for determining antibody sequences *de novo*.

### Methods

Nivolumab (IgG4k) and dulaglutide (IgG4 based Fc-fusion peptide) were used as European union available drug products. They were reduced and carbamidomethylated prior to proteolytic digest by trypsin, elastase or chymotrypsin. After analytical LC -2.1x150 mm C18 column with 45 min gradient length and 60 min cycle time- they were subjected to PASEF analysis on the timsTOF. LC-MS/MS datasets were processed directly either in BioPharma Compass 4.0 (Bruker) where spectra were matched with the reference sequence (5 ppm MS tolerance, no enzyme) for confirmation of provided sequences or to Supernovo (ProteinMetrics) to establish sequences *de novo*.

### Sequence confirmation:

Proteins were digested by 3 enzymes individually. For nivolumab 98/99/87% sequence coverage and 93/97/85% fragment coverage were obtained from the individual digest analysis with Trypsin/Chymotrypsin/Elastase, respectively. For dulaglutide 100/100/100% sequence coverage were obtained. The merged peptide maps from the 2 proteins resulted in ~1000 peptides, sufficient to safely confirm the full sequences and to determine the nivolumab sequence *de novo*.

### De Novo Sequencing:

For nivolumab, the combined data from three digests were analyzed using Supernovo. The output consisted of the full heavy chain and light chain sequences, deduced *de novo* sequences, as well as metrics/visualizations to aid inspection. The deduced sequence was correct in all the CDR regions and differed from the known nivolumab sequence at three locations outside of the CDR regions. There was one arginine versus valine+glycine substitution (nearly isobaric), and there were two locations where extra residues were inserted: a single residue at one location and multiple residues at the other location. The metrics/visualizations, which include the confidence of each deduced residue, an aggregate fragmentation map, differences from germline, and a peptide inspection dashboard, helped to spot and fix the errors.

### Conclusions:

Use of trapped ion mobility as orthogonal separation dimension allowed to shorten chromatographic cycle time to 1 hour at increased sequence coverage. These conditions provided data qualities sufficient to obtain nearly full sequence coverage from 2-3 enzyme digest analyses of nivolumab and to reliably establish all 6 CDR sequences from *de novo* sequencing.

## POSTER SESSION A

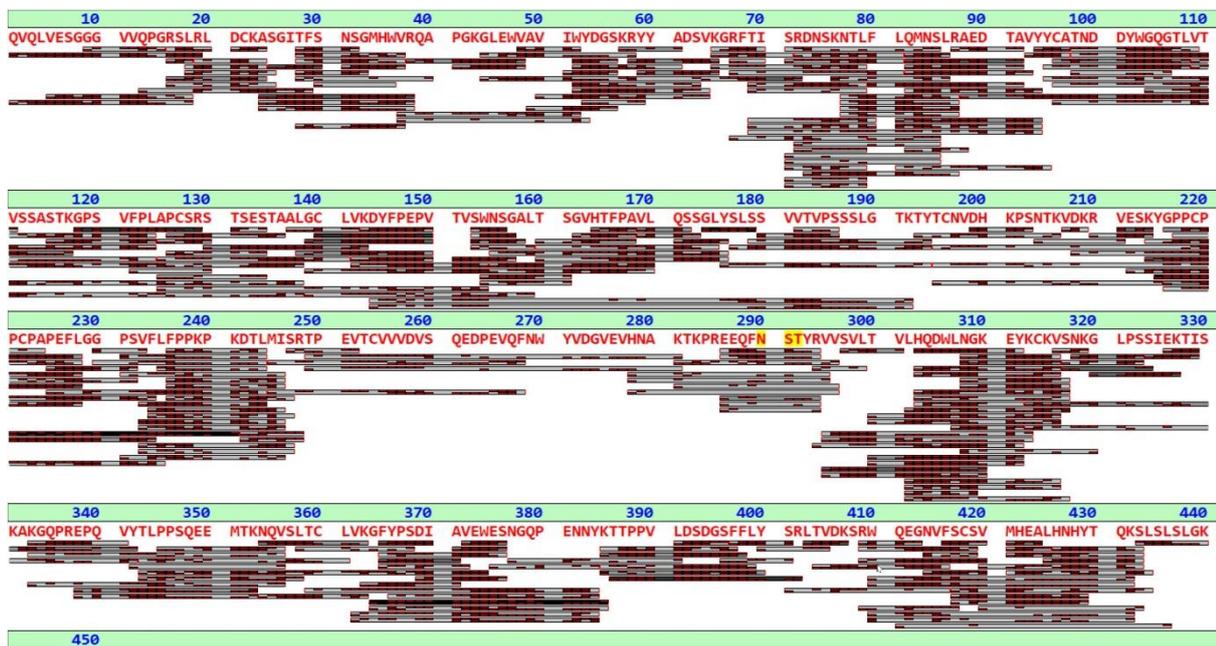
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### Please explain why your abstract is innovative for mass spectrometry?

Use of 2-3 different enzyme digests for trapped ion mobility-enhanced LC-MS/MS datasets for fast and thorough sequence coverage with *de novo* sequencing quality.

### Co-authors:

Stuart Pengelley, Bruker Daltonics GmbH & Co. KG  
Waltraud Evers, Bruker Daltonics GmbH & Co. KG  
Eckhard Belau, Bruker Daltonics GmbH & Co. KG  
Ilker Sen, Protein Metrics Inc.  
Wilfred Tang, Protein Metrics Inc.  
Alain Beck, Centre d'Immunologie Pierre-Fabre



Sequence coverage map of nivolumab HC from 3 individual digests

Poster number: **FP-PA-002**

## **AUTOMATED ANNOTATION OF CLIPPING RELATED HETEROGENEITIES IN VEDOLIZUMAB**

Abstract ID: **137**

**Presenting author: Christian Albers, Bruker Daltonics GmbH & Co KG**

### **Introduction**

For Biopharmaceuticals, degradation of the active drug substance during production, formulation or storage poses a threat to drug efficacy and safety and is a critical quality attribute (CQA). The detection of such truncated protein species -clipping variants- can be difficult to achieve through classic peptide mapping with trypsin. In this case study, a vedolizumab biosimilar was characterized after SpeB treatment, which cleaves IgG1 predominantly in the hinge region. Minor cleavage products with uncharacterized specificity have also been observed. We analyzed the reduced SpeB digest products with LC-MS using a high isotopic fidelity QTOF instrument to characterize the enzyme specificity and evaluate the performance of our clipping variants detection workflow. Middle-Down sequence validation measurements were subsequently performed to confirm the findings.

### **Methods**

Vedolizumab biosimilar sample (Polpharma) was measured after treatment with FabULOUS and IgGZERO enzyme kits (Genovis) followed by reduction. Concentration was approximately 0.25 mg/ml. The sample was measured with a UHPLC system (Elute) coupled to a QTOF mass spectrometer (maXis II ETD, both Bruker Daltonics). Injected sample amounts were 0.25 µg (intact mass) and 2 µg (Middle-Down), using a BEH C4 column (Waters, 300Å 1.7 µm, 2.1 x 100 mm) and utilizing a 33.5 min gradient for antibody subunit separation. Data were processed with Biopharma Compass software to assign possible clipping variants and to confirm sequences based on fragment spectra.

### **Preliminary data (results)**

All expected subunits molecular weights (Fd, Fc/2 and Light Chain) were determined with a mass accuracy better than 2 ppm and the Middle-Down analysis provided an average sequence validation percentage (SVP) of 50 % from LC-ETD and 37% from LC-CID. Besides the expected and chromatographically predominant three vedolizumab subunits four additional chromatographic peaks were observed. The Clipping Variant functionality of BioPharma Compass provided an initial list of vedolizumab clipping candidates based on the determined monoisotopic intact masses of these additional peaks. Subsequent LC-CID and LC-ETD dataset were acquired for Middle-Down sequencing to provide sequence information on the protein subunits and to identify the clipping variant from the initial list of clipping candidates. The combined approach to qualify candidates by intact mass measurements for Middle-Down sequencing yielded 6 more minor-cleavage sites, 3 of which were confirmed by direct ETD and CID analysis, 2 were validated by their complementary nature and mass accuracy and one by mass accuracy and isotopic fidelity alone. The developed workflow was efficient in the detection and validation of antibody clipping products. Clipping variant candidates were detected by reliable monoisotopic mass determination and isotopic fidelity. Suggested candidates were validated by Top-Down sequencing on a single instrument and software platform. The workflow proved to be quite efficient in the detection of the expected SpeB cleavage sites and revealed additional modest unspecific enzyme side reactions.

### **Please explain why your abstract is innovative for mass spectrometry?**

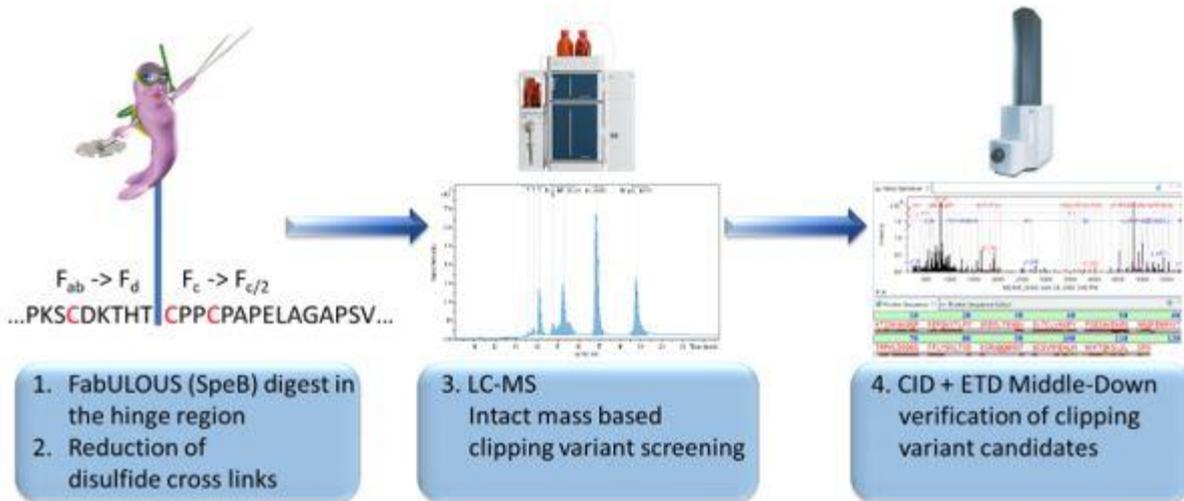
Automated assignment of protein clipping variants based on intact mass measurements with high mass accuracy and isotopic fidelity combined with Middle-Down experiments for sequence confirmation

### **Co-authors:**

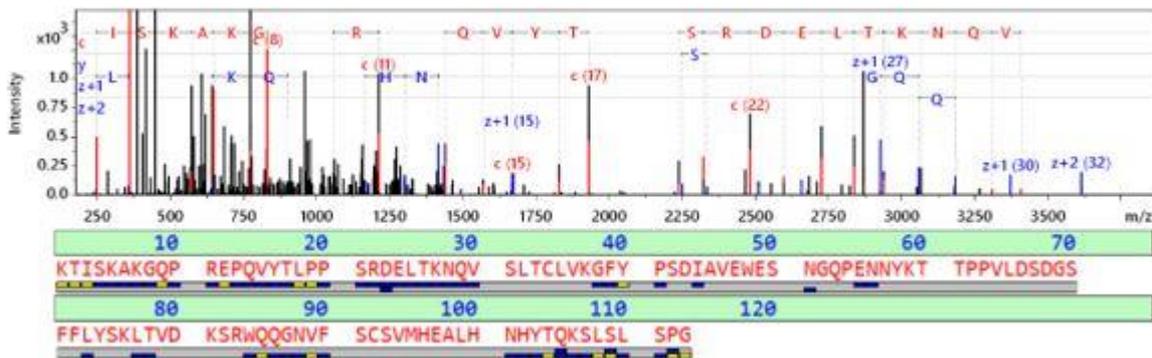
*Detlev Suckau, Bruker Daltonics GmbH & Co KG*  
*Tomasz Goździewicz, Polpharma Biologics S.A.*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Experimental design for the vedolizumab protein clipping analysis



Annotated ETD Spectrum of Heavy Chain degradation product [338-450]

Poster number: **FP-PA-003**

## **FORCED DEGRADATION STUDY OF A MONOCLONAL ANTIBODY BY HRMS AT INTACT, SUBUNIT AND PEPTIDE LEVELS**

Abstract ID: **149**

**Presenting author: Geert Van Raemdonck, AnaBioTec**

### **Introduction**

Monoclonal antibodies (mAbs) are inherently heterogeneous molecules. Protein post-translational modifications (PTMs) can arise at different stages of antibody manufacturing or during drug product storage. Even though forced degradation studies are performed at relatively harsh conditions within a short time period, the information gathered can provide highly relevant data of the mAbs to predict effects of real time environmental conditions. The most commonly observed pathways during forced degradation studies on monoclonal antibodies are aggregation, fragmentation, deamidation and oxidation.

Liquid chromatography coupled with high resolution mass spectrometry (LC–HRMS) analysis has been widely utilized for protein therapeutic development and characterization. It is one of the most sensitive technologies providing site-specific identification and quantitation of PTMs in biotherapeutics such as mAbs.

### **Methods**

Samples of adalimumab were assessed at the intact protein, subunit and peptide level by using a high end Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer to identify the sites of oxidation and deamidation within the primary sequence. Subunit analysis was performed by using IdeS protease digestion including denaturation and reduction. Subsequently, the three subunits (Fc/2, LC and Fd') were separated chromatographically. For the peptide mapping experiments, enzymatic digestion with trypsin was performed followed by chromatographic separation of the resulting peptides.

### **Preliminary data (results)**

Here, we investigate the susceptibility of oxidation and deamidation by subjecting the IgG mAb adalimumab to either oxidative stress by exposing the mAb to 1% hydrogen peroxide and to basic hydrolysis by incubation with 0.1M NaOH during 24 hours.

Four methionine residues, prone for oxidation after oxidative stress, could be localized at intact, subunit and peptide levels. Peptide mapping analysis of samples exposed to basic hydrolysis showed clearly higher levels of deamidated peptides. These results indicate that LC-HRMS is a powerful tool in the development and quality control of (bio)therapeutics.

### **Please explain why your abstract is innovative for mass spectrometry?**

HRMS has become a powerful tool during forced degradation studies that has been commonly used to evaluate critical quality attributes (CQA) evaluation and identification of product variants.

### **Co-authors:**

*Florencia Linero, AnaBioTec*

*Jessika Wynendaele, AnaBioTec*

*Koen Iterbeke, AnaBioTec*

Poster number: **FP-PA-004**

## **ACCELERATED SOLVENT EXTRACTION AND ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH HIGH-RESOLUTION MASS SPECTROMETRY FOR THE ANALYSIS OF ADDITIVES IN POLYMERS FOR BIOMANUFACTURING PROCESSES**

Abstract ID: **574**

**Presenting author: Sandra Rontree, Thermo Fisher Scientific**

### **Introduction**

Single-use bioprocessing bags are increasingly used in biopharmaceutical manufacturing. Despite their advantages, these plastic assemblies draw concern because they are a potential source of contamination due to extractable and leachable compounds (E&Ls). Characterising E&Ls from such materials is a necessary step in establishing their suitability for use. Therefore, there is an urgent need for sensitive methods to identify and quantitatively assess compounds in plastic materials. Accelerated solvent extraction (ASE) is a powerful technique that can be reliably used for this purpose.

### **Methods**

In this study, ASE followed by liquid chromatography and Orbitrap-based High-Resolution Accurate Mass (HRAM) mass analysis was found to be an efficient and versatile method for the determination of additives in different multilayer polymer systems from singleuse bags. ASE optimisation was performed using a design of experiments approach.

### **Preliminary data (results)**

More than 100 additives and degradation products were confidently identified by HRAM MS. The type of solvent, temperature, swelling agent addition, static time and a number of cycles were the selected variables. Optimum conditions were dependent on the type of plastic film. Ethyl acetate and cyclohexane were selected individually as optimum solvents. Optimum temperatures were 90–100 °C. The pressure was set at 1500 psi and extraction time was 30 min in 2 cycles. Swelling agent addition was necessary with polar extraction solvents. Correlations between the type and levels of identified additives and the type of polymer system were established. In addition, degradation behaviour and pathways for some additives can be addressed.

### **Please explain why your abstract is innovative for mass spectrometry?**

Automated solvent extraction can be reliably used for the analysis of additives in singleuse polymeric bioprocessing bags in biomanufacturing processes using Ultra-High Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry.

Poster number: **FP-PA-005**

## **COMPREHENSIVE PROFILING OF MAB GLYCOFORMS USING THE TSKGEL FcR-III A AFFINITY COLUMNS: FROM ONLINE NATIVE MASS SPECTROMETRY TO OFFLINE N-GLYCANS ANALYSIS**

Abstract ID: **743**

**Presenting author: Elena Kumm, Tosoh Bioscience GmbH**

### **Introduction**

Glycosylation is a crucial factor determining the pharmacological properties of biotherapeutics, including monoclonal antibodies (mAbs). Therefore, characterizing the heterogeneity of N-glycan moieties in mAbs is part of their manufacturing and quality control. However, the different possible structures of glycans lead to a vast number of glycoisoforms for a given protein, making their identification a crucial analytical challenge. Tosoh has developed a novel stationary phase utilizing a recombinant FcγIIIa protein to profile and analyze glycan-dependent changes in Fc receptor affinity. Using this stationary phase, we present two workflows: inline native ESI-MS and offline released N-glycans analysis by HILIC-MS/MS.

### **Methods**

Glycoform profiling of mAbs was conducted on the TSKgel FcR-III A-NPR column using UV detection, and native ESI MS. Chromatographic separation was done using a linear pH gradient of 6.5 to 4.5. Native mass spectrometry detection was achieved on a SCIEX X500B QTOF. Further analysis of N-glycans composition was conducted using a two-step procedure. The mAbs were first fractionated on the TSKgel FcR-III A-5PW column. The collected peak fractions were subjected to enzymatic N-glycans release, fluorophore-labeling, and clean-up. Labeled and released glycans were then analyzed using HILIC-MS on the TSKgel Amide-80 column with MS/MS detection.

### **Preliminary data (results)**

Using several mAbs with unique protein sequences and glycosylation patterns, we established a direct correlation between the N-glycan nature, structure, content, and elution profile on the FcR-III A columns. Furthermore, we found a direct correlation between higher ADCC activity and binding affinity on the FcR-III A columns, indicating that the complexity of mAb glycosylation patterns is reflected in the observed FcR-III A elution profiles.

### **Please explain why your abstract is innovative for mass spectrometry?**

The FcγIIIa affinity ligand separates antibodies by functional characteristics. It can be combined with in-line ESI-HR-MS detection to additionally determine structural causes in a one-step method.

### **Co-authors:**

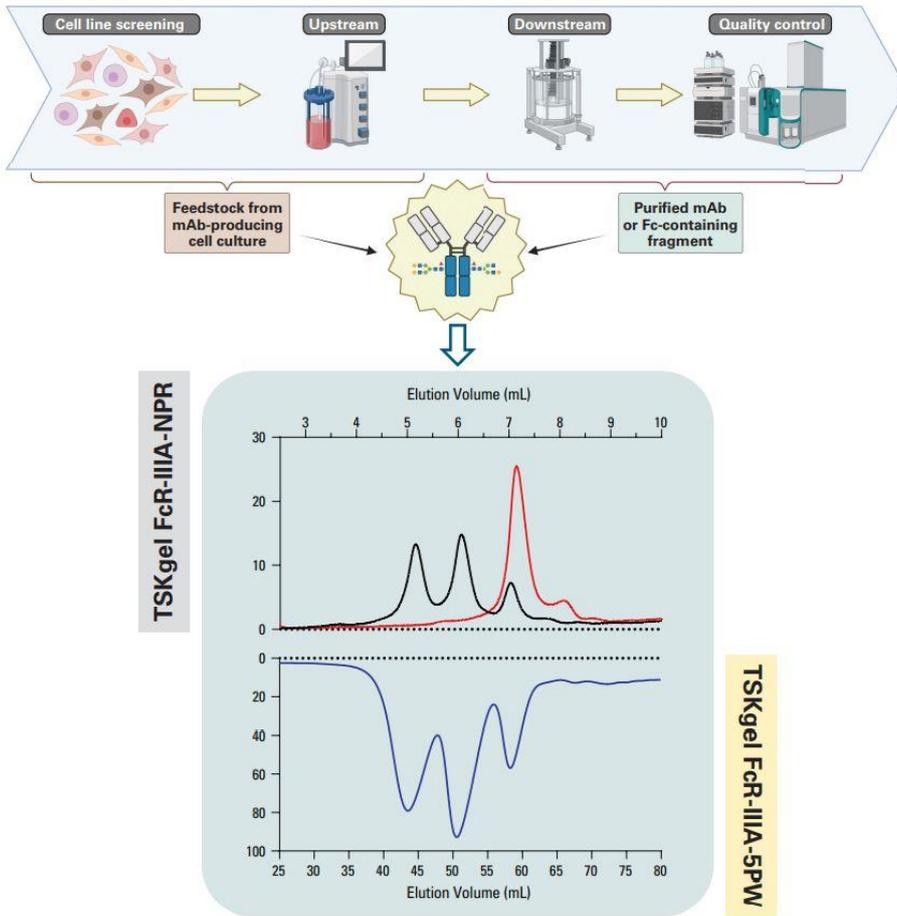
*Andrea Krumm, Tosoh Bioscience GmbH*

*Heidi Vitrac, Tosoh Bioscience LLC*

*Scott L. Melideo, Tosoh Bioscience LLC*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



FcR affinity analysis of monoclonal antibodies

Poster number: **FP-PA-006**

## **BIOSIMILAR PEPTIDE MAPPING CHARACTERIZATION AND MAM WORKFLOW USING A BENCHTOP QTOF WITH AN APP-BASED ACQUISITION AND DATA PROCESSING PLATFORM**

Abstract ID: **889**

**Presenting author: Emma Harry, Waters Corporation**

### **Introduction**

Peptide mapping is one of the gold standard techniques for post-translational modification (PTM) assessment not only for characterization, but for development and comparability, of biologics and biosimilar drugs. This rigorous characterization is crucial to ensure safety and efficacy of biopharmaceuticals. In recent years, multi-attribute method (MAM) has gained traction as a promising workflow in which digested samples (stressed or biosimilars, for example) are compared against a reference sample to assess differences or impurities among them. A successful workflow must be robust, easily implemented, and GMP-compliant. This study demonstrates the implementation of a peptide characterization and monitoring method for infliximab biosimilars on a benchtop QTOF instrument with compliance-ready app-based data acquisition and processing.

### **Methods**

Samples of reduced/alkylated Infliximab innovator (Remicade®) and biosimilars (Inflectra®, Avsola®, & Renflexis®) samples were trypsin-digested prior to analysis via RPLC-MS on a CSH C18 column (ACQUITY™ Premier CSH, 1.7µm, 2.1 x 100 mm) maintained at 60 °C for a 50-minute linear gradient of 1-35% B (80-minute total time). Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. DIA MS detection (MS<sup>E</sup>) was performed on a benchtop QTOF mass spectrometer. Data analysis was carried out via UNIFI™ and Peptide MAM apps in the waters\_connect™ platform.

### **Preliminary data (results)**

The goal of using this peptide mapping and MAM workflow was two-fold—First, to assess the comparability of infliximab innovator and three biosimilar drug products. Second, the innovator and one biosimilar were thermally stressed to assess critical quality attributes (CQAs) over time. In addition, the ability to employ intelligent data capture<sup>1</sup> allows for real-time noise reduction and overall file size reduction, which promotes more high throughput data acquisition and processing.

Once the peptide mapping data is generated, lists containing the high confidence peptide matches are generated using a built-in peptide mapping data processing tool. The comparison data shows the change in relative percent of the CQA peptides and flags them as “New Peaks” when the difference between the innovator and biosimilar sample exceeds a pre-defined threshold.

Over 90% sequence coverage is achieved for innovator and all biosimilars, and the detected CQA sites for infliximab are consistent with previous reports<sup>2,3</sup>. Deamidation hotspots include light chain N158 and heavy chain N57, and N392, all of which showed increases in deamidation upon thermal stress. Oxidation results did not show significant differences or increases after thermal stress. The N-glycoprofile and C-terminal lysine peptides were also detected and compared between innovator and biosimilars. Minor differences in the N-glycoforms are apparent between the samples, and the C-terminal lysine variant was found to be significantly lower in the biosimilar Renflexis. This peptide mapping workflow performed on a QTOF system allows for high quality characterization and simplified MAM data processing in a fully compliant setup.

### **Please explain why your abstract is innovative for mass spectrometry?**

Biosimilar peptide map characterization workflow with the use of a new benchtop QTOF with app-based data acquisition and processing platform.

### **Co-authors:**

*Samantha Ippoliti, Waters Corporation*

*Ying Qing Yu, Waters Corporation*

Poster number: **FP-PA-007**

## **A ROBUST WORKFLOW FOR BIOSIMILAR COMPARABILITY ASSESSMENT VIA INTACT AND SUBUNIT RPLC-MS AND NATIVE IEX-MS WITH A BENCHTOP QTOF**

Abstract ID: **890**

**Presenting author: Ronan O'Malley, Waters Corporation**

### **Introduction**

Over the last few decades, biosimilar monoclonal antibody (mAb) drug products have become increasingly sought after by pharmaceutical companies. As patents for innovator drugs expire, options for less expensive biosimilars are intriguing for both patients and drug developers. The FDA requires analytical data, animal studies, and clinical studies to prove biosimilarity<sup>1</sup>. Since biologics are much more complex than small molecules, for example, it is challenging to demonstrate comparability<sup>2</sup>. Some of the important data points are charge variant profile and post-translational modifications, which can be characterized by LC-MS techniques. We demonstrate a robust workflow for reversed-phase (RP-MS) and native ion exchange (IEX-MS) analysis of intact/subunit characterization of infliximab biosimilars via prototype QTOF with a dedicated workflow for intact protein mass confirmation.

### **Methods**

Infliximab innovator (Remicade®) & 3 biosimilars (Inflectra®, Avsola®, Renflexis®) were analyzed as intact and IdeS-digested subunits via reversed-phase chromatography (RPLC) and ion exchange chromatography (IEX) coupled to a new benchtop QTOF mass spectrometer. RPLC-MS analysis was performed on a BEH C4 column (ACQUITY™ Premier BEH C4, 1.7µm, 2.1 x 100 mm). Mobile phase A was 0.1% formic acid in water, mobile phase B was 0.1% formic acid in acetonitrile. IEX-MS analysis was performed on a strong cation exchange column (BioResolve™ SCX mAb, 3µm, 2.1 x 100 mm) at 30 °C with IonHance™ CX-MS pH concentrates A & B.

### **Preliminary data (results)**

The use of orthogonal analysis methods for biosimilar characterization allows us to achieve a more comprehensive picture of the quality attributes. RPLC-MS is a sensitive method for intact and subunit mAb confirmation of mass along with relative quantitation for post-translational modifications (PTMs) such as N-glycosylation, glycation, oxidation, and C-terminal lysine variants.

IEX-MS gives the opportunity to probe and monitor various charge variants of biopharmaceuticals. The UV chromatogram is used for quantitation and comparison of charge variants, with the additional benefit of online MS detection to investigate species under each peak. For intact mAb IEX-MS, an optimized gradient of 47-67% B was used to separate the main species (Infliximab with 0 C-terminal lysine (0K) species in all cases) from a variety of acidic and basic variants. After investigation of MS data, it is observed that the 3 main populations in the chromatogram correspond to Infliximab 0K, 1K, and 2K, each with its own sub-population of acidic species. Comparison of the innovator and 3 biosimilars shows that Renflexis has significantly less C-terminal lysine variants than rest. Analysis of the non-reduced IdeS digest via IEX-MS helps to further localize the acidic variants. It confirms the 3 C-terminal lysine variants are indeed present on the Fc, and that the acidic variants of the Fc are consistent with acidic N-glycoforms published previously<sup>2</sup>. The Fd and/or LC acidic variants likely stem from deamidation or conformational variants, as a significant mass shift is not observed for the pre-peak of this species.

### **Please explain why your abstract is innovative for mass spectrometry?**

A robust workflow for mAb biosimilar comparability via intact and subunit RP-MS and native IEX-MS via next generation QTOF MS.

### **Co-authors:**

*Samantha Ippoliti, Waters Corporation*

*Ying Qing Yu, Waters Corporation*

## Session: Imaging in Food & Pharma

Poster number: **FP-PA-008**

### **MICROPLASTIC CHARACTERIZATION IN SEAFOOD BY MASS SPECTROMETRY IMAGING**

Abstract ID: **342**

**Presenting author: Lidia Molina Millán, Maastricht Multimodal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University**

#### **Introduction**

Microplastics (MPs) have raised great ecological concerns since they can threaten the environment and human health. Presenting non-biodegradable and lightweight properties, these emerging pollutants can enter the aquatic environment. Some aquatic organisms, mainly filter-feeding, are unable to select the ingested particles and, consequently, MPs can easily bioaccumulate in their tissues. This phenomenon may have negative implications for human health due to the ingestion of contaminated seafood. In this work, we applied mass spectrometry imaging (MSI) technologies to provide a comprehensive insight into the toxicological effects of MPs in marine specimens.

#### **Methods**

- Limit of detection (LOD) of plastics on tissue: Snap-frozen fresh mussels were cryosectioned and sprayed with Norharmane and Ag-Dithranol matrices before being analyzed using MALDI-MSI on a RapifleX Tissue typer. Data were analyzed using FlexImaging and SciLS Lab software. All measured tissue sections were stained with hematoxylin and eosin (H&E) and their images were scanned using Aperio CS2 scanner. Polystyrene and polyethylene glycol were used as standards.

- Toxicity in a seafood model: polystyrene particles (200 µm) were used in toxicity experiments in a *Orzyias latipes* model. Tissue sections were analyzed by MALDI and MALDI-2 imaging using Ag-Dithranol matrix.

#### **Preliminary data (results)**

Cryosectioning process was optimized considering several parameters, such as section thickness, temperature and composition of the embedding media. H&E images showed that the optimal conditions to analyze mussels by MALDI-MSI were gelatin as embedding material (5% concentration) and section thickness of 20 µm at -22 °C. In addition, different concentrations of polymer standards were evaluated on mussel tissue to determine the LOD of the method down to 10 µg/µl. The optimized method was used for the analysis of dosed animals. Polystyrene standards were detected by MALDI and MALDI-2 instrumentation with specific locations in whole body sections from *Ozyias latipes* fish.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Our results suggest that the methods we have developed, based on MALDI-MSI technology, might be useful to analyze MPs in fish and seafood.

#### **Co-authors:**

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*Eulalia María Beltrán, Laboratory for Ecotoxicology, Department of the Environment, National Institute for Agricultural and Food Research and Technology (INIA)*

*María Victoria Pablos, Laboratory for Ecotoxicology, Department of the Environment, National Institute for Agricultural and Food Research and Technology (INIA)*

*Ron M. A. Heeren, Maastricht Multimodal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University*

*Berta Cillero Pastor, Maastricht Multimodal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University*

Poster number: **FP-PA-009**

## **SAMPLE PREPARATION STRATEGIES OF MUSHROOM AS A MODEL FOR FOOD QUALITY ASSESSMENT USING MASS SPECTROMETRY IMAGING**

Abstract ID: **350**

**Presenting author: Mudita Vats, Maastricht Multimodal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry, Maastricht University**

### **Introduction**

Food analysis is significant for both producers and consumers. Besides profit, producers should keep in mind the quality and safety of their products. MALDI (matrix assisted laser desorption ionization), DESI (desorption electrospray ionization) and REIMS (rapid evaporative ionization mass spectrometry) have been reported to analyze the metabolites, nutrients, toxins, acidity, and safety of food samples. However, sample preparation of mushrooms has not been explored so far. In this work we used mushroom (approximately 2cm in diameter) purchased from local supermarket as a model for food authentication, food origin and quality assessment using mass spectrometry imaging (MSI).

### **Methods**

Snap frozen mushrooms were sectioned on a cryostat. Embedding in 2% CMC and tape transfer method using conductive copper tape were tested. Also stamping was evaluated where half cut mushroom was pressed against ITO slide (figure 1) or against copper tape placed over an ITO slide.

**MALDI imaging:** Data acquisition on a Bruker RapifleX was done in positive and negative ion modes.

**DESI imaging:** A Waters xevo q-ToF system was employed for analysis.

**REIMS acquisition:** Fresh mushroom cap, stipe, gills and inner flesh were profiled using 3D MS Scanner with LA-REIMS (figure 2) coupled to XEVO G2 XS qToF (Waters) .

### **Preliminary data (results)**

**MALDI and DESI imaging:** Dry stamps of mushrooms with and without copper tape resulted as best sample preparation methods for small molecules. Choline (at  $m/z$  104.1 Da  $\pm$  0.05 Da) was found to be abundant in both mushroom cap and stipe. Stamping mushroom with solvents was not convenient to handle and sample preparation was not optimal.

**REIMS acquisition:** PCA and LDA models showed clear separation between different tissue areas based on their molecular profiles. The cap and the white flesh could be separated, indicating that there are molecules present that are specific for the brown layer on the mushroom. Different molecular distributions could be visualized on the mushroom cap using the 3D MS Scanner.

For samples like mushrooms, ambient ionization techniques are most suitable as they require very less or no sample preparation preserving the metabolic composition of the sample. But as MALDI imaging is commonly used in research so to broaden its application stamping the mushroom over the slide is more effective than sectioning.

### **Please explain why your abstract is innovative for mass spectrometry?**

Application of MALDI, DESI and REIMS was extended to mushroom analysis. Stamping procedure to prepare samples having high water content is a better alternative to sectioning.

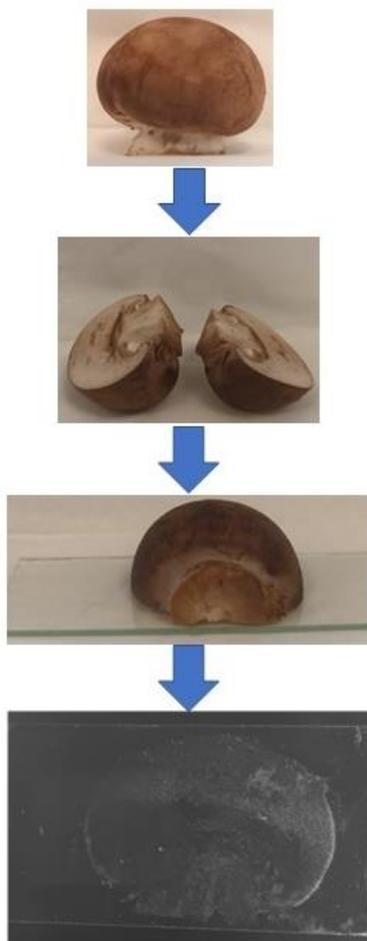
### **Co-authors:**

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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Maastricht University  
Ron M.A Heeren, Maastricht Multimodal Molecular Imaging Institute (M4I), Division of Imaging Mass Spectrometry,  
Maastricht University



Procedure of stamping

Mushroom cut into two halves was pressed against ITO slide

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Mushroom placed on the sample holder  
of 3D MS scanner

REIMS acquisition

Poster number: FP-PA-010

## SPATIAL LOCATION OF MONOTERPENOID INDOLE ALKALOIDS IN RAUWOLFIA TETRAPHYLLA BY MALDI-MSI

Abstract ID: 437

Presenting author: Marcus Lorensen, University of Copenhagen

### Introduction

*Rauwolfia tetraphylla* (Apocynaceae) is a plant that is known for its synthesis of various compounds for medicinal purposes, including reserpine. Reserpine is a monoterpene indole alkaloid (MIA) used to counter high blood pressure, however even though its existence has been known since 1952 the biosynthetic pathway is not fully understood. The purpose of the study is to understand the underlying pathways for MIAs with the aim of integrating them into other organisms such as yeast.

Initially, to understand which enzymes takes part in the MIA pathways, it is required to know where the pathway takes place. Therefore, in this study we focus on discovering the spatial location of reserpine and its theoretical intermediates by mass spectrometry imaging (MSI).

### Methods

Plant materials were sectioned into thin (10µm) slices using a cryo-microtome, whereafter the sliced plant material were stored at -80 °C. When ready for use, the samples were freeze-dried overnight. After freeze-drying, samples were coated with matrix using a pneumatic matrix sprayer. Samples were then analyzed by a SMALDI-5 (TRANSMIT GmbH, Giessen, Germany) coupled to a Thermo QExactive mass spectrometer. Raw files of the analyses were converted to imzML files by RawToImzML (TRANSMIT). Images were generated using the software MSiReader.

### Preliminary data (results)

Based on a proposed biosynthetic pathway of reserpine, a number of intermediates were imaged in tissues of *Rauwolfia tetraphylla*. The tissues investigated were roots, stem, leaf and berry.

In root sections, intermediates were found to be mostly present in the cortex area outside the endodermis and pith area. However, reserpine was observed in the cortex, endodermis and epidermis.

In stem sections, most intermediates were observed in the around the pith region and the vascular tissue. Some of the intermediates are only observe around the pith region, whereas some are also present in the cortex and epidermis.

Leaf sections were made of both young and old leaves. New leaves were acquired from the topmost branches and the old leaves were acquired from the bottommost part of the stem. Most intermediates were observed in both stages of leaf tissue, however two intermediates, namely RPI2 and RPI5 were not observed in the young leaves.

Berry sections were made from both immature and mature leaves. Immature berries were acquired as small green berries and the mature berries were acquired as wrinkly purple berries. The most noticeable difference in these two stages is that strictosidine/vincoside in young berry tissue was observed in the pericarp and endocarp, whereas in the old berry tissue it is observed in the epidermis. Another major difference is that reserpine in young berry tissue observed in the pericarp and seed, whereas it is only observed in the pericarp.

### Please explain why your abstract is innovative for mass spectrometry?

By utilizing MALDI-MSI, compounds and intermediates in a multicellular biosynthetic pathway were localized at high resolution.

### Co-authors:

Nanna Bjarnholt, University of Copenhagen

Sarah O'Connor, Max Planck Institute for Chemical Ecology

Vincent Courdavault, Université de Tours

Christian Janfelt, University of Copenhagen

Poster number: **FP-PA-011**

## **PHARMACOKINETICS OF CATIONIC AMPHIPHILIC ANTIHISTAMINES IN CANCER MODELS STUDIED BY WHOLE-BODY DESI-MSI**

Abstract ID: **557**

**Presenting author: Christian Janfelt, Dept. of Pharmacy, University of Copenhagen**

### **Introduction**

Cationic amphiphilic drugs (CADs) emerge as promising anti-cancer chemotherapeutics as their anti-cancer activities have extensively been demonstrated in in vitro and in vivo cancer models as well as in epidemiological studies. However, how CADs upon administration reach tumors in vivo and exert their anti-cancer activities remains largely understudied. We here demonstrate that CAD antihistamines upon oral administration poorly reach xenograft tumors in mice due to extensive hepatic metabolism but that their metabolites reach the tumors while retaining anti-cancer properties.

This was demonstrated by DESI-MSI of whole-body cryo-sections from mice orally dosed with ebastine and loratadine and confirmed by LC-MS of extracts of homogenized tumor samples.

### **Methods**

Immunodeficient NOG female mice were inoculated with MCF-7 cells into the axillary mammary fat pad. Once the tumors reached ~7 mm in diameter, the mice were orally treated with 30 mg/kg/day of ebastine and the same amount of loratadine for three consecutive days. 24 hours after the last dose, the mice were sacrificed. The mice were cut in whole-body cryosections, which were imaged using a custom-built DESI-MSI ion source mounted on a Thermo QExactive Orbitrap mass spectrometer. Imaging was performed in full-scan mode providing images of drugs, metabolites and endogenous lipids.

### **Preliminary data (results)**

Ebastine and loratadine were detected in positive ion mode as their protonated ions at  $m/z$  470.3054 and  $m/z$  383.1521, respectively. They were detected with high intensities in the period of 1-6 hours after the last given dose and predominantly in the stomach area of cryosections. At 24 hours from the last given dose, ebastine and loratadine were detected with lower intensity than the earlier time points and no longer found in the stomach area or in any other areas of the cryosections, except that ebastine was detected in the lungs. Given that the two CADs predominantly appeared in the stomach but not much elsewhere, these results suggest that they did not reach the tumor in high quantities after p.o. administration. However,  $H^+$  adducts of the metabolites OH ebastine ( $m/z$  486.3003) and carebastine ( $m/z$  500.2795), as well as desloratadine ( $m/z$  311.1309) and OH desloratidine ( $m/z$  327.1259) were detected at different locations in the body. Overall, the study of CAD-administrated mice with WB-DESI-MSI indicates that the parent CADs are mainly found in the stomach, while their metabolites reach a variety of tissues including the tumor. These findings were confirmed by quantitative LC-MS analysis of the tumor tissue.

### **Please explain why your abstract is innovative for mass spectrometry?**

Whole-body mass spectrometry imaging is applied to study the pharmacology and anti-cancer treatment and determine whether the pharmacological effect is caused by the drug or its metabolites.

### **Co-authors:**

*Jano Dicroce Giacobini, Danish Cancer Society Research Center*

*Tuula Kallunki, Danish Cancer Society Research Center*

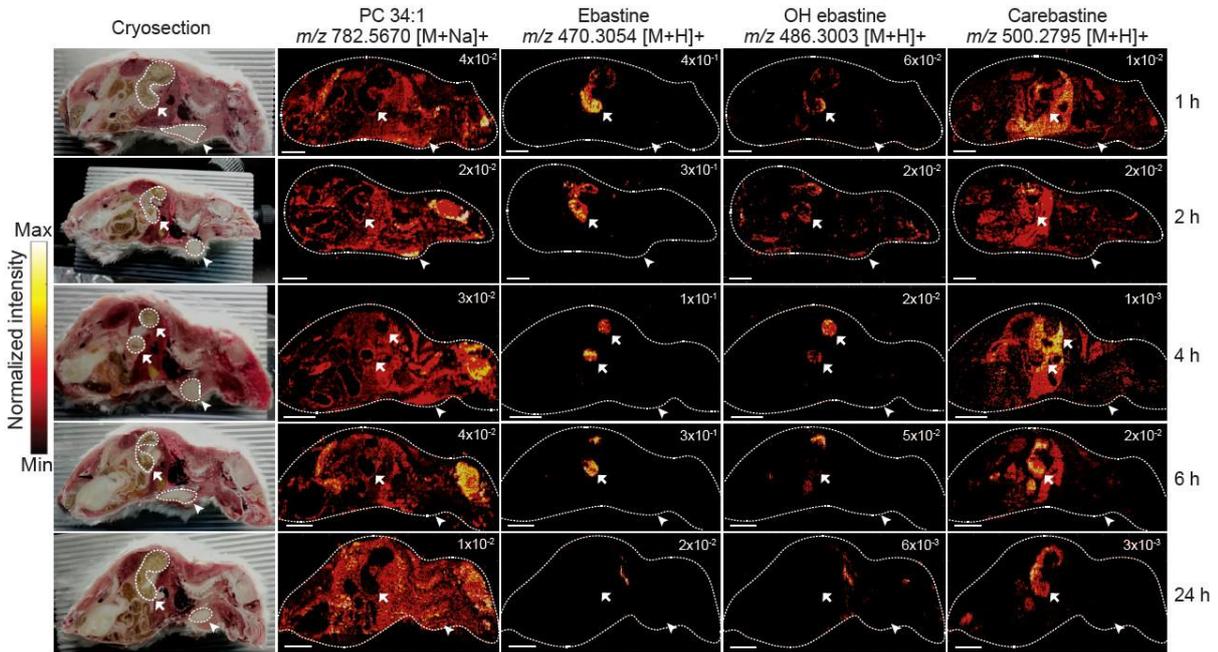
*Mesut Bilgin, Danish Cancer Society Research Center*

*Kenji Maeda, Danish Cancer Society Research Center*

*Marja Jäättelä, Danish Cancer Society Research Center*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
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Whole-body DESI-MSI of ebastine and its metabolites in tumor-bearing mice.

Poster number: **FP-PA-012**

## **DETERMINATION OF N-NITROSO SALBUTAMOL IN SALBUTAMOL PRODUCTS BY LC-MS/MS**

Abstract ID: **911**

**Presenting author: Jing Quan Lim, Health Sciences Authority**

### **Introduction**

In Dec 2021, GSK recalled the three batches of Ventolin 2mg tablets containing salbutamol, due to the detection of a new mutagenic impurity, N-Nitroso salbutamol. In response to this urgent recall, our laboratory developed and validated an LC-MS/MS method to determine N-Nitroso Salbutamol in salbutamol products (active ingredient: Salbutamol and Ipratropium).

### **Methods**

The instrument used was SCIEX QTRAP 7500 coupled with ExionLC, with a Hypersil GOLD analytical column (150x2.1 mm, 3 µm). Mobile phases A and B were 0.1% formic acid in deionized water and methanol, respectively. The injection volume was 5 µL. Flow rate was 300 µL/min. The mass spectrometer was operated in MRM (Multiple Reaction Monitoring) mode using negative ESI. The MRM transitions were 267.1/151.1 (Quantifier), 267.1/219.2 (Qualifier) and 267.1/162.9.

### **Preliminary data (results)**

The method LOD and LOQ were 0.1 µg/g and 0.2 µg/g, with respect to salbutamol. The method was found to have demonstrate excellent linearity (0.2 - 20 ng/mL), accuracy (80-110% recoveries), and precision (RSD <2%). Three salbutamol products were tested using this method. The content of N-nitroso salbutamol was found to be 0.2-2.05 µg/g.

### **Please explain why your abstract is innovative for mass spectrometry?**

An LC-MS/MS method was developed for the quantitative detection of N-nitroso salbutamol impurity in Salbutamol products. Method validation results indicated that the method was fit for its intended use.

Poster number: **FP-PA-013**

## **INVESTIGATION OF DRUG DISTRIBUTION IN RENAL TISSUE USING HIGH RESOLUTION MALDI MS IMAGING**

Abstract ID: **927**

**Presenting author: Eva-Maria Stemp, Universität Bayreuth**

### **Introduction**

The efficacy of therapeutic drugs depends on their ability to reach their site of action and their specific targets, for example proteins. Thus, *in situ* measurements are necessary to visualize the distribution of drugs in tissue. MALDI mass spectrometry imaging (MALDI MSI) is a versatile tool for visualization of drug compounds due to the label-free detection of a wide range of analytes. The application of this method can contribute to the elucidation of the effectiveness of (potential) therapeutical drugs and to a better understanding of (patho-)physiological processes in general.

### **Methods**

This project focuses on the development of a mass spectrometry imaging workflow to evaluate the distribution of the antidiabetic drug linagliptin *in situ* in renal tissue. Linagliptin is >75 % bound to its target protein, DPP-4. Since DPP-4 is mainly expressed in the proximal tubules of the nephrons, linagliptin is expected to be present at these sites. All MS imaging experiments were performed using an atmospheric pressure MALDI imaging source (AP-SMALDI<sup>5</sup>) in combination with an orbital trapping mass spectrometer (Q-Exactive HF) to provide high mass resolution and mass accuracy to reliably identify analytes and also allow high spatial resolution.

### **Preliminary data (results)**

For the first time, linagliptin ( $m/z$  473.24080) could be imaged in rat kidney tissue sections with high mass resolution ( $R > 100000$  FWHM) and high mass accuracy (RMSE <3 ppm). The detailed distribution in the fine structures of the kidney could be visualized with a spatial resolution of 10  $\mu\text{m}$  pixel size. The identity of the drug could be further confirmed by comparing tandem MS spectra generated on-tissue and from the drug standard.

### **Please explain why your abstract is innovative for mass spectrometry?**

This study reports the first successful MALDI MS imaging of linagliptin in rat kidney tissue with high resolution in mass and space.

### **Co-authors:**

*Andreas Römpp, Universität Bayreuth*

## Session: Toxicology and metabolism

Poster number: FP-PA-014

### **IDENTIFICATION AND CHARACTERIZATION OF IN VIVO, IN VITRO AND REACTIVE METABOLITES OF ZORIFERTINIB USING LIQUID CHROMATOGRAPHY ION TRAP MASS SPECTROMETRY**

Abstract ID: 3

**Presenting author: Nasser Ashakliah, Department of pharmaceutical chemistry, college of pharmacy, King Saud University, Saudi Arabia, Department of pharmaceutical chemistry, college of pharmacy, Aden University, Yemen**

#### **Introduction**

Zorifertinib is a novel, potent, oral, small molecule used to treat non-small cell lung cancer (NSCLC). Zorifertinib is an Epidermal Growth Factor Receptor (EGFR) inhibitor and has good blood–brain barrier permeability for (NSCLC) patients with EGFR mutations. Zorifertinib is currently at a phase II/III clinical trial. The current research reports the characterization and identification of in vitro, in vivo and reactive intermediates of zorifertinib.

#### **Methods**

Prediction of susceptible sites of metabolism and reactivity pathways (cyanide and GSH) of zorifertinib were performed by Xenosite web predictor tool. In-vitro metabolites of zorifertinib were performed by incubation with rat liver microsomes (RLMs) and isolated perfused rat liver hepatocytes. Extraction of zorifertinib and its in vitro metabolites from the incubation mixtures were done by protein precipitation. In vivo metabolism was done by giving a single oral dose of zorifertinib (10 mg/Kg) to Sprague Dawley rats in metabolic cages by using oral gavage.

#### **Preliminary data (results)**

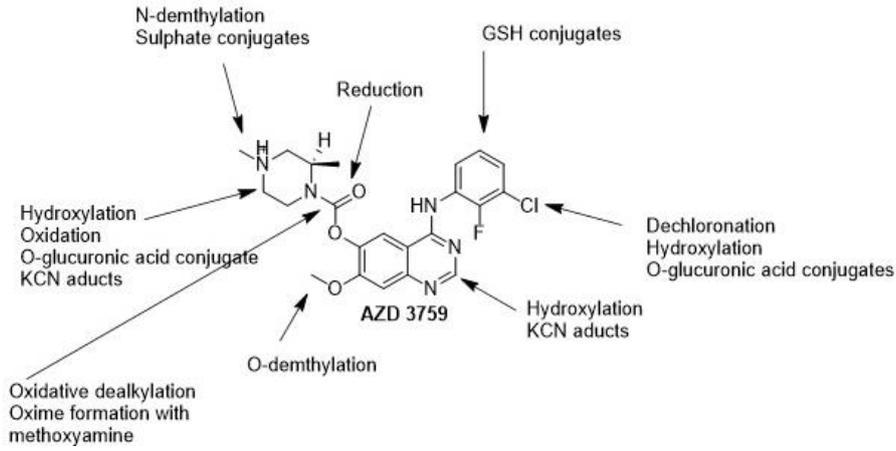
Urine was gathered and filtered at specific time intervals (0, 6, 12, 18, 24, 48, 72, 96 and 120 hr) from zorifertinib dosing. A similar volume of ACN was added to each collected urine sample. Both layers (organic and aqueous) were injected into liquid chromatography ion trap mass spectrometry (LC-IT-MS) to detect in vivo zorifertinib metabolites. N-methyl piperazine ring and quinazoline group of zorifertinib undergo metabolism forming iminium and electro-deficient conjugated systems respectively, which are very reactive toward nucleophilic macromolecules. Incubation of zorifertinib with RLMs in the presence of 1.0 mM KCN and 1.0 mM glutathione were made to check reactive metabolites as it is often responsible for toxicities associated with this drug. For in vitro metabolites there were nine in vitro phase I metabolites, four in vitro phase II metabolites, eleven reactive metabolites (three cyano adducts, five GSH conjugates, three methoxy metabolites) of zorifertinib were detected by LC-IT-MS. For in vivo metabolites there were eight in vivo phase I, ten in vivo phase II metabolites of zorifertinib were detected by LC-IT-MS. In vitro and in vivo phase I metabolic pathways were N-demethylation, O-demethylation, hydroxylation, reduction, defluorination and dechlorination. In vivo phase II metabolic reaction was direct conjugation of zorifertinib with glucuronic acid and sulphate.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Powerful structural characterization provided by LC-MS technique has greatly facilitated the identification of metabolites from biological matrices, with little purification owing to speed, inherent sensitivity of MS, and increased selectivity.

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Poster number: FP-PA-015

## A SENSITIVE LC-ESI-MS/MS METHOD FOR THE QUANTITATIVE DETERMINATION OF 54 PYRROLIZIDINE ALKALOIDS AND TWO TROPANE ALKALOIDS IN COWS' MILK

Abstract ID: 33

**Presenting author:** Lisa Klein, Chair of Food Safety and Analytics, Faculty of Veterinary Medicine, Ludwig-Maximilians-University of Munich, Schoenleutnerstr. 8, 85764 Oberschleissheim, Germany, Chair of Analytical Food Chemistry, TUM School of Life Science Weihenstephan, Technical University of Munich, Maximus-von-Imhof-Forum 2, 85354 Freising, Germany

### Introduction

1,2-unsaturated pyrrolizidine alkaloids (PA) and tropane alkaloids (TA) are toxic secondary plant metabolites. Contaminations with PA-, their corresponding *N*-oxides-(PANO)- or TA-containing plants led to multiple cases of acute food and feed poisoning. Moreover, PA/PANO exhibit chronic hepatotoxic and genotoxic effects to humans and livestock. Plant-based foods are considered to be the main source of PA/PANO and TA in human nutrition. However, carry-over of PA/PANO and TA into the milk of dairy cows has been shown. Today, the analytical capability to quantify a large set of different PA/PANO as well as atropine and scopolamine in milk is limited to a few published methods and little is known about the occurrence of these toxins in milk purchased directly from dairy farms.

### Methods

In this study, a sensitive analytical approach for detection and quantification of 54 PA/PANO as well as for the TA atropine and scopolamine in raw milk of dairy cows is presented. Milk samples were extracted using liquid-liquid extraction with 2% formic acid and hexane, followed by a cation-exchange solid phase extraction for purification. Analysis was performed with a sensitive target-orientated liquid chromatography tandem mass spectrometry method (LC-MS/MS). The method was applied to 15 milk samples obtained from milk vending stations at farms and from a local marketer in Bavaria, Germany.

### Preliminary data (results)

In-house validation of the final method showed low limits of detection and quantification from 0.005 to 0.054 µg/L and from 0.01 to 0.13 µg/L, respectively. The recovery rates ranged from 64.4% to 127.0% for 51 of the 54 PA/PANO and both TA. For 53 PA/PANO and both TA, precisions were below 15% at concentration levels of 0.05 and 0.5 µg/L and below 5% at a concentration level of 3 µg/L. To the best of our knowledge, the presented analytical method is the first one enabling the simultaneous sensitive analysis of PA/PANO as well as TA in cows' milk. The results for the 15 milk samples will be reported.

### Please explain why your abstract is innovative for mass spectrometry?

The developed method enables the simultaneous analysis of PA/PANO and TA in cows' milk that is unique at the current state.

### Co-authors:

*Angelika Gabler, Chair of Food Safety and Analytics, Faculty of Veterinary Medicine, Ludwig-Maximilians-University of Munich, Schoenleutnerstr. 8, 85764 Oberschleissheim, Germany*

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*Christoph Gottschalk, Chair of Food Safety and Analytics, Faculty of Veterinary Medicine, Ludwig-Maximilians-University of Munich, Schoenleutnerstr. 8, 85764 Oberschleissheim, Germany, Current address: German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Unit Plant toxins and Mycotoxins, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany*

*Florian Kaltner, Chair of Food Safety and Analytics, Faculty of Veterinary Medicine, Ludwig-Maximilians-University of Munich, Schoenleutnerstr. 8, 85764 Oberschleissheim, Germany, Current address: Justus Liebig University of Giessen, Institute of Food Chemistry and Food Biotechnology, 35392 Giessen, Germany*

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Monday 29 August 2022 from 14:00 to 15:30 hours  
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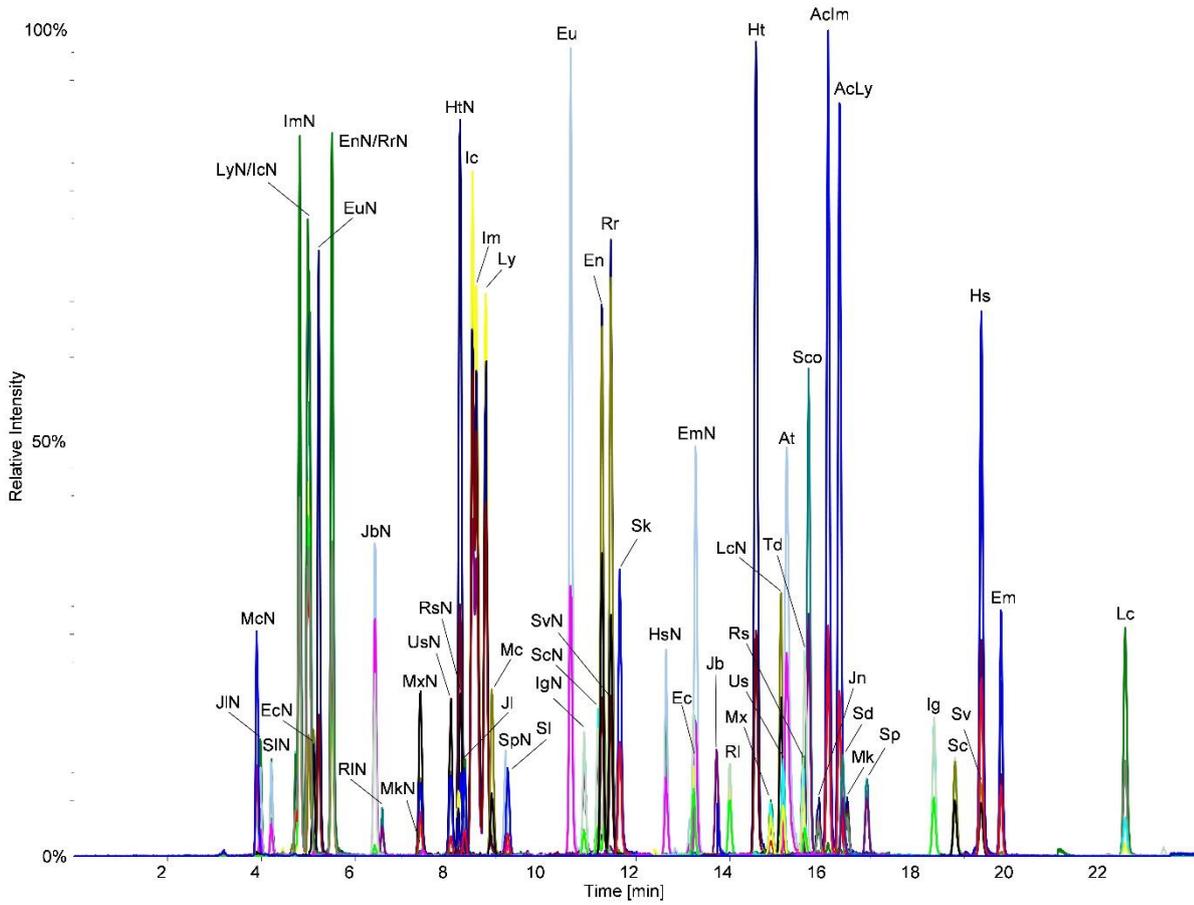


Figure: LC-MS/MS chromatogram of a raw milk sample artificially contaminated with 54 pyrrrolizidine alkaloids/ pyrrrolizidine alkaloid *N*-oxides and two tropane alkaloids ( $c = 0.5 \mu\text{g/L}$  per analyte)

Poster number: **FP-PA-016**

## **FATE OF TOXIC TROPANE AND PYRROLIZIDINE ALKALOIDS DURING FOOD PROCESSING OR STORING: CURRENT STATUS AND PERSPECTIVES**

Abstract ID: 67

**Presenting author: Florian Kaltner, Institute of Food Chemistry and Food Biotechnology, Justus Liebig University of Giessen, 35392 Giessen, Germany**

### **Introduction**

Tropane (TAs) and pyrrolizidine alkaloids (PAs) are plant secondary metabolites with toxic properties in humans and livestock. They can enter the food chain predominantly due to co-harvesting of weed plants or honeybees foraging nectar and pollen. Moreover, via contaminated feed TAs and PAs can also be transferred to milk and dairy products. During processing or storage of contaminated food raw materials or foodstuff, the toxic alkaloids may undergo certain molecular modifications, such as degradation or binding to food components, potentially affecting their analytical traceability as well as their toxicological properties. Thus, the current study aimed to investigate the fate of toxic TAs and PAs in two model systems (yoghurt fermentation, honey storage) and to unveil potential transformation products.

### **Methods**

Milk (UHT) and honey (floral, foraged in 2021) were spiked with standard solutions of selected TAs and PAs. Subsequently, milk was fermented to yoghurt employing several commercially available yoghurt cultures and honey was spiked and stored for few weeks. Non-spiked controls of the samples were also produced accordingly. Samples were extracted under acidic conditions and purified using C18 SPE. Extracts were analysed by LC-TripleQuad-MS (targeted) and, if the levels of alkaloids have changed, by UHPLC-QToF-MS (untargeted). Potential transformation products will be identified by comparing untargeted data of samples by means of multivariate analytics.

### **Preliminary data (results)**

Preliminary results showed a structure-dependant decrease of certain PA-*N*-oxides in stored honey, which prove findings of earlier studies. In contrast, the contents of corresponding PAs increased only slightly. In yoghurt, the PA-*N*-oxides also showed reduced levels during fermentation, but solely when probiotic microorganisms were incorporated. In contrast to honey samples, this observation was accompanied with greatly increased levels for the corresponding PAs. So far, neither storing of honey nor fermentation of yoghurt showed any influence on the spiked contents of TAs, which were thus stated as persistent in the investigated matrices. The formation of potential transformation products of PA-*N*-oxides in stored honey and yoghurt is currently being investigated and its results will be reported. The relevance of these findings regarding the future proper assessment of toxic TAs and PAs in food will be discussed.

### **Please explain why your abstract is innovative for mass spectrometry?**

The behaviour of spiked alkaloid levels was monitored using targeted MS approaches, while transformation products will be innovatively determined by means of methods typically used in untargeted metabolomics.

Poster number: **FP-PA-017**

## **HIGH-THROUGHPUT RESIDUAL SOLVENT ANALYSIS ACCORDING TO USP 467 USING SIFT-MS**

Abstract ID: **75**

**Presenting author: Arnd Ingendoh, Syft Technologies GmbH**

### **Introduction**

Volatile impurities occur frequently in pharmaceutical products and packaging and are often of concern due their toxicity. The United States Pharmacopeia's Residual Solvents USP (467) general chapter defines the approach to residual solvent analysis for most of the global pharmaceutical industry. Described are GC techniques using static headspace (HS) for sample introduction and FID (flame ionization detection). There are alternative procedures possible according to (1467) which are permitted when they are fully validated and produce comparable results to the compendial methods or procedure. Here we describe SIFT-MS and its validation as an alternative procedure for USP<467> regulated residual solvent analysis.

### **Methods**

SIFT-MS uses eight reagent ions ( $\text{H}_3\text{O}^+$ ,  $\text{NO}^+$ ,  $\text{O}_2^+$ ,  $\text{O}^-$ ,  $\text{O}_2^-$ ,  $\text{OH}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ), generated by plasma microwave discharge from moist air. These reactants exhibit a significant variety of ion-molecule reactions, i.e. react differently with each compound, thus enhance specificity and allow detection of a wide range of VOCs, including separation of isomers. The reagent ion selection is done in a first quadrupole with switching times in the millisecond range. The selected reagent ion interacts with the sample in a gas flow tube under well-defined conditions. Product ions and unreacted reagent ions are detected in a second quadrupole.

### **Preliminary data (results)**

Rapid switching between the eight reagent ions provides high selectivity in the absence of chromatographic separation or high-resolution mass spectrometric detection. The key benefit is not primarily the number of reagents ions, but the multiple reaction mechanisms that provide additional independent measurements of each compound. This delivers unparalleled selectivity and detection of an extremely broad range of compounds in real-time. Utilizing a compound library with known rate coefficients, the software instantaneously calculates each analyte's absolute concentration from the specific reagent and product ion count rates. Typical run times for individual samples are less than one minute. Focusing on quantitative testing of 28 Class 2 solvents listed in (467), very reasonable data was achieved. Linearities were  $R > 0.93$  for all compounds, with only four product ions having  $R < 0.99$ . Repeatability was generally  $\text{RSD} < 10\%$  with elevated values for solvents with higher water affinity. Similar behaviours were observed for accuracy and recovery, with acceptance criteria being met more consistently for compounds with lower water affinity. In addition to reporting the successful procedure validation for determination of most of the residual solvents evaluated, it is also discussed how direct MS might support quality-by-design and continuous manufacturing objectives through on-line analysis of residual solvents. It demonstrates that SIFT-MS has the potential of being used as an alternative procedure to those in (467) in compliance with acceptance criteria.

### **Acknowledgements**

Study was generated and guided by Edmond Biba, PhD, United States Pharmacopeia, Rockville, MD, USA, [exb@usp.org](mailto:exb@usp.org)

### **Please explain why your abstract is innovative for mass spectrometry?**

SIFT-MS has the potential of being used as an alternative procedure matching the acceptance criteria of the USP 467 directive. Benefits are higher throughput and the potential for online analysis.

### **Co-authors:**

*Christopher Pfaff, Syft Technologies GmbH*  
*Vaughan Langford, Syft Technologies Ltd.*  
*Mark Perkins, Anatune Ltd.*

Poster number: FP-PA-018

## MICROWAVE-ASSISTED DESULFATION OF THE HEMOLYTIC SAPONINS EXTRACTED FROM HOLOTHURIA SCABRA VISCERA

Abstract ID: 110

Presenting author: Philippe Savarino, University of Mons

### Introduction

Saponins are plant and marine animal specific metabolites that are commonly considered as molecular vectors allowing chemical defense against unicellular and pluricellular organisms. Their toxicity is attributed to their membranolytic properties. Modifying the molecular structures of saponins by quantitative and selective chemical reactions is increasingly considered to tune the biological properties of these molecules (i) to prepare congeners with specific activities for biomedical applications, and (ii) to afford experimental data related to their structure–activity relationship. A better understanding and control of the membranolytic properties of these molecules could be of major industrial interest.

### Methods

The present study focuses on the sulfated saponins contained in the viscera of *Holothuria scabra*, a Malagasy sea cucumber abundantly consumed on the Asian food market. Using complementary mass spectrometry techniques (MALDI-MS and LC-MS), we first qualitatively and quantitatively assessed the saponin content. Microwave activation under alkaline conditions in aqueous solutions was developed and optimized to quantitatively and specifically induce the desulfation of the natural saponins, by a specific loss of H<sub>2</sub>SO<sub>4</sub>. To determine the importance of the sulfate function in the membranolytic process, the hemolytic activity of the saponins of both extracts is evaluated on bovine blood cells.

### Preliminary data (results)

Investigations start with the saponin multi-step extraction of saponins from *H. scabra* viscera. After flash chromatography purification, MALDI-MS analysis (Figure 1a) demonstrates the presence of four groups of [M+Na]<sup>+</sup> signals. Exact mass measurements (MALDI-HRMS) establish that the detected ions possess the elemental compositions of sulfated and non-sulfated saponins with four or two sugars, generating a total of ten different elemental compositions.

Afterwards, LC-MS analyses demonstrated the natural saponin extract exclusively contains sulfated saponins with five elemental compositions, demonstrating that non-sulfated saponins were produced in source during the MALDI ionization, leading to a total of 26 different sulfated saponins. Molar ratios of the different isomers were determined by integrating the LC-MS profiles and the saponin composition of the saponin extract was determined and expressed in %-weight, using an internal standard.

Sulfated saponins of the natural extract were exposed to a basic treatment under microwave activation (pH 14 - 100 °C - 5 min). The new extract was then analyzed by MALDI-MS (Figure 1b) and signals assigned to sulfated saponins are no longer detected, demonstrating that saponins have been chemically modified. LC-MS analysis confirmed that the microwave-assisted desulfation of the saponins is quantitative and specific for all the different saponin congeners. We suggest that the formal H<sub>2</sub>SO<sub>4</sub> loss from the natural saponins corresponds to an elimination reaction involving a vicinal hydrogen atom and the creation of a C=C double bond.

Hemolytic activity assays showed that the cytotoxicity effect is directly related to the presence of the sulfate function (Figure 2).

### Please explain why your abstract is innovative for mass spectrometry?

Use of complementary mass spectrometry techniques for saponin structural characterization and chemical modification.

### Co-authors:

Emmanuel Colson, University of Mons

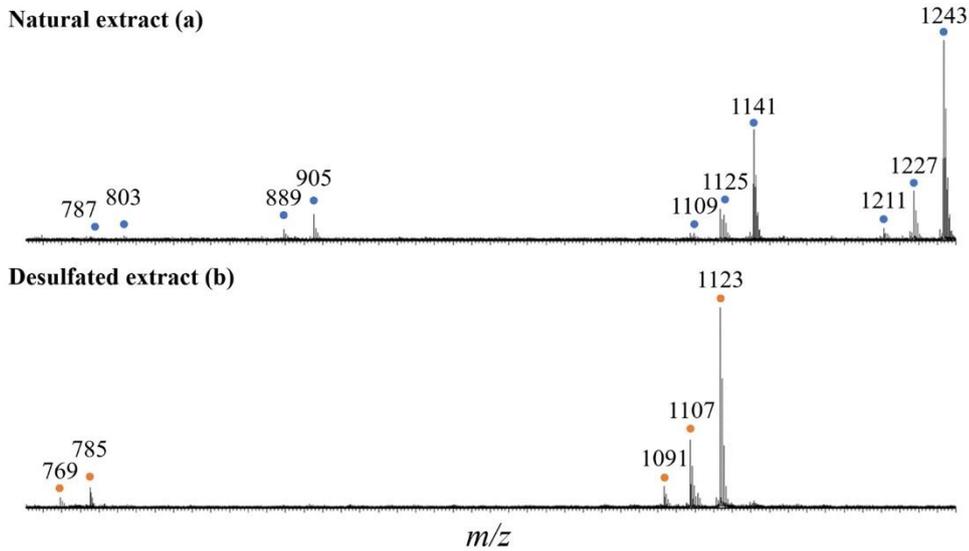
Guillaume Caulier, University of Mons, University of Toliara

Igor Eeckhaut, University of Mons, University of Toliara

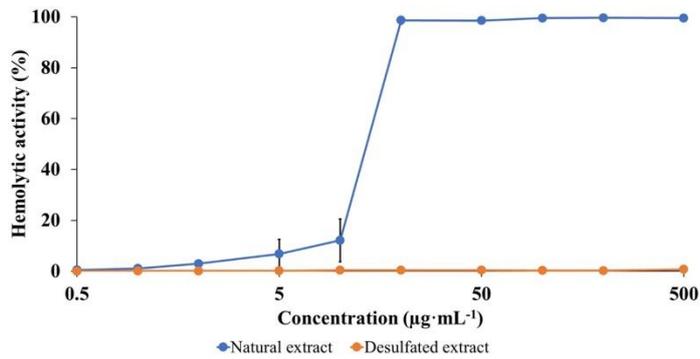
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Patrick Flammang, University of Mons  
Pascal Gerbaux, University of Mons



MALDI-MS analyses: (a) Natural extract, and (b) Desulfated extract.



Hemolytic activity: (●) Natural extract, and (●) Desulfated extract.

Poster number: **FP-PA-019**

## **ELIMINATION PROFILE OF MICRODOSED ZILPATEROL MIMICKING CONSUMPTION OF CONTAMINATED CATTLE MEAT**

Abstract ID: **119**

**Presenting author: Luisa Euler, Institute of Biochemistry/Center for Preventive Doping Research, German Sport University Cologne**

### **Introduction**

The synthetic  $\beta$ 2-adrenoreceptor agonist Zilpaterol is legitimately used as an animal feed supplement in some countries as it is known to increase lipolysis and protein synthesis as well as to decrease lipogenesis and protein degradation. Yet, these effects of Zilpaterol also make it a potent doping agent. The World Anti-Doping Agency (WADA) has thus banned the use of Zilpaterol in competitive sports. However, cattle are commonly fed Zilpaterol in the weeks before they are slaughtered. This leads to residues of the drug in edible tissues and, eventually possibly in the urine of consumers. The aim of this work is therefore to investigate the pharmacokinetics and the detection times of Zilpaterol in humans following a 'contamination' scenario e.g. due to ingestion of Zilpaterol-containing meat.

### **Methods**

In order to determine whether ingestion of Zilpaterol below or at the ADI level of 0.04  $\mu$ g/kg bodyweight can result in an adverse analytical finding in a doping control, healthy volunteers were administered single or multiple oral doses of 0.5  $\mu$ g or 3  $\mu$ g Zilpaterol to mimic the ingestion of contaminated cattle meat. Urine samples of the volunteers were collected prior to and post-administration of Zilpaterol over a period of up to ten days. These samples were then hydrolyzed, extracted with TBME and analyzed using a validated HPLC-MS/MS method.

### **Preliminary data (results)**

All volunteers excreted peak concentrations of Zilpaterol 2-5 h after ingestion. No accumulation of Zilpaterol was detected after intake on multiple days. Urinary metabolites described in previous studies on rats and cattle were not detected. Maximum concentrations of >6 ng/mL were measured after the intake of 3  $\mu$ g of Zilpaterol. In consideration of WADA's minimum reporting level for Zilpaterol in urine samples, the risk of adverse analytical findings resulting from the above-mentioned scenario necessitates further investigations. Based on the LOD, residues of Zilpaterol were detected up to 96 h after the last intake.

In order to investigate the enantiomer-specific elimination of Zilpaterol, an HPLC-UV method for enantiomeric separation of Zilpaterol with subsequent fractionation and mass spectrometric analysis of the fractions will be developed.

### **Please explain why your abstract is innovative for mass spectrometry?**

The study provides new insights into the pharmacokinetics of zilpaterol in humans using mass spectrometric detection techniques.

### **Co-authors:**

*Felicitas Wagener, Institute of Biochemistry/Center for Preventive Doping Research, German Sport University Cologne  
Andreas Thomas, Institute of Biochemistry/Center for Preventive Doping Research, German Sport University Cologne  
Mario Thevis, Institute of Biochemistry/Center for Preventive Doping Research, German Sport University Cologne,  
European Monitoring Center for Emerging Doping Agents (EuMoCEDA)*

Poster number: **FP-PA-020**

## **EFFICIENT CHARACTERISATION AND RATIONALISATION OF METABOLITE STRUCTURES FOR BIOTRANSFORMATION STUDIES USING THE SELECT SERIES™ CYCLIC™ IMS INSTRUMENT**

Abstract ID: **428**

**Presenting author: Jonathan Fox, Waters Corp**

### **Introduction**

LC-MS/MS is used routinely to detect and characterise metabolites to support biotransformation studies; here fragmentation data provides structural information to help localise the biotransformation. Whilst this approach provides key data, a major limitation exists in the characterisation of isomeric metabolites as the approach relies on adequate chromatographic selectivity and sufficiently different MS/MS spectra to distinguish these isomeric metabolites. These spectra rarely allow assignment of exact structures, more a confident localisation of the biotransformation. For definitive structures, efforts are made around metabolite isolation, identification using NMR or synthesis of multiple putative metabolite standards for comparison. An alternative method is to use the orthogonal separation of ions based on their collisional cross section (CCS) provided by ion mobility mass spectrometry coupled with UPLC™.

### **Methods**

Warfarin was incubated in rat hepatocytes and samples were analysed by UPLC-MS on the SELECT SERIES Cyclic IMS (Waters Corp, UK). The increased ion mobility resolving power and analytical flexibility afforded by this instrument was used to provide new data and critical insights, allowing potential metabolite structures to be rationalised in a pragmatic, data-led manner that is less resource intensive. This ultimate mobility resolution not only afforded separation of intact protonated metabolite isomers, but also the separation of relevant isomeric fragment-ions for further characterisation and rationalisation of metabolite structures.

SELECT SERIES, UPLC and Cyclic are trademarks of Waters Technologies Corporation

### **Preliminary data (results)**

Three hydroxylated metabolites were observed at around 3% of parent response, which were assigned tentatively as the 4, 7 and 10 hydroxy metabolites by chromatographic comparison with respective standards and inspection of low-energy mass spectra. Analysis of the hepatocyte incubation and metabolite standards using multiple passes of the Cyclic IMS revealed that the tentative assignment was incorrect for one of the observed metabolites when compared with the authentic standards.

When comparing fragment-ion data, two of the hydroxy metabolites showed common fragments and, whilst comparison with authentic standards or NMR analysis would be the conventional approach to assigning a definitive structure, here the alternative approach was to compare their measured CCS as an additional compound identifier with the predicted CCS of putative structures. In a second experiment, the fragment ion common to both isomeric warfarin metabolites was interrogated using multiple passes of the Cyclic IMS, where the data revealed distinguishable mobility responses for the same isomeric fragment ion from each of the two metabolites, suggesting differences in the structural localisation of the oxidation. Good precision was shown for replicate measurements ( $n=3$ ) of the isomeric fragment CCS, giving increased confidence when comparing to their predicted CCS obtained using a machine learning based model. These preliminary data suggest multiple-pass ion mobility experiments, high CCS precision and the mining of fragment-ion mobility data could provide a more effective approach for characterisation and rationalisation of likely metabolite structures in a resource efficient manner, giving a novel and effective alternative to more traditional approaches.

### **Please explain why your abstract is innovative for mass spectrometry?**

Multiple-pass ion mobility experiments, high CCS precision and fragment-ion mobility data could provide a more effective approach for characterisation and rationalisation of likely metabolite structures in a resource efficient manner.

**Co-authors:**

*David Higton, Waters Corp*  
*Daniel J Weston, GSK R&D*  
*David Berry, GSK R&D*  
*Jo-Anne Riley, Waters Corp*

Poster number: **FP-PA-021**

## **APPLICATION OF MICRO PILLAR ARRAY COLUMNS FOR METABOLITE IDENTIFICATION**

Abstract ID: **496**

**Presenting author: Arnaud Lubin, Janssen Pharmaceutica**

### **Introduction**

Two of the main reasons why drugs fail during the clinical phase are safety and efficacy. Over the last three decades a shift has been initiated in the pharmaceutical industry to try and answer those issues as early as possible during the drug development process. Nowadays, metabolite identification is commonly performed during early-stage drug discovery where a high analytical throughput is required. Current state of the art UHPLC (ultra-high performance liquid chromatography) columns offer a viable solution for shorter generic gradients. However, base peak separation cannot always be achieved on complex samples. The potential of micropillar array columns, enabling the operation of long columns at moderate pressures was evaluated to achieve maximal separation with no additional method development.

### **Methods**

Buspirone and Verapamil were incubated in human hepatocytes at a concentration of 10  $\mu$ M for 180 min. 40  $\mu$ L incubation volume was quenched with two volumes of DMSO. Samples were centrifuged for 10 min at 4000 rpm before analysis.

A  $\mu$ PAC C18 (50 and 200 cm; ThermoScientific) and Acquity BEH C18 (100x2.1 mm; Waters) column were compared using simple linear gradients (mobile phase A: water + 0.1% formic acid and mobile phase B: acetonitrile + 0.1% formic acid).

ddMS<sup>2</sup> data were recorded on an IDX system (ThermoScientific) and data processing was performed using Compound Discoverer (ThermoScientific).

### **Preliminary data (results)**

Two pharmaceutical compounds, buspirone and verapamil, with known metabolism were selected for this experiment. Buspirone is eliminated primarily by oxidative metabolism and with multiple sites available for hydroxylation, its phase I metabolism results in complex mixtures of +O, +2O and +3O metabolites. Verapamil has five sites available for glucuronidation following demethylation which creates complex mixtures of glucuronide conjugates with various degrees of demethylation. Both compounds were incubated in human hepatocytes to generate a substantial amount of phase I and phase II metabolism.

Generic linear gradients used for high throughput metabolite identification on UHPLC systems are not able to fully resolve such complex mixtures of isobaric compounds. An achievement inaccessible without advanced and time-consuming LC method development. As an example, Buspirone +O metabolites cannot be fully separated (Fig. 1) with a short 10 min gradient and even by tripling the gradient length to 30 min little improvement was observed (Fig. 1). However, by simply transposing the gradient to nano-LC with the use of a 50 cm micro pillar array column and 90 min gradient, base peak separation can be easily obtained (Fig. 2). In the example of Buspirone +O, a 200 cm column with 360 min gradient was also tested but did not provide any additional information (Fig. 2).

Similar examples for other isobaric metabolites of buspirone and verapamil highlight the power of the micro pillar arrays columns to fully separate complex mixtures of isobaric metabolites without the need of further LC method development.

**Please explain why your abstract is innovative for mass spectrometry?**

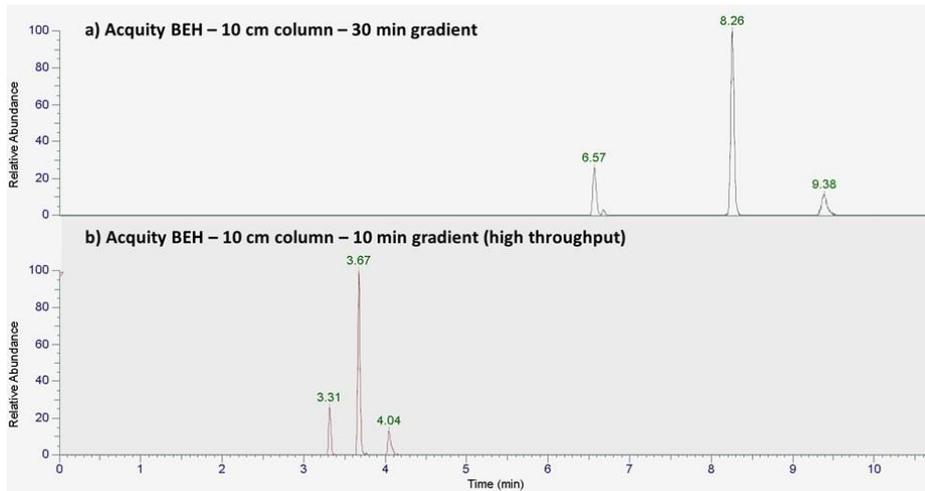
**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

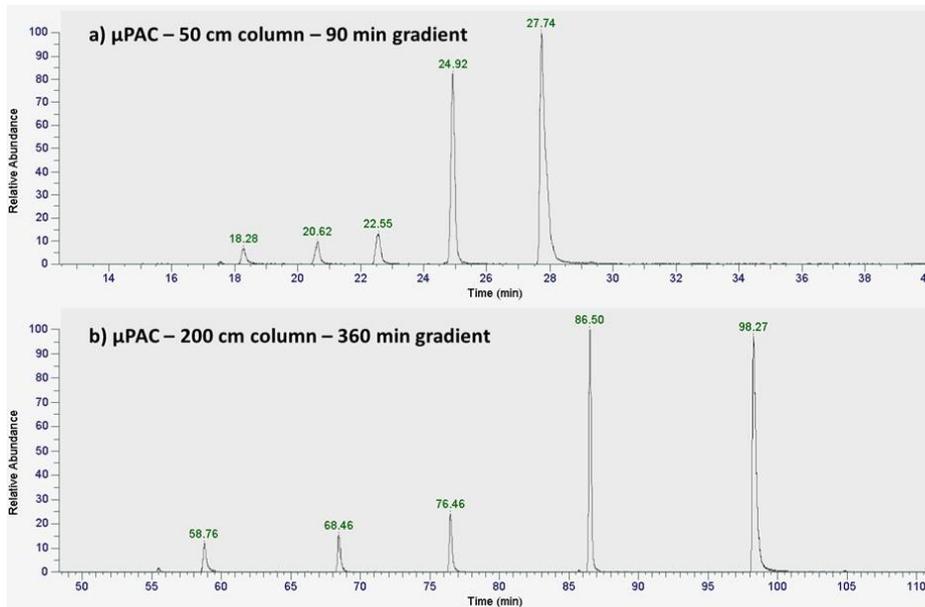
Complex mixtures of isobaric metabolites are often not separable by UHPLC-MS. New generation micro-pillar-array columns showed potential to fully resolve complex mixtures without the need for time consuming method development.

**Co-authors:**

*Ronald De Vries, Janssen Pharmaceutica*  
*Filip Cuyckens, Janssen Pharmaceutica*



Extracted ion chromatogram of buspirone on a standard UHPLC column



Extracted ion chromatogram of buspirone on micro pillar array columns

Poster number: **FP-PA-022**

## **IN VITRO IDENTIFICATION OF PHASE I BIOTRANSFORMATION PRODUCTS OF NEW PLASTICIZERS WITH SUSPECT AND NON-TARGET SCREENING WORKFLOWS ON HIGH RESOLUTION MASS SPECTROMETRY**

Abstract ID: **685**

**Presenting author: Adrian Covaci, University of Antwerp, Toxicological Center**

### **Introduction**

Three plasticizers, bis (3,5,5-trimethylhexyl) phosphate (TMHPh), di(propylene glycol) dibenzoate (DiPGDB), and tri-n-butyl trimellitate (TBTM), were recently identified and reported in high concentrations in indoor dust from Belgian homes. In this study, we investigated for the first time the formation of Phase I biotransformation products.

### **Methods**

Incubation of the plasticizers was performed using human liver microsomes (HLMs) following an in-house developed protocol. Liquid chromatography coupled to time of flight mass spectrometry (LC-QTOF-MS) was employed to investigate the Phase I biotransformation products. Data analysis was performed using a combination of suspect screening (*in silico* predicted biotransformation products obtained using the Meteor Nexus software) and non-targeted analysis (feature table obtained from MS-DIAL v. 4.48 was used to select relevant features between the different experimental conditions in MetaboAnalyst).

### **Preliminary data (results)**

Following the incubation of the plasticizers to HLMs, various biotransformation products have been identified as a result of hydroxylation, oxidative O-dealkylation and hydrolysis of acyclic carboxylic esters. For TMHPh, hydroxylation reactions on one or two positions in the structure resulted in five biotransformation products. For DiPGDB, biotransformation products were formed after hydrolysis of carboxylic esters and oxidative O-dealkylation. For TBTM, biotransformation products were formed through hydrolysis of the different carboxylic esters of the molecule, in agreement with studies on structurally similar compounds. The generated results can contribute to biomonitoring studies creating new knowledge on human exposure to emerging compounds and the metabolism of xenobiotics.

### **Please explain why your abstract is innovative for mass spectrometry?**

Suspect and non-target screening workflows were developed for high resolution mass spectrometry (QTOF) for the identification of new biotransformation products.

### **Co-authors:**

*Christina Christia, University of Antwerp, Toxicological Center*  
*Katyeny Manuela da Silva, University of Antwerp, Toxicological Center*  
*Giulia Poma, University of Antwerp, Toxicological Center*  
*Alexander van Nuijs, University of Antwerp, Toxicological Center*

Poster number: **FP-PA-023**

## **INVESTIGATIONS INTO THE CONCENTRATION AND METABOLITE PROFILES OF ANABOLIC STEROIDS IN BLOOD PLASMA AND SEMINAL FLUID USING LIQUID CHROMATOGRAPHY- HIGH-RESOLUTION MASS SPECTROMETRY.**

Abstract ID: **841**

**Presenting author: Johanna Breuer, Institute of Biochemistry, Center for Preventive Doping Research, German Sport University Cologne,**

### **Introduction**

The complexity of modern doping analysis is increasing with technical progress, leading to the development of even more sensitive methods. As a consequence, distinguishing between intentional doping and those adverse analytical findings (AAFs) resulting from inadvertent exposure to prohibited substances (e.g. through contaminated dietary supplements or exchange of body fluids) has become an additional challenge to address. One recently argued scenario is the contamination of female urine with seminal fluid containing banned substances, which has warranted considerations regarding analytical evidence to verify or falsify such claims.

### **Methods**

A method was established to study the concentration and metabolite profiles of selected anabolic agents included in category S1 of the World Anti-Doping Agency's (WADA's) Prohibited List in blood plasma and seminal fluid. For seminal fluid, the established analytical approaches were characterized concerning specificity, recovery, linearity, precision and identification capability. For proof-of-concept, an animal administration study (boar) with the anabolic agents stanozolol and LGD-4033 was conducted, and blood plasma as well as seminal fluid samples were screened for the presence of the aforementioned substances and related metabolites.

### **Preliminary data (results)**

The detection of the anabolic agents was accomplished in both matrices, blood plasma and seminal fluid, with a limit of detection at 0.1 ng/mL. In both matrices, the intact drugs were quantifiable in the specimens sampled 24 h post-administration. Also, various metabolites of different metabolic pathways were identified in seminal fluid and blood plasma.

To the best of our knowledge, no data exist on the presence of stanozolol and LGD-4033 and corresponding metabolites in ejaculate. In this exploratory investigation, the banned substances and biotransformation products were identified in seminal fluid, suggesting that a contamination scenario of female (doping control) urine with ejaculate and, hence, drug residues after sexual intercourse cannot be excluded. However, in the animal administration study the analyte concentrations observed in the seminal fluid were particularly low, and the plausibility of a contamination scenario considering the parameters of urine volume, potential volumetric load of ejaculate, and urinary drug (or metabolite) concentrations needs to be assessed at the result management level. Interestingly, the distribution of stanozolol and LGD-4033 appeared to be different in blood plasma compared to the seminal fluid. Hence, further studies investigating different substances are needed to further furnish the knowledge of matrix distribution and elimination profiles especially concerning seminal fluid.

### **Please explain why your abstract is innovative for mass spectrometry?**

This study is among the first to address the substance transfer scenario of seminal fluid into female urine using liquid chromatography / high-resolution mass spectrometry (LC-HRMS).

### **Co-authors:**

*Andreas Thomas, Institute of Biochemistry, Center for Preventive Doping Research, German Sport University Cologne, Philippe Delahaut, CER Groupe*

*Hans Geyer, Institute of Biochemistry, Center for Preventive Doping Research, German Sport University Cologne, Mario Thevis, Institute of Biochemistry, Center for Preventive Doping Research, German Sport University Cologne,, European Monitoring Center for Emerging Doping Agents (EuMoCEDA)*

Poster number: FP-PA-024

## AMINO FUNCTIONALIZED METAL–ORGANIC FRAMEWORK FOR MAGNETIC SOLID PHASE EXTRACTION COMBINED WITH HPLC–MS/MS FOR THE DETECTION OF PARABENS

Abstract ID: 859

Presenting author: Hui-Ling Lee, Fu Jen Catholic University

### Introduction

Parabens are esters of p-hydroxybenzoic acid. These compounds are widely used as preservatives and antimicrobial agents in food, cosmetics and personal care products. However, parabens have weak estrogenic properties, which leads to endocrine disruption and may affect human health adversely. Due to the trace level of parabens in real sample or matrix effect, sample preparation is an efficient approach for capturing these analytes before real sample analysis. In this study, an amino functionalized metal–organic framework ( $\text{Fe}_3\text{O}_4@\text{MIL}-101\text{-NH}_2$ ) is used as the adsorbents for magnetic solid phase extraction (MSPE) for the detection of nine parabens by HPLC–MS/MS.

### Methods

MSPE for nine parabens were carried out. Briefly, 10 mg  $\text{Fe}_3\text{O}_4@\text{MIL}-101\text{-NH}_2$  was dispersed into the mixed standard solution (adjusted pH 7.0). Then the mixture was vortexed for 5 min. After which, the adsorbents were separated from the aqueous phase by an external magnet and the supernatant was discarded. The adsorbents were eluted by ACN+0.1%  $\text{NH}_4\text{OH}$  and vortexed for 5 min. Subsequently, the supernatant was collected, filtered to eliminate particulate matter before HPLC–MS/MS analysis.

### Preliminary data (results)

MSPE are attractive materials used as sorbents in analytical microextraction application in food safety. To achieve the best extraction conditions such as comparison of different adsorbents, pH value, desorption solvent and solvent volume, were studied and optimized for parabens pre-concentration and clean-up process. First, we have synthesized the  $\text{Fe}_3\text{O}_4@\text{MIL}-101(\text{Cr})$ ,  $\text{Fe}_3\text{O}_4@\text{MIL}-101@\text{NH}_2$  and  $\text{Fe}_3\text{O}_4@\text{MIL}-101@\text{SO}_3\text{H}$ , used as sorbents for magnetic solid phase extraction. The  $\text{Fe}_3\text{O}_4@\text{MIL}-101\text{-NH}_2$  were characterized by Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD), transmission electron microscopy (TEM), Superconducting Quantum Interference Device Magnetometer (SQUID) and thermogravimetric analysis (TGA) in detail. According to the results,  $\text{Fe}_3\text{O}_4@\text{MIL}-101\text{-NH}_2$  was displayed as the most favorable adsorbent, via  $\pi\text{-}\pi$  and hydrogen bonding interaction of the analytes. Under the optimized condition of extraction procedure including the pH value of 7.0, 10 mg of  $\text{Fe}_3\text{O}_4@\text{MIL}-101\text{-NH}_2$ , 5 mins extraction time, and 400  $\mu\text{L}$  desorption solvent volume, good responses were demonstrated.

### Please explain why your abstract is innovative for mass spectrometry?

In the present study, a new, rapid, and efficient  $\text{Fe}_3\text{O}_4@\text{MIL}-101\text{-NH}_2$  by LC-MS/MS was established for the extraction and sensitive detection of nine parabens

Poster number: **FP-PA-025**

## **PROFILING OF LIPID METABOLITE IN LIPID DROPLET REVEALS THAT LIPID DROPLETS ARE ESSENTIAL ORGANELLE IN HCV REPLICATION**

Abstract ID: **945**

**Presenting author: So-Young Park, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unuit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Korea**

### **Introduction**

Lipid droplets (LD) are essential organelles as they contribute to the energy metabolism and regulation of various signaling in our bodies. But it is like a double-sided knife because it is deeply related to the viral life introduced to the outside. Recent studies reveal that RNA viruses could regulate cellular architecture for their replication through lipid droplet. Since viral proteins play an essential role as the bridge between replication compartments (RC) and lipid droplets to provide lipid molecules into the RCs, an understanding of lipolysis machinery should be required for further antiviral therapy. We investigated how viral replication compartments of HCV virus could be regulated by LD, interacting with the viral proteins, NS5A.

### **Methods**

Lipid metabolites were analyzed in LD using liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS).

### **Preliminary data (results)**

As a result, it was confirmed that phosphatidylcholine (PC), plasmalogen PC (plasmalogen PC and plasmalogen PC) were increased in HCV replication group compared to the control group. These results corresponded to previous studies that PC in lipid droplets could be supplied to the enteroviral compartment via the lipolysis machinery. It is believed to be increased to protect against cytotoxicity from ROS produced from viral proteins. However, changes in these lipid species by our inhibitor had no effect. In conclusion, we mentioned that lipid droplets are essential organelles in HCV replication. Through lipidomic analysis from the lipid droplets, we suggest that NS5A transports lipids from the lipid droplets to the RC and helps to sustain viral replication by interacting with the lipid droplets.

### **Please explain why your abstract is innovative for mass spectrometry?**

We hoped our lipidomic analysis could contribute to understanding the interaction of the virus with the host cells, thereby providing essential information for HCV therapeutics.

### **Co-authors:**

*Chulhwan Kwak, Department of Chemistry, Seoul National University, Seoul 08826, Korea*

*Hyun-Woo Rhee, Department of Chemistry, Seoul National University, Seoul 08826, Korea*

*Kwang-Hyeon Liu, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unuit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Korea, Mass Spectrometry Based Convergence Research Institute, Kyungpook National University, Daegu 41566, Korea*

Poster number: **FP-PA-026**

## **ENHANCING THE ASSESSMENT OF FORMATION OF REACTIVE METABOLITES BY USING MSXELERATOR WITH DIFFERENT UNLABELED: STABLE ISOTOPE LABELED GLUTATHIONE ON SEVERAL HIGH RESOLUTION MASS SPECTROMETERS PLATFORMS**

Abstract ID: **971**

**Presenting author: Marco Ruijken, MsMetrix**

### **Introduction**

In order to assess the formation of reactive metabolites in a discovery stage, trapping agents such as glutathione (GSH), cyanide and methoxyamine are used. The detection of the reactive metabolites is based on the characteristic high resolution isotopic signatures from mixing the labeled and unlabeled trapping agent. Here we propose a higher throughput assay that is enabled using MsXelerator software on several high resolution mass spectrometer platforms.

### **Methods**

Troglitazone (20 mM) were incubated with human liver microsomes (1 mg/mL) fortified with NADPH and GSH (1 mM) for 60 min. GSH was a 1:3, 1:1 or 3:1 mixture of unlabeled and labeled glutathione with N-15 and two C-13 atoms on the glycyl moiety. Supernatants were evaporated and reconstituted in methanol:water. The GSH trapping assay was performed on three different ultra-high resolution and accurate mass systems: LTQ-Orbitrap and Velos of Thermo Scientific and AB Sciex Triple TOF 5600, which allows us to evaluate the software of MsXelerator and have a direct comparison.

### **Preliminary data (results)**

More than ten GSH conjugates of troglitazone were detected. They were all within 5 ppm for all three instruments. Surprisingly, the accuracy of isotopic ratio for 1:3, 1:1 or 3:1 unlabeled:labeled GSH are very compatible for all three instruments except the ratio of 1:3, although there were only 1 or 2 scans in Orbitrap due to slow scan speed. There were more neutral losses of 129 detected on Sciex 5600, which indicates that 5600 offers better spectra of CID product ion scans. Of course, Velos is offering better quality than Orbitrap. It's also interesting to know that there were more GSH conjugates detected on Orbitrap and Velos than 5600.

We used to run several compounds per week, but our results demonstrate the power and efficiency of MsXelerator software to process data generated from Orbitrap, Velos and 5600, which allows us to run up to 30 samples per week. To save the cost of stable labeled GSH, 3:1 ratio is recommended.

### **Please explain why your abstract is innovative for mass spectrometry?**

Powerful New High Resolution Isotope Detection Algorithm

Applicable to any isotopic signature many research areas

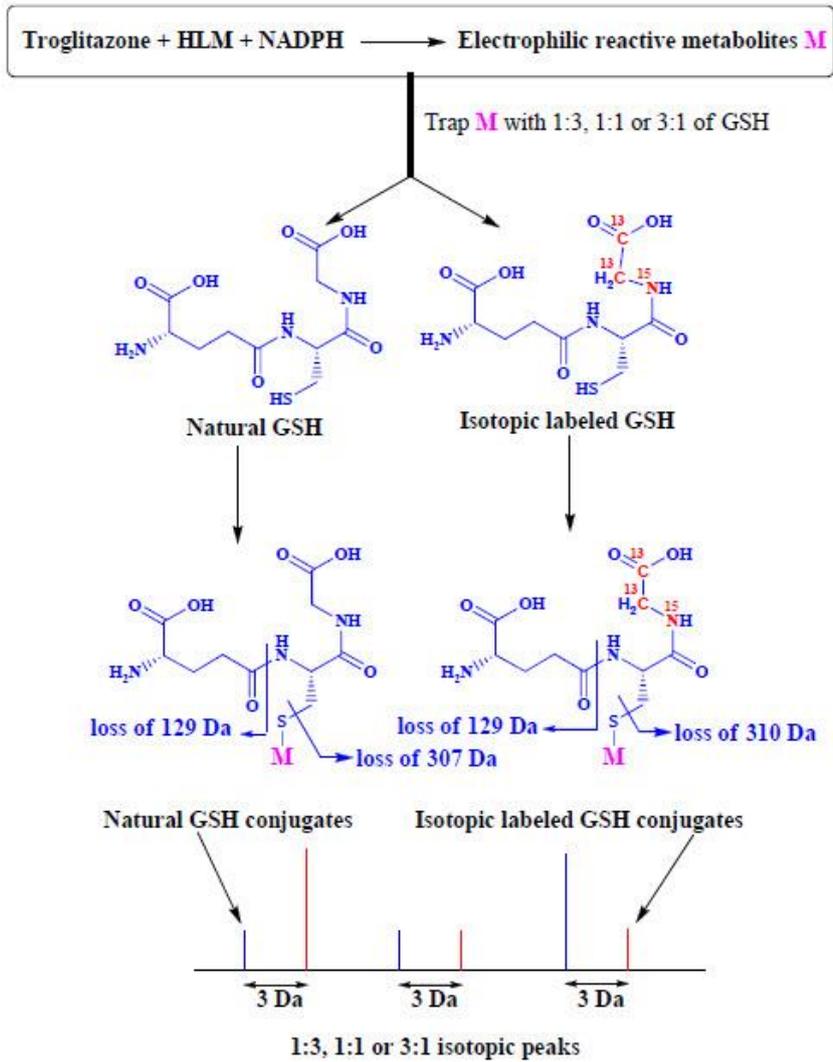
Combination of Different MS filters (Isotope, Mass Defect, EIC, Neutral Loss) to find optimal components

### **Co-authors:**

*Chenghong Zang, Genentech*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Non-labeled and labeled Glutathione and MSMS fragmentation scheme.

## Theme: Instrumentation and methods

### Session: Data sciences in MS/AI/Chemometrics/identification/modelling

Poster number: IM-PA-001

## A NOVEL HIGH-RESOLUTION MASS SPECTROMETRY TOOLBOX FOR UNRAVELLING THE CHEMICAL EXPOSOME

Abstract ID: 63

Presenting author: Saer Samanipour, UvA, UQ

### Introduction

Non-target analysis (NTA) combined with high resolution mass spectrometry (HRMS) is considered the most comprehensive monitoring approach for characterization of the chemical exposome. NTA has thus far not fulfilled its promise of comprehensive analysis and been adopted as a routine approach due to the richness of HRMS data, sample complexity, and the challenges faced by the analysts to perform NTA.

### Methods

NTA data are typically very large (3-4 GB per sample), noisy, and complex so analysts must perform multiple data pre-processing steps to be able to identify features in a sample. These steps may include feature detection, feature prioritization, and database matching. Due to the complexity of these datasets, each step taken in an NTA workflow is prone to error and thus false detection.

### Preliminary data (results)

Here we present a collection of open source/access tools that we have developed to specifically improve and simplify the NTA workflow from feature detection to identification, while maintaining maximum transparency. These tools have been tested on complex environmental samples such as wastewater influent and wastewater sludge and have outperformed commonly used methodologies. Additionally, we present the implementation of these tools within the framework of an open access web platform. This cloud-based web platform will provide both a hub for data processing and a repository for data. The repository facilitates future analysis (i.e. retrospective NTA and/or suspect screening). We envisage that the platform will be a focal point for future developments in NTA.

### Please explain why your abstract is innovative for mass spectrometry?

A set of open access/source data processing tools for the analysis of HRMS data of complex samples is presented.

### Co-authors:

*Mahieu Feraud, UQ*  
*Denice van Herwerden, UvA*  
*Jake O'Brien, UQ*  
*Phil Choi, UQ*  
*Kevin Thomas, UQ*

Poster number: **IM-PA-002**

## **HANS 1.0: A VENDOR-INDEPENDENT ALGORITHM FOR DETECTION OF IMPURITIES**

Abstract ID: **839**

**Presenting author: Kevin Kretschmer, TU Darmstadt**

### **Introduction**

The Buchwald-Hartwig amination is one of the most important reactions in the large-scale production of pharmaceuticals, natural products, and organic electronic materials. Possible side-reactions can lead to halogenated and multiple aminated byproducts. Halogenated compounds especially show a detrimental effect on organic light emitting diodes (OLEDs) or lead to undesired toxic properties when they occur in drugs. Early detection and characterization of these impurities with liquid chromatography - mass spectrometry (LC-MS) can determine whether a purification step was successful or not. Doing this automatically can save time and resources, helping the optimization process to prevent the occurrence of these molecules in the final product. For industry laboratories with multiple MS instruments in particular, a rapid, vendor-independent method that requires limited operator expertise, is a valuable tool.

### **Methods**

In this work, a JavaScript based algorithm called HaNS 1.0 (Halogen and Nitrogen Seeker) was developed to automatically detect halogenated and multiple aminated impurities in MestReNova-MS. Additional algorithms were developed to automatically detect peaks and export a report as a PDF.

Raw data of Buchwald-Hartwig products and educts were evaluated and compared on different instruments including a Bruker impact II TOF-MS, Thermo Fisher QExactive Plus Orbitrap-MS and an Agilent single quadrupole MS. For liquid chromatography, instruments including an Agilent Infinity II 1200 series, and a Thermo Fisher Scientific Vanquish LC system were used, each with a reverse phase C18 column.

### **Preliminary data (results)**

HaNS 1.0 was able to successfully analyze raw data of various instruments including a TOF-MS, an Orbitrap, and a single quadrupole-MS without converting it to other data formats. The calculation time varied depending on the instrument. For low-resolution data acquired on a single quadrupole instrument, the calculation took 10 seconds on a Dell Latitude 5490 laptop. For high-resolution data acquired on a TOF instrument, the calculation took up to 30 minutes on the same laptop. Algorithm parameters must be tuned for each instrument individually to improve sensitivity and selectivity.

Our algorithm essentially consists of two parts. The NitrogenDetector algorithm can detect over-aminated compounds using the nitrogen rule. The HalogenDetector algorithm detects chlorinated or brominated compounds by their isotopic distribution pattern. Experimentally, the NitrogenDetector has been tested for masses up to 1300 Da, whereas the HalogenDetector has been tested for masses up to 700 Da.

With a custom peak-detector, relevant peaks are assigned and labeled as over-aminated/chlorinated/brominated impurities in an extracted ion chromatogram and later exported with a custom layout in a PDF-file. Absence of these impurities will result in a comment in the report. This automated method can be operated with minimal user knowledge of mass spectrometry data-analysis. This is beneficial for routine laboratories, saving resources and time.

### **Please explain why your abstract is innovative for mass spectrometry?**

A vendor-independent, automated tool that can be operated with minimal user knowledge to detect impurities in LC-MS-analytics was developed.

### **Co-authors:**

*Ulrich Engel, Merck KGaA*

*Kevin Eckey, Merck KGaA*

*Frederik Lermyte, TU Darmstadt*

Poster number: **IM-PA-003**

## **A STATISTICAL FRAMEWORK FOR DECONVOLUTED ION CHROMATOGRAM FOR THERAPEUTIC OLIGONUCLEOTIDES**

Abstract ID: **860**

**Presenting author: Piotr Prostko, Hasselt University**

### **Introduction**

Synthetic oligonucleotides have emerged as a successful drug modality as recently witnessed by an increased number of oligo-based medicines under active development or already approved. To meet high-quality demands imposed by regulatory agencies or GMP standards, mass spectrometry is the preferred platform (due to, among others, its inherent sensitivity) for quantitative characterization of oligo-based therapeutics. However, potential process impurities and degradation products present in drug product can hardly be chromatographically resolved from the drug substance itself (full-length product, FLP), resulting in convolved, overlapped LC-MS signals. Therefore, we present a targeted approach for estimating the proportions of the FLP and impurities in mixture samples using both mass-to-charge and retention-time (RT) dimensions.

### **Methods**

A linear combination of the theoretical isotope distributions of FLP and known impurities (pre-computed with the BRAIN algorithm) is fitted to the observed spectrum to disentangle and quantify the FLP and impurity contributions. These contributions evolve over RT, and hence we estimate coefficients of the linear combination by employing fast linear constrained optimization to each LC scan and charge state separately. The individual estimates are plotted against RT, giving rise to a deconvoluted chromatogram of the FLP and impurities. The RT-specific proportions are then integrated over RT yielding final estimates that account for often complex chromatographic profiles.

### **Preliminary data (results)**

The proposed approach was applied to LC-MS1 data from a controlled experiment of mixture samples of two commercially available oligonucleotides that mimics a real-life setting of overlapping FLP and impurities. These oligonucleotides were spiked at 7 different ratios, ranging from 0% to 33%. Deconvoluting 5 selected charge states of FLP and the impurity from one LC-MS data file took less than 1 minute with our algorithm run on a local PC. Very good agreement between our across-RT proportion estimates and the expected values was found as indicated by a calibration curve.

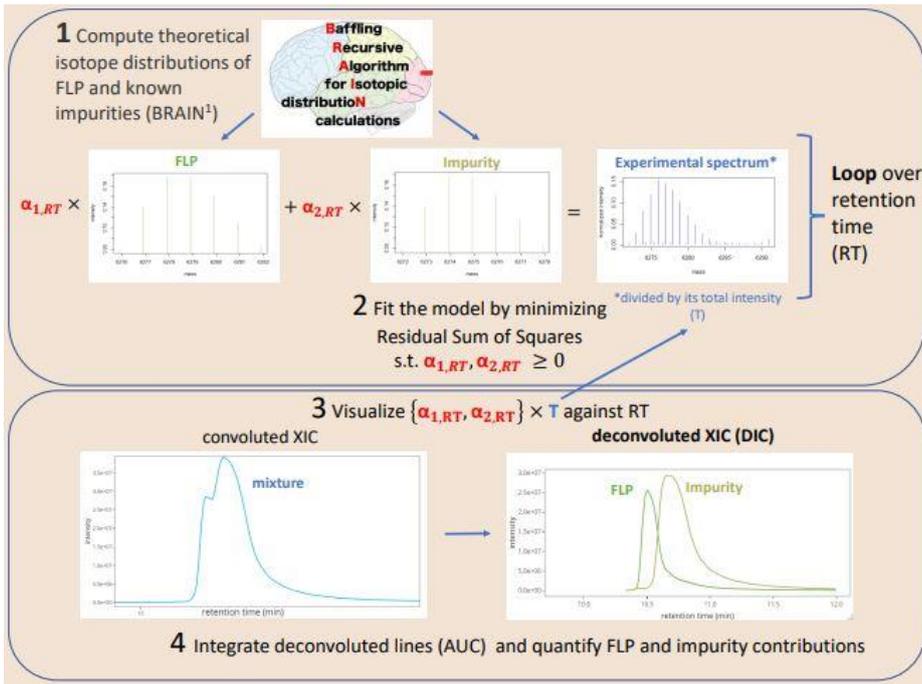
Future research will focus on incorporating statistical inference on the RT-specific and across-RT impurity proportion estimates. Furthermore, the current approach provides separate estimates for each charge state, and therefore we will investigate how to efficiently combine results across multiple charges.

### **Please explain why your abstract is innovative for mass spectrometry?**

Fast and targeted construction of deconvoluted extracted ion chromatogram of known oligonucleotides.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Deconvoluted ion chromatogram-based quantification of overlapping LC-MS signals

Poster number: **IM-PA-004**

## **POINTLESS4DNA: PREDICTION OF THE ISOTOPE DISTRIBUTION FOR AVERAGE DNA AND RNA MOLECULES**

Abstract ID: **879**

**Presenting author: Melvin Geubbelmans, Hasselt University**

### **Introduction**

Oligonucleotides are gaining much interest as therapeutic agents for human health. Mass spectrometry is the preferred technology to assess the quality of the production process and handle regulatory affairs due to its open discovery framework and high sensitivity. Bioinformatics tools must be adjusted to accommodate this oligo-based data flood from the mass spectrometer. The first step in characterizing the observed spectrum is to compare the obtained isotope pattern of the molecule to the one theoretically expected based on its elemental composition. However, this is not straightforward when the molecule's identity under investigation is unknown. We present a modeling approach for predicting the aggregated isotope distribution of an average DNA or RNA molecule when a particular monoisotopic mass is available.

### **Methods**

A theoretical database of possible DNA/RNA oligonucleotides up to 25kDa is created. The theoretical aggregated isotope distribution for the database is generated using the BRAIN algorithm. A pseudo-isotope is designed ensuring that isotope information is compositional. The additive log-ratio transformation cast the compositional isotope probabilities into Aitchison geometry. A classical linear regression model is trained, predicting Aitchison ratios based on monoisotopic mass. The model's prediction is back-transformed into probabilities. The figures-of-merit, testing the performance is mean squared error (MSE) and a modified version of Pearson's  $\chi^2$  goodness-of-fit measure on experimental oligonucleotides synthesized via solid-phase chemical synthesis and LC-MS.

### **Preliminary data (results)**

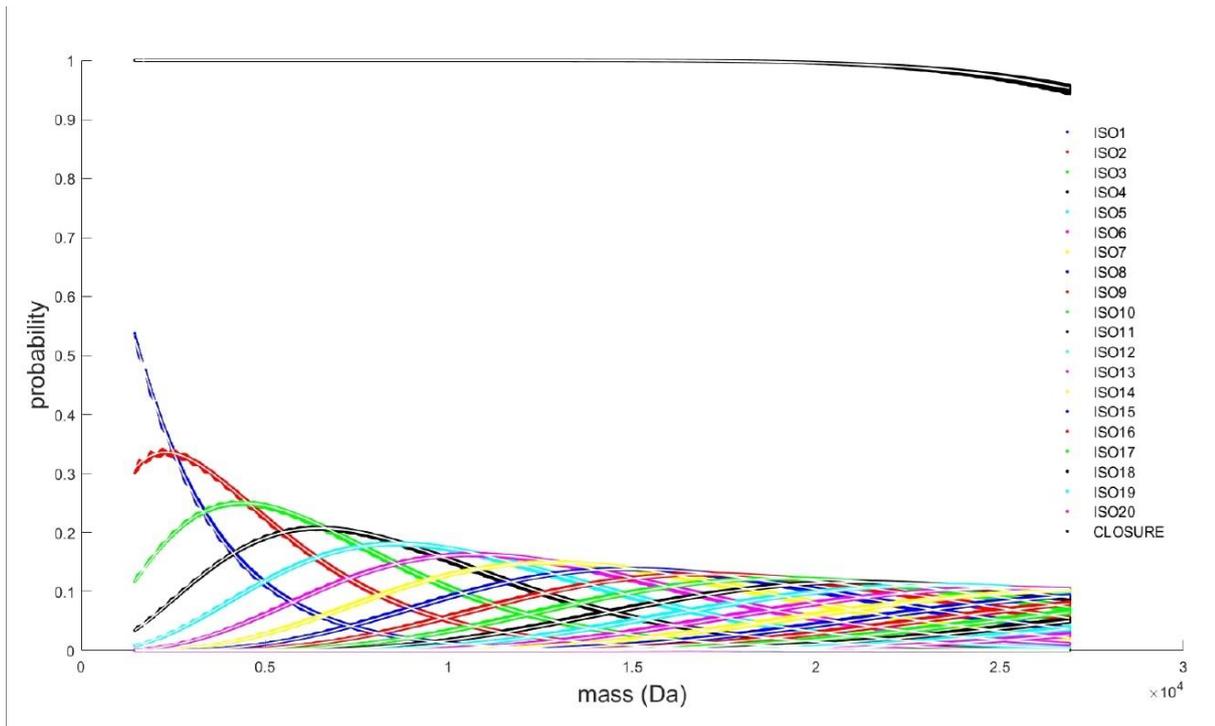
The first 20 aggregated isotope variants of the DNA and RNA compounds are computed, covering up to 95% of the aggregated isotope distribution for the highest molecular weight DNA/RNA molecules. Forward model selection using minimal MSE on an independent test set is conducted to determine the optimal model flexibility. As a result, a univariate weighted polynomial regression model of order ten is fitted to predict the first 20 isotope peaks based on the monoisotopic mass for DNA and RNA molecules separately. The entire method is made available as an R shiny interface called POINTLESS4DNA. Our figures-of-merit are evaluated on experimental data of known composition. The observed isotope distribution is compared to the theoretical one from BRAIN and the predicted one from POINTLESS4DNA. Given the measurement error present in the data, no significant difference could be detected between the BRAIN and POINTLESS4DNA methods. This result indicates that the prediction for an average oligonucleotide is equally accurate as the computed isotope distribution derived from the element composition of the DNA/RNA molecule. Moreover, it is demonstrated that model misspecification leads to severely inflated errors. This principle can be adopted for the inclusion in an automated processing workflow as a label-free DNA/RNA classifier. The prediction model is simple and not demanding in computational resources as it only requires matrix multiplication.

### **Please explain why your abstract is innovative for mass spectrometry?**

A compositional model to predict the isotope distribution for oligonucleotides.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



First 20 isotopes of all possible DNA molecules in range.

Poster number: **IM-PA-005**

## IN-DEPTH ANALYSIS OF ELECTRON IONIZATION MASS SPECTRA OF MONOTERPENES WITH HELP OF MOLECULAR NETWORKS

Abstract ID: **891****Presenting author: Natan Horacek, Charles University Faculty of Science, Institute of Organic Chemistry and Biochemistry CAS**

### Introduction

Monoterpenes are structurally rich group of natural products defined by ten-carbon backbone composed from two isoprene units, eventually modified by cyclizations or substitutions. The modular origin and subsequent modifications can potentially lead to enormous numbers of structures sharing common building blocks. Gas chromatography coupled with mass spectrometry is the method of choice for monoterpene analysis because of their relatively low polarity and small molecular weight. However, another consequence of the modularity of terpenoids is the similarity of their electron ionization mass spectra, hampering their empiric structural resolution. Therefore, we decided to use the molecular network approach to cluster structurally similar compounds and pinpoint their diagnostic MS patterns, and vice versa, to relate the differences among mass spectra with particular differences among structures.

### Methods

We automatically searched the NIST library for all compounds with general molecular formula:  $C_{10-12}H_{12-26}O_{1-3}$  and then manually selected structures that can arise from two isoprene units, to end up with an initial dataset of 500 monoterpenes. We added to each spectrum following spectral features: intensities normalized on local ion current, averaged intensities of mass interval, summarization of modulations of 14, sum of characteristic losses and logarithmic intensities ratio for lower masses. We calculated cosine similarity among all spectral queries and visualised it as a molecular network. We then calculated Tanimoto similarity between chemical structures based on their molecular fingerprints.

### Preliminary data (results)

We created two molecular networks with similarity cut-off set to 0.8 and 0.94. We selected those values based on evaluation of the mass spectra and structures with different similarities. Spectra with values similarity higher than 0.8 were marked as structurally similar enough to have some interesting common fragmentations for the group. Spectra with similarity higher than 0.94 do not allow for consistent separation of the structures. Similarity cut-off 0.8 led to 96 different groups with 22 of them having more than one compound, thus potentially sharing some interesting spectral characteristics. We selected the two biggest groups and run a random forest supervised learning algorithm to create classification and search for spectral ions that have biggest impact on group differences. Most prominent ions were  $m/z$ : 84, 154, 111, 83, 71, 97, 112 and 139. Molecular network with cut-off 0.94 had 23 groups with more than one compound. Those had almost identical spectra. Interestingly, the molecular network calculated only from mass spectra had 82 clusters. If this is an artefact of increasing the number of variables with no spectral and structural background or the spectral features enhanced the resolution among very similar spectra currently remains unresolved.

### Please explain why your abstract is innovative for mass spectrometry?

Our results allow us to conclude that the molecular network technique is a promising tool to facilitate the separation and structural characterisations of monoterpenes based on their EI mass spectra.

### Co-authors:

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*Robert Hanus, Institute of Organic Chemistry and Biochemistry CAS*

Poster number: **IM-PA-006**

## **IMPROVED HPLC-MS IDENTIFICATION OF SHORT PEPTIDES IN COMPLEX FOOD SAMPLES USING SEQUENCE-BASED RETENTION TIME PREDICTIONS**

Abstract ID: **895**

**Presenting author: Boudewijn Hollebrands, Wageningen University, Unilever R&D**

### **Introduction**

There are several challenges associated with proteins in foods. Of particular concern is the hydrolysis of proteins into peptides. Peptides are an important group of compounds contributing to the desired, as well as the undesired taste of a food product. Their taste impressions can include aspects of sweetness, bitterness, savoury, umami and many other impressions depending on the amino acids present as well as their sequence. Identification of especially small peptides (less than 6 amino acids in length) is challenging. In this contribution analytical methods based on chromatography, mass spectrometry and in silico predictions are discussed that allow us to accurately identify small peptides in typical foods.

### **Methods**

Large sets of small peptides were generated using 'swopped-sequence' synthesis and by hydrolysis of protein standards. Peptides were separated by reversed phase chromatography and peptide MS2 data was acquired on an orbitrap system using Data-Dependent Acquisition (DDA). The identified peptide sequences and their experimental retention times were used to train and validate a Support Vector Regression model to predict retention times.

### **Preliminary data (results)**

Our predictive model used, next to common peptide descriptors (as e.g. length, MW, logP etc.), a set of amino acid indices that showed excellent performance in a sequence dependent model. We achieved a strong correlation between measured and predicted retention times, also for small homologous peptide structures. Our study reveals that particularly peptide sequences play an important role in the LC retention behavior of short peptides.

### **Please explain why your abstract is innovative for mass spectrometry?**

The identification of small peptides can be improved by accurate prediction of their retention time using our presented model.

Poster number: **IM-PA-007**

## **A MODERN APPROACH TO MASS SPECTROMETRY IMAGING DATA SEGMENTATION WITH SPATIAL-DGMM AND MASSERSTEIN**

Abstract ID: **906**

**Presenting author: Michał Ciach, Faculty of Mathematics, Informatics and Mechanics, University of Warsaw, Data Science Institute, Hasselt University**

### **Introduction**

Computational and statistical methods enable more advanced analyses of mass spectrometry images. One of the most commonly used methods is univariate image segmentation, which attempts to delineate regions with characteristic concentrations of a selected molecule. Typically, it is performed by clustering pixels with similar signal intensities. This approach is sensitive to overlapping isotopic envelopes, which can distort ion images by mixing the spatial distributions of several molecules. It is also sensitive to pixel-to-pixel variability of signal intensity, which can result in segments which have poor spatial homogeneity and, therefore, poor agreement with anatomical regions. Filtering out the overlapping isotopic envelopes would make the results more reliable, and improving the spatial homogeneity of segments would facilitate their interpretation in terms of tissues or other sub-structures.

### **Methods**

To improve the quality of MS image segmentation, we have combined two recently developed algorithms: masserstein and Spatial-DGMM.

The former resolves the overlapping isotopic envelopes by fitting a combination of reference spectra of the ions of interest. The latter algorithm identifies regions with distinct average intensities of ions in a spatially consistent manner by considering the ion intensities in a neighborhood of each pixel. This is accomplished by using Dirichlet Gaussian Mixture Models, a method rooted in Bayesian statistics, and post-processing the results with a filter specifically designed for MS images.

### **Preliminary data (results)**

We have validated our approach on both simulated and experimentally acquired data sets. The simulations, designed specifically to mimic signal distortions and interferences observed in real spectra, allowed us to compare our results to known ground truth. This, in turn, allowed us to demonstrate that peak-based segmentation may be misleading when overlapping isotopic envelopes are present, which occurs commonly in many fields of research, such as mass spectrometry imaging of unsaturated phospholipids. Segmentation based on monoisotopic peaks may identify spurious regions and leave out ones that actually do correspond to distinct ion intensities. Separating the overlapping envelopes, on the other hand, allows us to discover the true regions.

The experimentally acquired spectra allowed us to demonstrate the practical capabilities of our approach. For the sake of reproducibility of our results, we have used a publicly available mass spectrometric image of a mouse bladder tissue section. We have used masserstein to estimate the intensities of a selected set of lipids, segmented the image with Spatial-DGMM, and compared the results to ones obtained with K-means clustering. This case study demonstrates that incorporating spatial dependencies between pixels allows us to obtain segments that are more spatially consistent, and therefore easier to interpret and more meaningful biologically. We have also discovered that in over 60% of lipids, their monoisotopic peaks are potentially influenced by isotopic envelopes of lighter lipids. We have further confirmed our results on an MS image of mouse cerebellum.

### **Please explain why your abstract is innovative for mass spectrometry?**

Advanced algorithmic solutions result in MS image segments with a high degree of agreement with underlying anatomical regions.

### **Co-authors:**

*Dan Guo, Khoury College of Computer Sciences, Northeastern University*  
*Dirk Valkenburg, Data Science Institute, Hasselt University*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



*Olga Vitek, Khoury College of Computer Sciences, Northeastern University*  
*Anna Gambin, Faculty of Mathematics, Informatics and Mechanics, University of Warsaw*

Poster number: **IM-PA-008**

## **NEW CCS PREDICTION WORKFLOW TO EXTEND MS/MS SPECTRAL LIBRARIES ON THE FLY WITH CCS INFORMATION**

Abstract ID: **974**

**Presenting author: Heiko Neuweger, Bruker Life Sciences Mass Spectrometry Division**

### **Introduction**

Due to their great structural diversity, the identification of small molecules remains one of the major challenges in Metabolomics research. Trapped Ion Mobility Mass Spectrometry (TIMS) enables to determine the Collision Cross Section (CCS) of ions, which is characteristic for the three-dimensional composition of compounds. Besides retention time (RT), mass accuracy, isotope pattern fit and MS/MS spectra matching, the CCS value serves as an additional, orthogonal qualifier that can increase confidence in compound annotation.

Many publicly available databases and MS/MS libraries still lack reference CCS values though. The new automatic CCS-Predict workflow in the Spectral Library annotation presented here enables to extend MS/MS spectral libraries with predicted CCS values on the fly using a machine learning based CCS prediction model.

### **Methods**

When provided with chemical structure information, CCS-Predict calculates feature vectors of physico-chemical properties and makes use of support vector regression models to predict the CCS of compounds. MS/MS spectral libraries with structure information can be imported or generated within MetaboScape. The Spectral Library annotation enables to annotate compounds based on accurate mass, isotope pattern, MS/MS spectra and CCS value. For library spectra that do not provide CCS information, the CCS-Predict workflow predicts CCS values from structures to perform CCS matching.

### **Preliminary data (results)**

Here, we present a novel workflow for automated CCS prediction of spectral library compounds with attached structure information and without available CCS references. For this, CCS-Predict was fully integrated into the Spectral Library annotation workflow of the untargeted profiling software solution MetaboScape. As a result, CCS-supported Spectral Library annotation can be performed with any user-created or publicly available MS/MS libraries that contain structural information.

The addition of CCS matching for the annotation of reference compounds enables, for example, to increase confidence in annotations and to exclude false positive annotations, which is of great importance when applying large MS/MS libraries.

The new workflow does not equal predicted CCS values to CCS references. First, available CCS references will always be prioritized over predicted CCS values. Second, if the CCS deviation to a reference exceeds a threshold, this annotation will be disqualified and removed; exceeding CCS deviations to a predicted value will be clearly reported in the annotation quality, but the annotation remains active. The CCS deviation thresholds can be controlled by the user.

Since predicted CCS values are generated on the fly rather than precalculated, all mentioned annotation workflows will automatically benefit when the machine learning models are improved.

This new annotation workflow unlocks higher annotation confidence for existing MS/MS libraries even in the absence of CCS reference values.

### **Please explain why your abstract is innovative for mass spectrometry?**

CCS prediction workflow to extend MS/MS spectral libraries with CCS information.

### **Co-authors:**

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

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*Sofie Weinkouff, Bruker Life Sciences Mass Spectrometry Division*

*Lucy Woods, Bruker Life Sciences Mass Spectrometry Division*

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Poster number: **IM-PA-009**

## **MERGEION: A SOLUTION FOR BUILDING MULTIFUNCTIONAL R PIPELINES FOR PROCESSING, SEARCHING, AND ORGANIZING SMALL MOLECULE LC-MS/MS DATA**

Abstract ID: **983**

**Presenting author: Youzhong Liu, Janssen Pharmaceutica**

### **Introduction**

The structural elucidation of small molecules using tandem mass spectrometry (MS/MS) plays a crucial role in life science, bioanalytical and pharmaceutical research. There is a pressing need for laboratories involved to not only increase the throughput of compound identification, but also transform historical data into an information-rich spectral database. Meanwhile, molecular networking, a recent bioinformatic framework, provides global displays and system-level understanding of LC-MS/MS datasets of complex mixtures.

### **Methods**

We present meRgelON, a multifunctional, modular, and flexible R-based toolbox to streamline spectral database building, automated structural elucidation, and feature-based molecular networking. The toolbox offers diverse tuning parameters and the possibility to combine various advanced algorithms in the same pipeline (Figure 1).

### **Preliminary data (results)**

Using meRgelON, we have created an integrated spectral database covering diverse pharmaceutical compounds. This database was successfully applied to annotate drug-related metabolites from a published non-targeted metabolomics dataset. The chemical space behind this complex dataset was revealed by meRgelON through molecular networking. Moreover, meRgelON-based processing workflow was applied in pharmaceutical stress testing studies to annotate degradant products and to understand drug degradation pathways. meRgelON is freely available at: <https://github.com/daniellyz/MergeION2>.

### **Please explain why your abstract is innovative for mass spectrometry?**

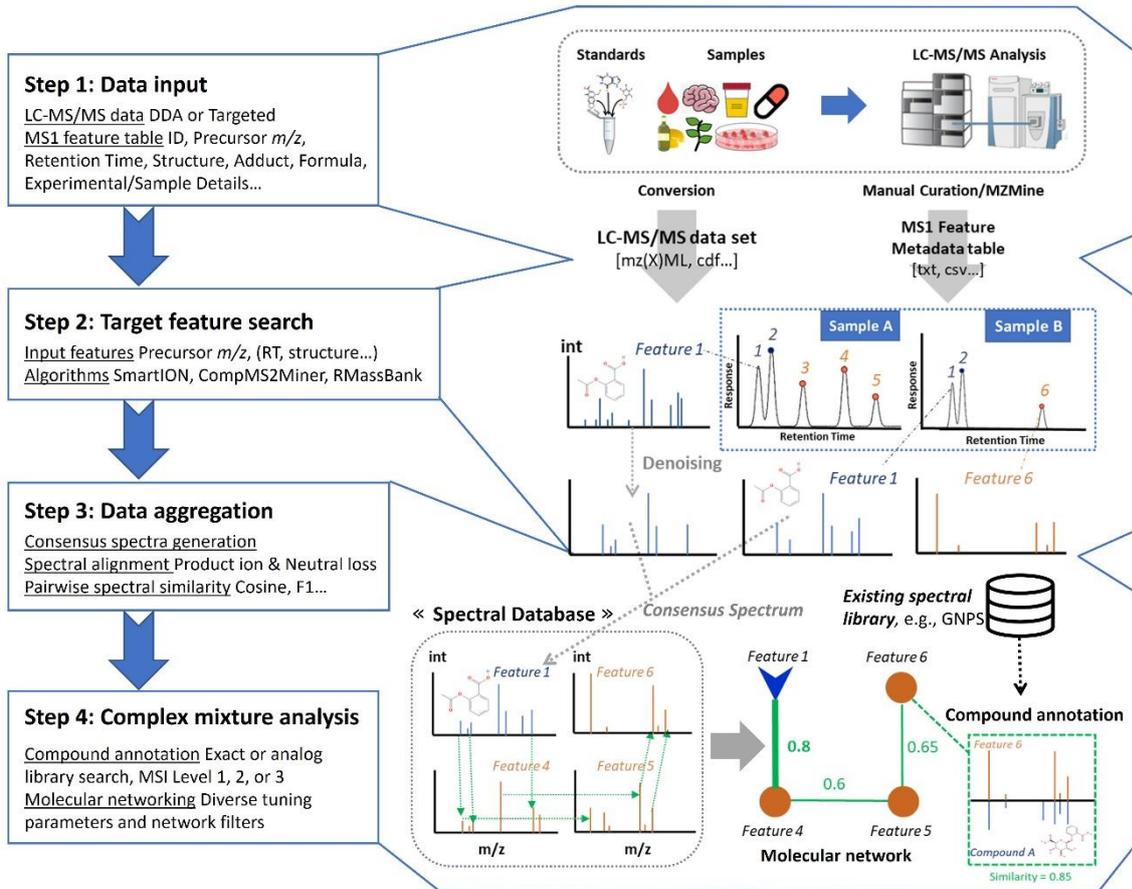
As an open-source R package, meRgelON is ideally suited for building spectral databases and molecular networks from privacy-sensitive and preliminary mass spectrometry data.

### **Co-authors:**

*Yingjie Zhang, Open Analytics*  
*Tom Vennekens, Janssen Pharmaceutica*  
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POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Standard LC-MS/MS data analysis workflow by meRgeION

Poster number: **IM-PA-010**

## **ENVEMIND: ACCURATE MONOISOTOPIC MASS DETERMINATION BASED ON ISOTOPIC ENVELOPE**

Abstract ID: 1011

**Presenting author: Piotr Radziński, University of Warsaw**

### **Introduction**

Nowadays, monoisotopic mass is used as an important feature in top-down proteomics. Knowing the exact monoisotopic mass is helpful for precise and quick protein identification in large protein databases. However, only in spectra of small molecules, the monoisotopic peak is visible. For bigger molecules like proteins, it is hidden in noise or undetected at all, therefore its position has to be predicted. By improving the prediction of the peak, we contribute to a more accurate identification of molecules, which is crucial in fields such as chemistry and medicine.

### **Methods**

We present envemind algorithm, which is a two-step procedure to predict monoisotopic masses of proteins. The prediction is based on an isotopic envelope. Therefore, envemind is dedicated to spectra where we are able to resolve the one dalton separated isotopic variants. Furthermore, only single molecule spectra are allowed, i.e. spectra that do not require prior deconvolution.

### **Preliminary data (results)**

The algorithm deals with the problem of off-by-one dalton errors, which are common in monoisotopic mass prediction. A novel aspect of this work is a mathematical exploration of the space of molecules, where we equate chemical formulas and their theoretical spectrum. Since the space of molecules consists of all possible chemical formulas, this approach is not limited to known substances only. This makes optimization processes faster and enables to approximate theoretical spectrum for a given experimental one. The algorithm is available as a Python package envemind on our GitHub page <https://github.com/PiotrRadzinski/envemind>.

### **Please explain why your abstract is innovative for mass spectrometry?**

Usage of the whole isotopic envelope for monoisotopic mass prediction. Usage of mathematical exploration of the space of molecules, where chemical formulas are equated with their theoretical spectra.

### **Co-authors:**

*Dirk Valkenburg, Hasselt University*

*Michał Startek, University of Warsaw, Johannes-Gutenberg University Mainz*

*Anna Gambin, University of Warsaw*

Poster number: **IM-PA-011**

## **PATROON 2.0: NON-TARGET SCREENING WORKFLOWS FOR AUTOMATED TRANSFORMATION PRODUCT SCREENING AND OTHER MAJOR IMPROVEMENTS**

Abstract ID: 153

**Presenting author: Rick Helmus, University of Amsterdam - Institute for Biodiversity and Ecosystem Dynamics**

### **Introduction**

Non-target screening (NTS) with high resolution mass spectrometry (HRMS) is increasingly used to systematically identify chemicals of emerging concern (CECs) in the environment. Microbial degradation, UV irradiation and other processes can transform CECs into transformation products (TPs), which may pose similar or higher risk. However, the complexity of environmental samples and the lack of suitable workflows and data sources often complicates comprehensive identification of TPs.

patRoom 2.0 is an open-source R based platform for comprehensive and flexible NTS workflows, now upgraded with automated TP screening and other novel functionality. Two studies will be used to demonstrate patRoom: biotransformation processes of residual CECs in wastewater effluent meant for re-use (Study I) and elucidation of UV by-products formed from CECs during drinking water preparation processes (Study II).

### **Methods**

Groundwater and soil samples for Study I were taken from the only Dutch directly and intentionally wastewater effluent irrigated site (Haaksbergen, the Netherlands), and incubated aerobically (3 months) and anaerobically (6 months). Analyses were performed with a liquid chromatograph (Shimadzu Nexera) coupled to QTOF HRMS (Bruker maXis 4G).

Samples for Study II consisted of drinking water with trace levels of pharmaceuticals. The UV degradation experiment was performed with online liquid chromatography (Agilent Infinity 1290), fractionation, UV exposure in a flow cell and finally HRMS detection (aforementioned QTOF) to sequentially isolate and irradiate the pharmaceuticals and detect their UV TPs.

### **Preliminary data (results)**

An automated TP screening workflow was developed that combines various approaches to find and identify parent/TP pairs (Figure 1). Feature data is first obtained from a 'classical' NTS workflow (A). Subsequently, suspect screening for known or predicted TPs (B), MS similarity between features (C) and/or (statistical) parent/TP classifications (D) are combined to link parent/TP features into components (E). The suspects for (B) are retrieved from automated prediction or library search of TP candidates. MS similarity is calculated by MS/MS spectral similarity score (cosine or Jaccard) or overlap of putative fragment formula or neutral loss assignments obtained with automatic annotation of feature MS/MS data. Feature classification (D) relies on fold changes or other commonly used statistical or data mining methods available from R. Finally, candidate TPs are prioritized, identified and summarized with interactive reports (F-H). Most of the workflow steps are optional and implemented via various (parametrized) algorithms, which allow flexible and customizable TP screening workflows to accommodate various study types.

Other major new functionality includes simultaneous processing, combining and interpretation of positive and negative ionization data, additional algorithms for detection of features, adducts and isotopes, interactive tools to curate workflow data and estimating feature identification confidence levels.

The new patRoom 2.0 workflows will be demonstrated using the HRMS data from Study I and II.

### **Please explain why your abstract is innovative for mass spectrometry?**

patRoom 2.0 implements automated TP screening, combining positive and negative ionization data and other data mining tools to improve comprehensive HRMS-based NTS workflows, demonstrated in two case studies.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Co-authors:

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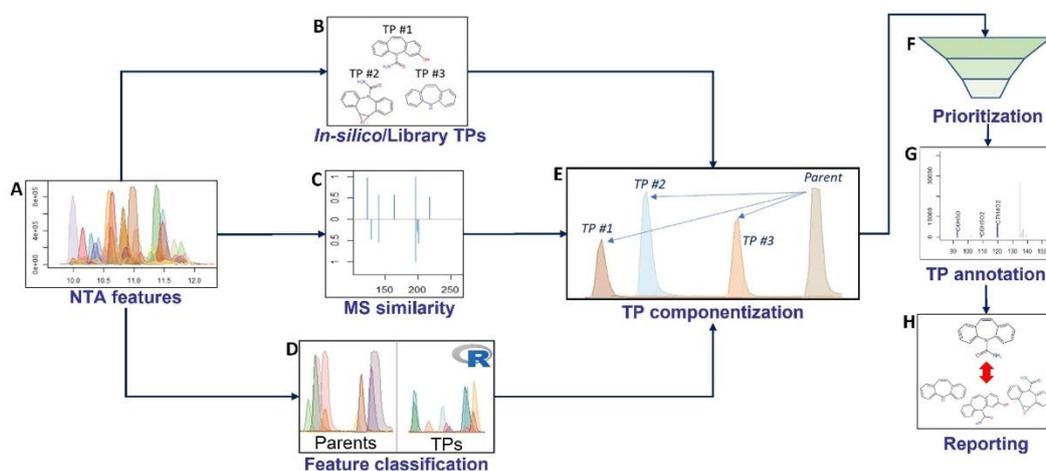
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Generalized transformation product (TP) screening workflow in patRoan 2.0.

Poster number: **IM-PA-012**

## **SPATIAL PROBABILISTIC MAPPING OF METABOLITE ENSEMBLES IN MASS SPECTROMETRY IMAGING**

Abstract ID: **237**

**Presenting author: Denis Abu Sammour, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, Institute for Medical Technology, Heidelberg University and Mannheim University of Applied Sciences**

### **Introduction**

Mass spectrometry imaging (MSI) has evolved into a label-free core technology for visualization and spatially-resolved analysis of digested proteins, drugs, glycans and metabolites, incl. lipids, in basic, clinical and pharmaceutical research. Despite enormous advances in speed, sensitivity and spatial resolution of MSI instruments, the fundamental concept in MSI data representation, the use of so-called ion images for visualization and molecular analysis, has not changed since the inception of the technology. These false color renderings of signal intensities lack objectivity and are often prone to input- and perceptual user-bias. Here, we report *moleculaR* (<https://github.com/CeMOS-Mannheim/moleculaR>), a computational framework that introduces probabilistic mapping and user-unbiased point-for-point statistical testing for the presence of metabolites in tissue.

### **Methods**

*moleculaR* provides molecular probability maps (MPMs) as the basis for computing statistically-validated spatial distributions of metabolites by comparing their distribution to data-derived complete spatial randomness which acts as the spatial null hypothesis (Figure 1). These MPMs are capable of highlighting areas of significant spatial clustering of metabolite signals which are unlikely to occur if generated by a random spatial process. Moreover, *moleculaR* provides data-integrating probability maps of metabolite ensembles such as entire lipid classes, potassium adducts of lipids or any other user-defined set of metabolites, which paves the way for visualization and exploration of integrated MSI data.

### **Preliminary data (results)**

In a neurooncology example, MPMs of individual lipids demonstrated how spatial probabilistic mapping of analytes aids in outlining the significant presence or absence of analytes relative to vital tumor regions, as inferred from a neuropathologist's annotation of a fresh-frozen tissue section of isocitrate dehydrogenase (IDH) wild-type glioblastoma (GB). Moreover, by projecting groups of metabolites into the same image space we were able to show the collective probabilistic spatial distribution of potassium and sodium adducts of all detectable Glycerophospholipids in an IDH wild-type GB tissue showing higher abundance of potassium adducts in vital tumor and vascularized areas while sodium adducts were more pronounced in necrotic tissue showing significant absence (analyte coldspot) in vital tumor.

### **Please explain why your abstract is innovative for mass spectrometry?**

*moleculaR* provides the scientific community with solutions for molecular spatial probability mapping and for collective visualization and analysis of molecular ensembles in MSI.

### **Co-authors:**

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*Jonas Cordes*, Faculty of Computer Science, Mannheim University of Applied Sciences, Institute for Medical

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Technology, Heidelberg University and Mannheim University of Applied Sciences  
Christian Marsching , Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences  
Mirco Friedrich , Department of Neurology, MCTN, Medical Faculty Mannheim, Heidelberg University  
Michael Platten , Department of Neurology, MCTN, Medical Faculty Mannheim, Heidelberg University, DKTK Clinical Cooperation Unit Neuroimmunology and Brain Tumor Immunology, DKFZ  
Ivo Wolf , Faculty of Computer Science, Mannheim University of Applied Sciences, Institute for Medical Technology, Heidelberg University and Mannheim University of Applied Sciences  
Andreas von Deimling , Department of Neuropathology, University Hospital Heidelberg, and, Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), German Cancer Consortium  
Christiane Opitz , DKTK Brain Cancer Metabolism Group, German Cancer Research Center (DKFZ), Department of Neurology and National Center for Tumor Diseases, Heidelberg University Hospital  
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Carsten Hopf , Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, Institute for Medical Technology, Heidelberg University and Mannheim University of Applied Sciences, Medical Faculty Heidelberg, Heidelberg University

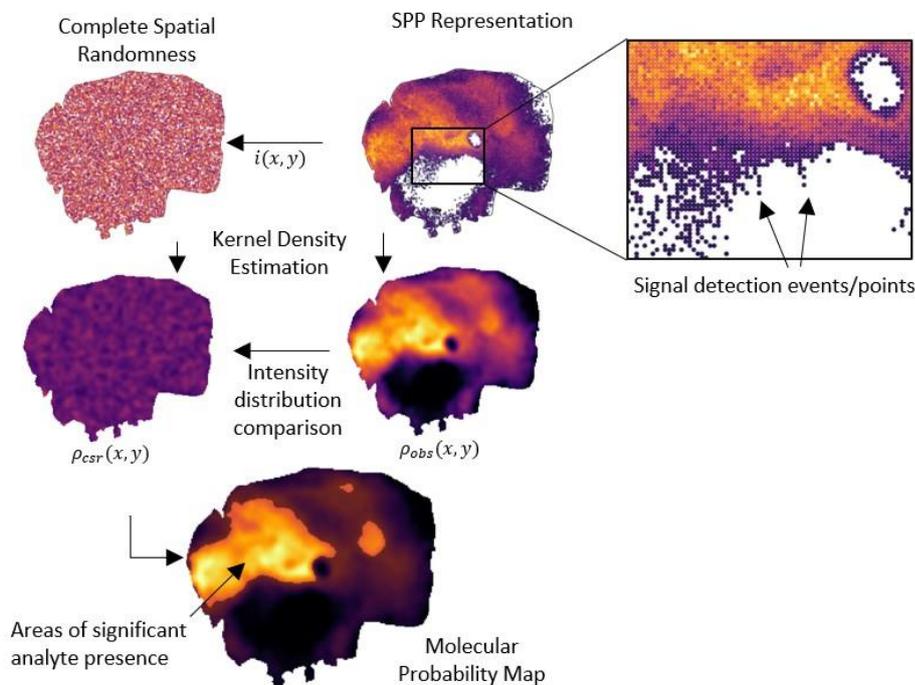


Figure1: The MPM method workflow.

Poster number: **IM-PA-013**

## **$\Delta$ MR: INTER-PEAK DISTANCE FOR SPECTRAL ALIGNMENT AND RESOLUTION ESTIMATION**

Abstract ID: **244**Presenting author: **Eduardo Jacobo Miranda Ackerman, Mpi-CBG**

### **Introduction**

As continuous developments in mass spectrometry strive to reach higher resolutions, the resolution information is not always conveyed to the end-user, for example when using open standards such as MZXML. The resolution information is very useful for grouping, averaging, and filtering scans; providing a consensus of the spectra, instead of sporadically occurring signals. To approximate the resolution of spectra we use the minimum distance between detected  $m/z$  values of peaks from multiple scans within the same spectra. The approximation is comparable to the reported FWHM resolution of Thermo (orbitrap) raw files. We also present some caveats of this approach, namely the positive correlation between the amount of data and the accuracy of the approach and how some high-intensity peaks can overlap and distort results.

### **Methods**

Delta mass resolution ( $\Delta$ MR) is what we call the approximation of the resolution, below is the pseudo-code we use to generate the  $\Delta$ MR

1. For each comparable scan
  1. On a quantile basis, split on 10% of the peaks, get the minimum distance ( $\text{min\_D}$ )
  2. Join all  $\text{min\_D}$  from each scan
2. From all  $\text{min\_D}$  generate a rolling average to remove outliers.
3. Use a monotonic filter across the  $m/z$  range to remove any dips in the resolution ( this assumes resolution only decreases as the masses increase)
4. Fit a curve to the data points that were kept to represent the  $\Delta$ MR

### **Preliminary data (results)**

To calculate the delta mass resolution ( $\Delta$ MR), we first read in multiple spectra files that should be analyzed jointly into a single data structure we call a MasterScan, we expect the spectra files to originate from the same machine with consistent settings, to provide a consistent result. The MasterScan is based on Python Pandas DataFrames for fast and efficient data processing and visualization. The MasterScan data structure is extensively used in LipidXplorer for lipid identification, quantification, and filtering. For the filtering, a software called PeakStrainer is used. PeakStrainer uses signal reproducibility to filter out sporadic (noise) signals. To define if a signal is repeating across multiple scans we require tolerance of signal variation, the tolerance is provided by  $\Delta$ MR. When the peak masses  $M$  from different scans occur consistently within the  $\Delta$ MR tolerance they are considered repeated signals. If a signal is repeatable it can be aggregated and used for identification and quantification, and the results are a summary of the consensus data. In Figure 1 we show how  $\Delta$ MR closely tracks the reported resolution at full-width-half-maximum of selected peaks. Peaks are selected if they are not low intensity and repeatable. Peaks may overlap after centroiding, which can lead to outliers in the IPD, mitigated by using a rolling average over IPDs. If there are not enough peaks to establish a congruent minimum peak distance, a dip in the estimated resolution will occur, this is mitigated by using a monotonic function. This approach provides a valid baseline for resolution estimation.

### **Please explain why your abstract is innovative for mass spectrometry?**

Approximation for resolution information is used to group signals and provide a consensus summary of the group, and potentially avoid combining signals from different origins.

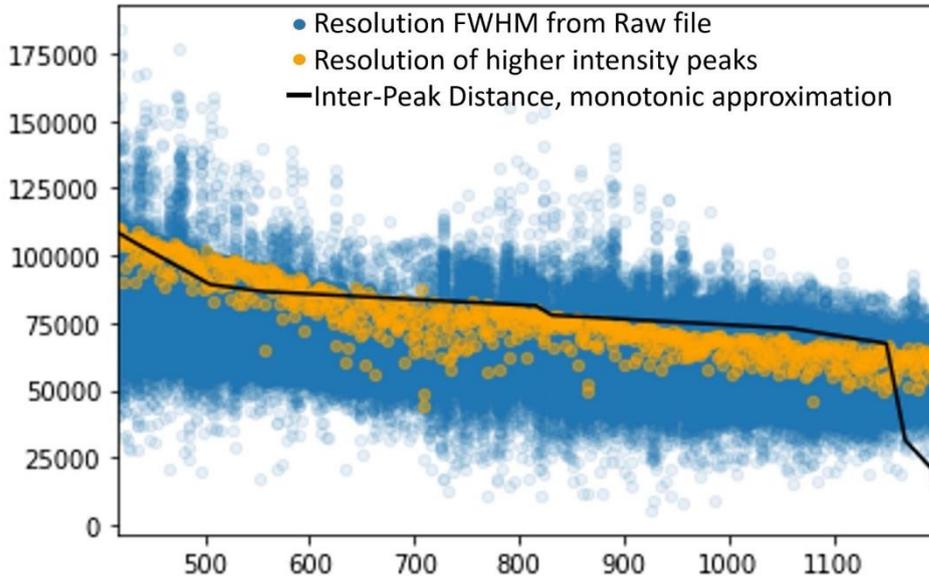
### **Co-authors:**

*Kai Schuhmann, Mpi-CBG**Henrik Thomas, Mpi-CBG*

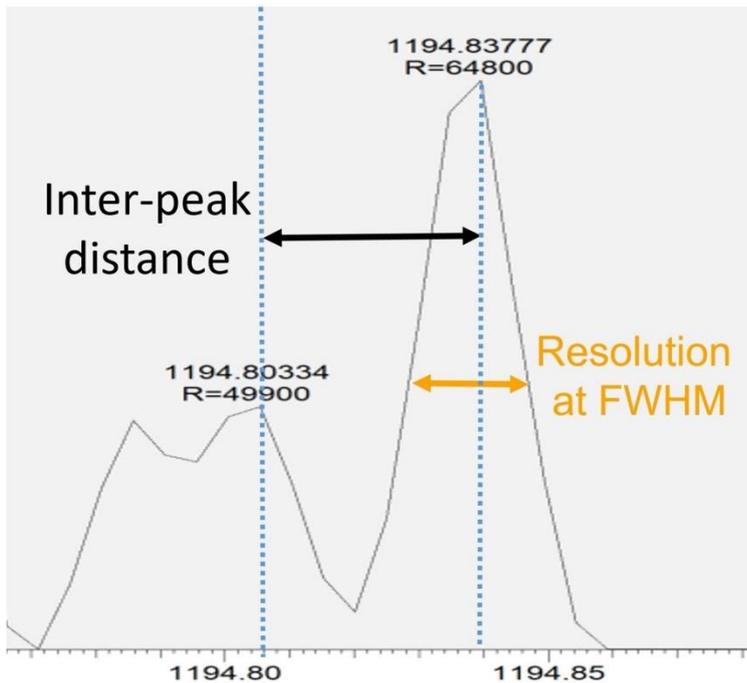
**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Nils Hoffmann, Centrum für Biotechnologie (CeBiTec), Universität Bielefeld  
Andrej Shevchenko, Mpi-CBG



Resolution from a single Thermo raw file and  $\Delta MR$  approximation



The distinction between FWHM resolution and inter-peak distance (IPD)

Poster number: **IM-PA-014**

## **VENDOR-NEUTRAL, BROWSER-BASED MS DATA PROCESSING**

Abstract ID: **281**

**Presenting author: Shahriar Jahanbakht, ACD/Labs**

### **Introduction**

Analytical data processing is moving to the Internet browser. Browser-based apps are easy to access, and many such apps are cloud-based and available from anywhere. In addition, cloud deployments provide computing power on-demand, cutting costs and time.

In response, several browser-based LC/MS and GC/MS processing tools have emerged. But until now, commercial tools have not been vendor-neutral.

With most scientific labs owning chromatography and MS instruments from multiple vendors, software complexity increases. Each vendor outputs data in its own format and provides its own processing tools. Scientists must learn and switch between multiple processing interfaces.

Here, we present a simplified software landscape: Spectrus JS, a *vendor-neutral* browser-based tool for processing MS data. Now, scientists can handle all their data in one interface.

### **Methods**

LC/MS and GC/MS data in multiple major instrument vendor formats was imported into Spectrus JS. Peaks were detected and integrated, and chemical structures were assigned. The processed data was saved, and an auto-generated report was created from the results.

### **Preliminary data (results)**

Spectrus JS successfully handled peak detection and integration. It also recorded all actions in an audit trail, allowing processing steps to be traced for data integrity. The software worked with major vendor formats and all major browsers (Chrome, Safari, Firefox, Edge, and Opera).

NMR, chromatography, and hyphenated chromatography-MS data could also be analyzed in Spectrus JS, further reducing software learning requirements for scientists who use NMR, chromatography, and MS.

Spectrus JS was designed for both cloud and on-premise deployment. Cloud deployments employ clustering technology to address scalability and load demand. This flexibility allows organizations to choose the model that fits their size, use patterns, and security requirements.

### **Please explain why your abstract is innovative for mass spectrometry?**

A vendor-neutral browser-based tool allows scientists to process any MS data from one interface, decreasing the data-handling barrier for MS labs.

### **Co-authors:**

*Anne Marie Smith, ACD/Labs*

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*Rostislav Pol, ACD/Labs*

Poster number: **IM-PA-015**

## **ANNOTINE: AUTOMATED WORKFLOW FOR MS1 ANNOTATION AND COMPARISON ACROSS IMAGING MASS SPECTROMETRY EXPERIMENTS.**

Abstract ID: **295**

**Presenting author: Lukasz Migas, Delft Center for Systems and Control, Delft University of Technology, Delft, The Netherlands**

### **Introduction**

Imaging Mass Spectrometry (IMS) has transitioned into a versatile analytical technique to spatially resolve the chemical composition of biological tissue samples. One major advantage is its untargeted nature, enabling IMS to concurrently detect and explore hundreds of molecular species throughout a tissue sample. Recent advances adding ion mobility (IM) as a separation dimension, further increase the number of detectable ion species or features per experiment. However, this also exacerbates the complexity of such data, increasing the need for directed (molecular species-aware) means of exploring and filtering these datasets. Here, we present a new workflow to automatically annotate ion peaks in IMS and IM-IMS measurements with their potential molecular species names, using criteria based on accurate mass, isotopic pattern fit, spatial/spectral correlation, and collision cross-sections.

### **Methods**

Annotine is developed in Python and supports (IM-)IMS data in imzML format. First, a public or user-curated local database is established as a source of annotation data. Second, features are detected in the mean or individual spectra of an IMS experiment. Third, these features are tentatively matched to entries in the database using accurate mass. All annotations are accompanied by image metrics, and summarized for human interpretation or further filtering. IMS data were acquired on a Bruker timsToF fleX at 10- $\mu$ m pixel size. Tissues were cryo-sectioned at 10  $\mu$ m, mounted on ITO-slides, desiccated, and coated with 1,5 DAN.

### **Preliminary data (results)**

Due to the ever-increasing dataset sizes for (IM-)IMS experiments, the ability to rapidly annotate observations and offer means for filtering down thousands of spectral features to more manageable levels becomes essential. Annotine fulfills this role by providing a scalable, parallelizable, and customizable MS1 annotation pipeline that delivers tentative species-database matches, accompanied by figures of merit based on mass accuracy, isotopic fit, and image/mobilogram correlation.

Classical identification of compounds in an IMS experiment is commonly performed post-IMS using on-tissue fragmentation or LC-MS, and can often be practically performed on only a limited subset of IMS-detected m/z-features. Annotine is complementary to such identification, aiming to provide tentative annotations using only MS1, but for all features in an IMS dataset.

*Annotine* is highly configurable, allowing public (e.g. LipidMaps) and custom-built databases, arbitrary chemical formulae (e.g. [M-A+X], [2M-NL+A] with A=adduct, NL=neutral loss, and X=mass offset), and matching isotopes (e.g. [M+0]), and adducts (e.g. [M+Na]+). In addition to annotation, Annotine enables comparative analysis across multiple datasets (e.g. across patients, tissue sections) or multiple tissue subsections (e.g. healthy vs diseased, tissue functional units). This comparative analysis is not limited to annotine's own annotations, but can also explore annotations provided by other platforms such as Metaspacer, by providing an interface to annotated datasets on the Metaspacer portal. Annotine is demonstrated on a case study of 13 human kidney tissues from the HuBMAP program (positive+negative mode, MS-only, lipid-focus) and publicly available data from Metaspacer.

### **Please explain why your abstract is innovative for mass spectrometry?**

A new automated annotation engine for IMS and IM-IMS datasets with built-in support for tentative lipid and metabolite annotation.

**Co-authors:**

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Elizabeth Neumann, Department of Biochemistry, Vanderbilt University, Mass Spectrometry Research Center, Vanderbilt University*

*Martin Dufresne, Department of Biochemistry, Vanderbilt University, Mass Spectrometry Research Center, Vanderbilt University*

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*Leonoor Tideman, Delft Center for Systems and Control, Delft University of Technology, Delft, The Netherlands*

*Angela Kruse, Department of Biochemistry, Vanderbilt University, Mass Spectrometry Research Center, Vanderbilt University*

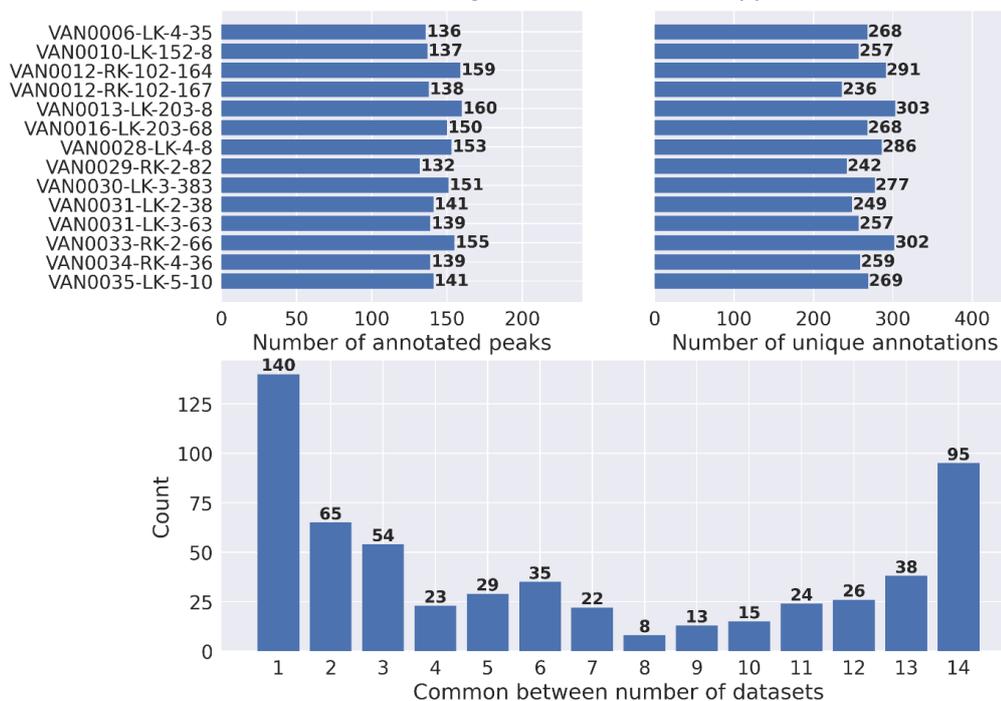
*Katerina Djambazova, Department of Biochemistry, Vanderbilt University, Mass Spectrometry Research Center, Vanderbilt University*

*Richard Caprioli, Department of Biochemistry, Vanderbilt University, Mass Spectrometry Research Center, Vanderbilt University, Department of Pharmacology, Vanderbilt University, Department of Medicine, Vanderbilt University, Department of Chemistry, Vanderbilt University*

*Jeffrey Spraggins, Department of Biochemistry, Vanderbilt University, Mass Spectrometry Research Center, Vanderbilt University, Department of Cell and Developmental Biology, Vanderbilt University*

*Raf Van de Plas, Delft Center for Systems and Control, Delft University of Technology, Delft, The Netherlands, Department of Biochemistry, Vanderbilt University, Mass Spectrometry Research Center, Vanderbilt University*

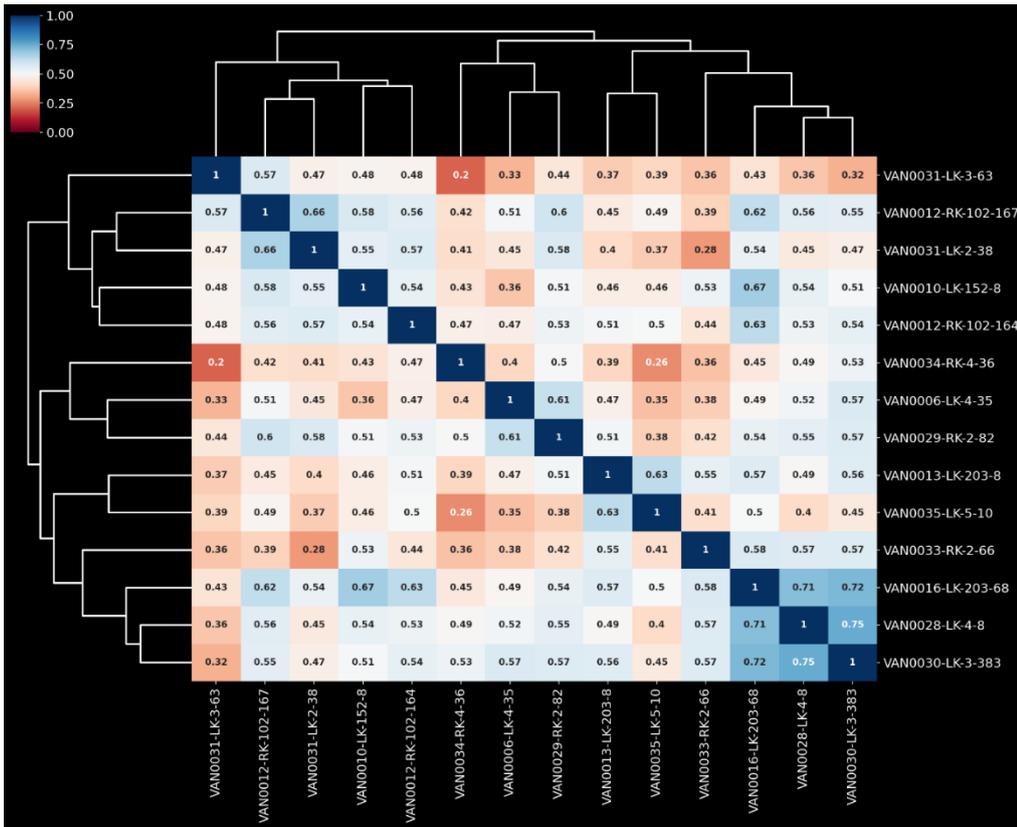
Annotating 14 datasets with  $\pm 3$  ppm limit.



Summary view of a multi-dataset annotation workflow.

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Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Hierarchical clustering of all annotations remaining after false-discovery rate filtering.

Poster number: **IM-PA-016**

## ACCURATE PREDICTION OF FRAGMENT INTENSITIES WITH N-DIMENSIONAL POLYNOMIAL REGRESSION FOR LIPID QUANTIFICATION

Abstract ID: **373**

**Presenting author: Kai Schuhmann, MPI-CBG - Max Planck Institute of Molecular Cell Biology and Genetics**

### Introduction

The cellular lipidome comprises a large number of structurally distinct lipid molecules resulting from the combinatorics of the fatty acid (FA) substituents. The characterization of these molecules is based on specific carboxylate anions (CA) generated from the precursor ions in Orbitrap HCD FT MS/MS. Furthermore, CAs intensities can be utilized for quantification if the analytes fragment similarly. We found however, significant differences especially for unsaturated analytes. To compensate for the fragmentation differences between the analytes and thus increase the accuracy of quantification of bottom-up lipidomics, we investigated a novel n-dimensional polynomial regression approach. Its special feature is that polynomial models can be derived using a limited number of fragmentation data, and yet still allows for a very accurate prediction of fragment intensities for lipids.

### Methods

Break down curves for synthetic PCO- and PC standards were acquired by HCD FT MS/MS with a normalized collision energy (NCE) of 10-70% on QExactive. Fragment intensity data were extracted generated \*.mzXML files, normalized to the total ion current at NCE 10-14% and averaged prior to n-dimensional polynomial regression. In the regression, the NCE and the chemical features of the analytes, fatty acid chain carbons, number of double bonds (DB) and their location, were used as function variables. The polynomial degree/model complexity was separately adjusted for each variable in the regression method

### Preliminary data (results)

In our previous work, we observed from the HCD FT MS/MS data that the fragment intensities of CA from lipids strongly depended on the selected NCE, CA chain length, number of DB, and their position. However, data integration was challenging because the correlation of these parameters was unclear.

To replace the existing empirical model with a mathematical model, we decided to integrate the available data using n-dimensional polynomial regression. Major advantages of this approach compared to neural networks commonly used today are that 1.) we obtain a unique polynomial function that reflects the correlations of the input variables and 2.) the function can represent the measured data with a very high accuracy, with deviations of less than 5%, from a small input data set.

The observed trends within the data were reproduced as expected. As shown in Figure 1 for PCO-, the fragment intensity of CA with 20 carbon atoms and 3 DBs decreases the closer the CA is located to its carboxyl group. To reproduce the function, it is important to have the data of the extrema of each analyte group to describe it as complete as possible. In addition to allow a lipid class to be described with sufficient data, the presence of a function allows the transfer of recognized relationships to other lipid classes with less available data, which is important to overcome the limited availability of standards for lipidome analysis.

### Please explain why your abstract is innovative for mass spectrometry?

Fragment intensities in MS/MS spectra were integrated for the first time by n-dimensional polynomial regression. Compared to conventional neural networks, the polynomial models is more robust, accurate and transferable.

### Co-authors:

*Sachin Krishnan Thekke Veetil, CASUS - Center for Advanced Systems Understanding, HZDR - Helmholtz-Zentrum Dresden-Rossendorf e.V.*

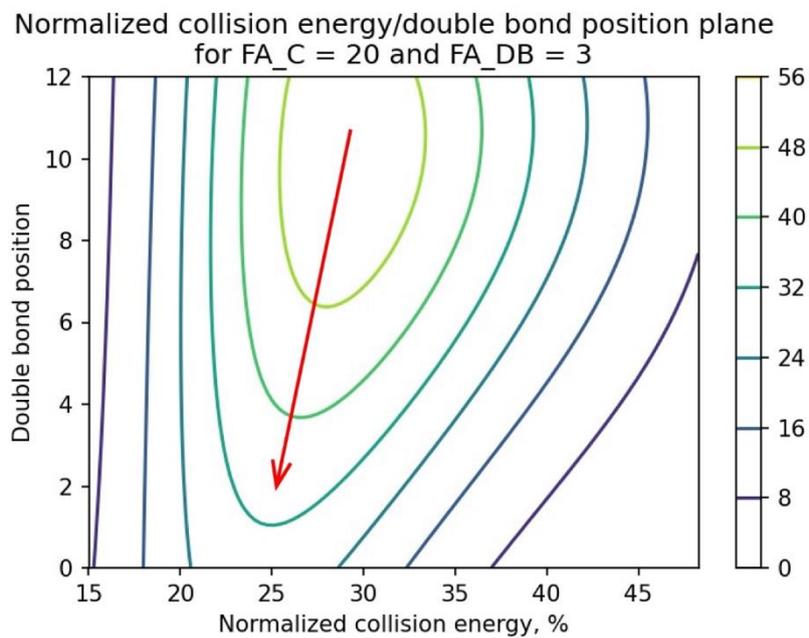
*Michael Hecht, CASUS - Center for Advanced Systems Understanding, HZDR - Helmholtz-Zentrum Dresden-Rossendorf*

**POSTER SESSION A**

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Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

e.V.

Andrej Shevchenko, MPI-CBG - Max Planck Institute of Molecular Cell Biology and Genetics



Fragment intensities calculated from the specified polynomial for PCO- isomers.

Poster number: **IM-PA-017**

## **PEAKONLY: A PRECISE ALGORITHM FOR DETECTING PEAKS IN LC-MS DATA USING NEURAL NETWORKS**

Abstract ID: **408**

**Presenting author: Vadim Yanshole, Laboratory of Proteomics and Metabolomics, International Tomography Center SB RAS**

### **Introduction**

We report the development of a novel method based on modern machine learning technology for the detection of peaks (features) in raw high-resolution liquid chromatography – mass spectrometry (LC-MS) data. Despite the wide range of LC-MS-based applications and recent developments in analytical instrumentation, LC-MS data processing still faces a number of challenges. One of the most critical bottlenecks is the processing of raw data. Mainstream applications for the automated analysis of untargeted metabolomics data (eg. *XCMS*, *MZmine2*) often tend to produce many false positive signals or to incorrectly integrate peaks. The amount of false positives can reach 20-50% of the total peak amount, what can lead to false scientific discoveries or complicate the interpretation of real ones.

### **Methods**

The improved peak detection can be achieved by the application of deep learning, particularly of convolutional neural networks (CNNs) that are widely used in computer vision tasks such as image classification and object recognition. We developed an open source algorithm *peakonly*. The code is written in *Python*. Neural networks were constructed and trained using *PyTorch* framework.

### **Preliminary data (results)**

*Peakonly* implements the following steps (Figure 1): (1) detection of regions of interest (ROIs) in raw LC-MS data, by slightly modified *centWave* algorithm; (2) classification of ROIs by a first CNN into two classes (peaks, noise); (3) segmentation of ROI by a second CNN to find borders of peaks, and their subsequent integration. The CNNs were trained on manually annotated dataset of more than 6000 ROIs from real LC- and GC-MS data with the admixture of synthetic data. The developed approach has shown to process data more reliably and more efficiently than existing programs, achieving the highest possible precision of 97%, and shown to work with many different LC-MS and GC-MS data.

*Peakonly* is available with graphical user interface, and is able to process multiple files (batch), including peak matching among different files and automated zero fillings. The *Python* source code can be downloaded from GitHub (<https://github.com/arseha/peakonly>)

*Acknowledgements:* The algorithm development was funded by Russian Science Foundation, Project 22-24-00390.

### **Please explain why your abstract is innovative for mass spectrometry?**

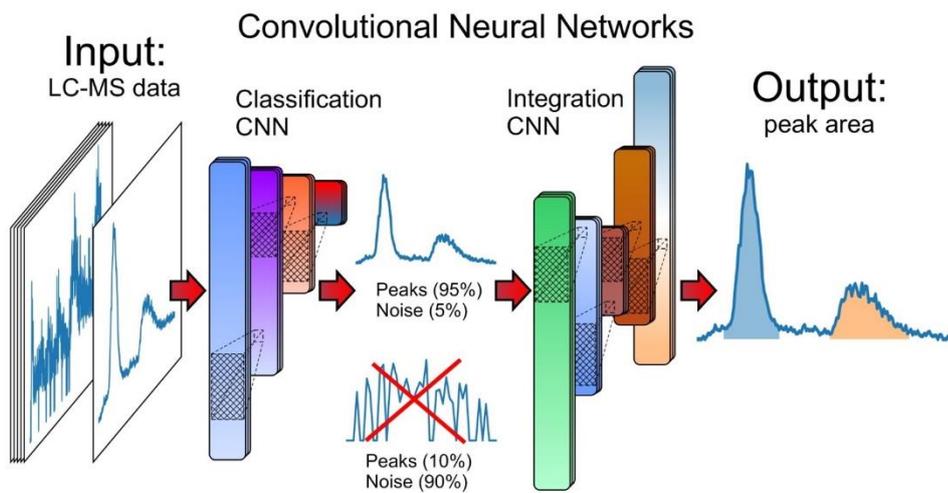
An algorithm based on convolutional neural networks is implemented to detect peaks in raw high-resolution LC-MS and GC-MS data with high precision

### **Co-authors:**

*Arsenty Melnikov, Laboratory of Proteomics and Metabolomics, International Tomography Center SB RAS*

**POSTER SESSION A**

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Tuesday 30 August 2022 from 14:00 to 15:30 hours



Graphical representation of *peakonly* algorithm operation

Poster number: **IM-PA-018**

## **INCREMENTAL PRINCIPAL COMPONENT ANALYSIS WITH LOW MEMORY USAGE FOR LARGE MASS SPECTROMETRY IMAGING DATASET ANALYSIS**

Abstract ID: **500**

**Presenting author: Kasper Krijnen, M4I**

### **Introduction**

Development of higher spatial resolution, mass resolution, and throughput Mass Spectrometry Imaging (MSI) instruments causes increasingly large data. Conventional analysis methods used in MSI, such as principle component analysis (PCA), usually rely on random-access memory for data processing. However, the ability of modern MSI instruments to collect data far exceeds the current capabilities of storing all data sets in memory. Solutions are usually either (A) downsampling-based or (B) "incremental PCA"-based [1], which utilizes less memory with increased runtime. We present an incremental PCA [2] implementation that produces PCA analyses of very large datasets in comparable runtimes to conventional PCA. Further, we work to apply our implementation on both microscope mode MSI as well as microprobe-mode MSI data and compare our results with alternative methods.

### **Methods**

Our incremental PCA implementation was written in the programming language Rust and is based on an algorithm in the scikit-learn Python library [2]. Benchmarking datasets include the public MSI dataset [3] obtained from the PRIDE database, images collected with a prototype Bruker Daltonics (Bremen, DE) timsTOF flex, and images collected with a microscope-mode MSI instrument equipped with an Amsterdam Scientific Instruments TPX3CAM detector (Amsterdam, NL). Benchmarks were performed on a workstation class desktop PC with 64 GB of 2933 MHz RAM and a Intel i7-10700k processor with 16 cores running at 3.800GHz base speed, 5.00 GHz boost speed.

### **Preliminary data (results)**

Our implementation produced the same results principal components as conventional PCA methods when applied to the "Iris" dataset. Similarly, our method produced results that were highly similar to those generated by SCiLS-lab (Bremen, DE) with the exception of slightly different orderings of principal components (Figure 1). Preliminary and unverified benchmarks of the runtimes of PCA of the publically-available MSI dataset were comparable between our method (1.2 s), SCiLS-lab (2.0 s), Lipostar-MSI (8.0 s). A small, sparsely-sampled dataset of 3.3 GB and 2.1 million pixels and 3041 m/z bins per spectrum required approximately 2.6 hours to complete with our implementation and would not be feasible with other, non-incremental approaches due to memory limitations. A larger dataset of 1.2 billion pixels (but far fewer relevant m/z bins) has been collected on the microscope-mode MSI instrument but has not yet been analysed with our method.

### **References**

1. Race, A.M., et al., *Memory efficient principal component analysis for the dimensionality reduction of large mass spectrometry imaging data sets*. *Anal Chem*, 2013. **85**(6): p. 3071-8.
2. Pedregosa, F., et al., *Scikit-learn: Machine Learning in Python*. *J. Mach. Learn. Res.*, 2011. **12**: p. 2825–2830.
3. Rompp, A., et al., *Histology by mass spectrometry: label-free tissue characterization obtained from high-accuracy bioanalytical imaging*. *Angew Chem Int Ed Engl*, 2010. **49**(22): p. 3834-8.

### **Please explain why your abstract is innovative for mass spectrometry?**

Analysis of the large dataset of 1.2 billion pixels that has been collected on the microscope-mode MSI instrument is not possible with current PCA implementations for MSI.

**Co-authors:**

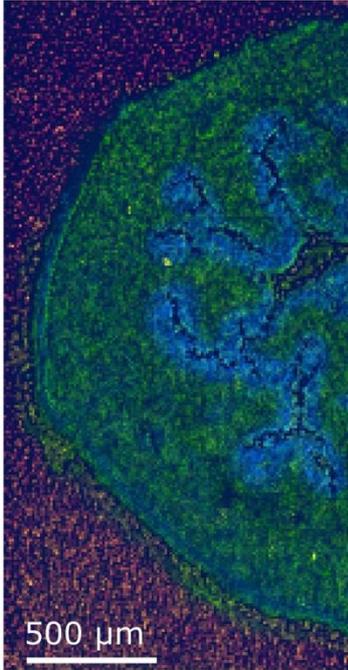
**POSTER SESSION A**

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Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

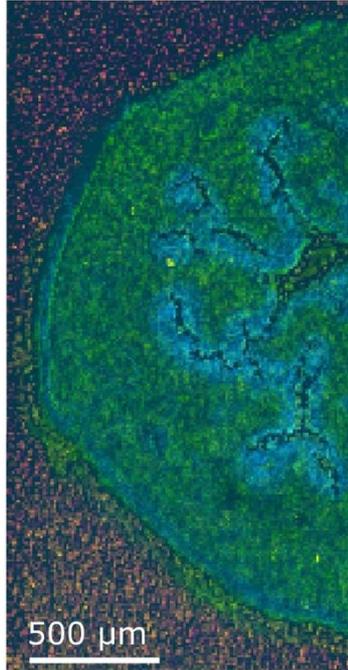
*Ian Anthony, M4I*  
*Ron Heeren, M4I*

## Hyperspectral Visualization PCA

Our Implementation



SCiLS Lab



Hyperspectral visualization of the different PCA results.

Poster number: **IM-PA-019**

## **A UNIFYING, SPECTRUM-CENTRIC APPROACH FOR THE ANALYSIS OF PEPTIDE TANDEM MASS SPECTRA**

Abstract ID: **534**

**Presenting author: Martin Frejno, MSAID GmbH**

### **Introduction**

Mass spectrometry-based proteomics data is acquired using data dependent (DDA), data independent (DIA) or targeted acquisition (PRM) methods. Typically, the former is analyzed using spectrum-centric algorithms assuming that it generates non-chimeric spectra, while the latter two are analyzed in a peptide-centric fashion. However, peptide-centric approaches often struggle to control for the contribution of multiple peptides to the experimental fragment ion intensity. Recently, we showed that DDA, and by extension PRM, spectra can be substantially chimeric and introduced an approach that deconvolutes spectra irrespective of isolation window size, thereby substantially boosting the number of identified peptides for DDA. Here, we demonstrate that the same approach generalizes to any chimeric MS2 spectrum, unifying the analysis of DDA, DIA and PRM data.

### **Methods**

Our acquisition-type agnostic, library-free and spectrum-centric algorithm compares predicted and experimental MS2 spectra using various intensity-based scores. During the main search, MS2 spectra are analyzed without pre-processing and all promising peptides in each MS2 isolation window are considered simultaneously and compete for experimental fragment ion intensity in one concerted step. Our algorithm aims at explaining as much experimental intensity with as few candidate peptides as possible and distributes the intensity of shared fragment ions to peptide spectrum matches given their estimated proportional contribution to the experimental MS2 spectrum, resulting in the deconvolution of chimeric spectra. FDR-control is performed using Percolator.

### **Preliminary data (results)**

First, we evaluated this approach on DDA data using a 1h HeLa cell lysate with a 1.3 Th isolation window, resulting in an identification rate of 80%, yielding a total of 114k PSMs, 61k peptides and 7.3k protein groups at 1% FDR. When we applied our algorithm to DDA data acquired with isolation windows from 0.4 Th to 8 Th, we observed a linear increase in the number of identified PSMs, which saturated at ~150k PSMs for an isolation window of ~6 Th, thus demonstrating its applicability to highly chimeric MS2 spectra.

Conceptually, a DDA MS2 spectrum is indistinguishable from a DIA or a PRM spectrum acquired with the same isolation window. Hence, we transferred our concept to both DIA and PRM data. For DIA, we analyzed a 1h HeLa cell lysate acquired with 14 Th isolation windows. Here, the algorithm yielded a total of 136k PSMs and 45k peptide groups at 1% FDR. These results are comparable with results obtained using DIA-NN in library-free mode. For PRM, we analyzed a 1h measurement of the UPS protein standard spiked into a HeLa cell lysate and achieved comparable quantitative accuracy as Skyline without the need for a spectral library.

Our spectrum-centric algorithm allows researchers to judge the quality of single PSMs and enables the visualization of deconvoluted chimeric spectra through a mirror plot. In addition, it permits the use of classical, validated FDR-estimation tools like Percolator, while being able to analyze data from all different acquisition methods.

### **Please explain why your abstract is innovative for mass spectrometry?**

A unifying, spectrum-centric approach for analyzing DDA, DIA and PRM data, fueled by the deep learning-based deconvolution of chimeric spectra.

### **Co-authors:**

*Daniel Zolg, MSAID GmbH*

*Tobias Schmidt, MSAID GmbH*

*Siegfried Gessulat, MSAID GmbH*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Michael Graber, MSAID GmbH*  
*Florian Seefried, MSAID GmbH*  
*Magnus Rathke-Kuhnert, MSAID GmbH*  
*Samia Ben Fredj, MSAID GmbH*  
*Patroklos Samaras, MSAID GmbH*  
*Bernhard Kuster, Technical University of Munich*  
*Mathias Wilhelm, Technical University of Munich*

Poster number: **IM-PA-020**

## AN END-TO-END MACHINE LEARNING WORKFLOW FOR MS-BASED PROTEOMICS

Abstract ID: **536****Presenting author: Siegfried Gessulat, MSAID GmbH**

### Introduction

In proteomics, machine learning augments various steps in the data analysis, from predicting peptide properties that serve as priors for experimental data, to training target/decoy classifiers for error estimation. How a model is integrated into production systems determines its requirements. However, generating, evaluating and integrating such models remains manual labor. Here we present a workflow that automates all steps from raw data to production-ready model. First, it imports identified spectra and transforms them to training datasets for various peptide properties. Second, a set of model architectures are trained, evaluated, and their hyperparameters optimized. Hyperparameter optimization automatically balances conflicting requirements such as speed and accuracy and is use-case specific. Third, the models are optimized and exported for different deployment scenarios.

### Methods

The importer scrapes PRIDE repositories by identifier, downloads raw data and search results, and stores their inferred relations in a data lake on Amazon Web Services (AWS). Data transformation jobs are executed on AWS Batch. The workflow implements several deep neural network architectures, while hyperparameters, layer sizes and their depth are abstractly defined. The optimization of model architecture and associated hyperparameters is automated via hyperband optimization on a GPU-cluster. Use-case-specific performance metrics are tracked and evaluated during and after training on holdout datasets. Final models are exported for different compute architectures, operating systems and with bindings to different programming languages.

### Preliminary data (results)

Within one week, we ingested >13,000 RAW files (>7 TB of data) using this workflow. The import step filters, compresses, and uploads ~500 RAW files per hour to AWS – faster than they are published on PRIDE. Through filtering and compression, the data is reduced to 336GB, resulting in storage costs of under 8\$ per month on AWS S3. The data transformations are specific for four different use cases including dataset-specific retention time refinement learning and collision energy-calibrated fragment ion intensity prediction. The computationally most expensive transformation job annotates  $\gamma$ -,  $b$ -, immonium, parent and the most frequent combinations of neutral loss ions in peptide tandem mass spectra. This transformation takes <5 hours for ~120M spectra in our data lake. The model architecture and hyperparameter optimization automatically generated and evaluated >2,000 distinct models within the last year. It identified a new fragment ion intensity model that combines capabilities to predict HCD, CID, label-free, and TMT-modified peptides in a single model. It matches Prosit – a publicly available neural network – in accuracy with a 4-fold reduction in memory footprint and a 3-fold increase in speed. In addition, the workflow identified a retention time model that has 65 times fewer parameters than Prosit while showing better accuracy. These models are optimized for NVIDIA GPUs, as well as Intel, AMD, and ARM CPUs. They can run on Linux or Windows. The system offers Python, Cpp, and GRPC bindings for these models to facilitate easy integration independent of the deployment scenario.

### Please explain why your abstract is innovative for mass spectrometry?

An end-to-end machine learning workflow that manages and transforms data, trains and evaluates models, and exports them to different environments.

### Co-authors:

*Tobias Schmidt, MSAID GmbH**Michael Graber, MSAID GmbH**Samia Ben Fredj, MSAID GmbH**Lizi Mamisashvili, MSAID GmbH**Patroklos Samaras, MSAID GmbH**Florian Seefried, MSAID GmbH**Magnus Rathke-Kuhnert, MSAID GmbH**Daniel Zolg, MSAID GmbH**Martin Frejno, MSAID GmbH*

Poster number: **IM-PA-021**

## **PREDICTION OF SCHEDULE AND GAS CHROMATOGRAPHIC RETENTION INDICES FOR GC/MS IDENTIFICATION OF CHEMICALS RELATED TO CHEMICAL WEAPONS CONVENTION BY MATCHED MOLECULAR PAIRS AND MACHINE LEARNING METHODS**

Abstract ID: **548**

**Presenting author: Albert Kireev, Skolkovo Institute of Science and Technology**

### **Introduction**

The involvement of retention indices (RI) together with mass spectra improves the accuracy of the GC/MS identification for factual findings during on-site verification activities with sampling and analysis conducted by technical Secretariat of Organization for the Prohibition of Chemical Weapons (OPCW). The problem of the OPCW database is that MS and RI data are limited. After addition in 2020 of four more entries to Schedules of Chemical Weapon Convention, including groups of so called "Novichok" toxic chemicals, the problem became even more challenging. For toxic chemicals, the data required to build models is limited, expensive, and resource-intensive, that's why it is important to have models to predict Schedule and RI for such chemicals and their precursors and degradation products.

### **Methods**

Data of OPCW central analytical database for Schedule 1A1, 1A2 and 1A3 is arranged by specific scaffold. Classification algorithms are used for Schedule prediction based on electron ionization mass-spectra. Scaffold analysis is combined with the matched molecular pair (MMP) formalism. Chemicals with pairs of R1O and R2P groups constitute MMPs. RI differences for MMPs are calculated and used for prediction. Classification and RI predictions are compared for methods such as deep learning, gradient boosting, and simple linear regressions. Homologous tree approach is used to automate the evaluation of scaffolds and MMPs from group of degradation products of scheduled chemicals.

### **Preliminary data (results)**

Applying different machine learning methods allowed to achieve classification accuracy up to 95% depending of sets of compounds. Calculation of RI differences in molecular pairs matched by R1O or R2P groups makes available RI prediction for the absent data by using additive scheme. Exhaustive leave-one-out cross-validation is performed to estimate accuracy of a predictive MMP model. Achieved accuracy: mean absolute and percentage errors 2 units and 0.001%. RI differences for Schedule 1A2, 1A3 and selected 2B4 chemicals are calculated for further utilization in the practice of analytical laboratories by using GC/MS.

MMP approach was compared with gradient boosting and simple linear regressions that were trained on molecular descriptors of scheduled chemicals as well as with novel deep learning-based predictions such as 1D-CNN and 2D-CNN that were trained on NIST-17 database. In case of Schedule 1A1, traditional machine learning methods and both neural networks exhibit similar performance, worse than for MMP. Achieved accuracy: mean absolute and percentage errors 20-27 units and 1.6-2.1%. The power of machine learning methods was used to predict RI for 1A13-16 toxic chemicals due to MMP method limitations, when possibility to calculate RI differences is absent. Instead of human knowledge of chemistry the fragmentation tree algorithm was used to find homologues tree, which became base for further scaffolds and matched molecular pairs analysis in group of bis(dialkylamino)sulfides and their homologues.

### **Please explain why your abstract is innovative for mass spectrometry?**

Improvement of GC/MS identification by Schedule and retention indices prediction with combination of matched molecular pairs for narrow classes, machine learning methods for wide classes, and homologues trees extraction.

### **Co-authors:**

*Sergey Osipenko, Skolkovo Institute of Science and Technology*

*Gary Mallard, Teal Consulting*

*Yury Kostukevich, Skolkovo Institute of Science and Technology*

*Evgeny Nikolaev, Skolkovo Institute of Science and Technology*

Poster number: **IM-PA-022**

## **ADDING A NEW DIMENSION TO SPATIAL OMICS: PYXIS, COMPREHENSIVE SOFTWARE FOR ION MOBILITY MS IMAGING DATA ANALYSIS**

Abstract ID: **564**

**Presenting author: Sara Tortorella, Molecular Horizon srl**

### **Introduction**

Mass spectrometry imaging (MSI) is a powerful analytical technique suited for simultaneously measuring and assigning functional role of multiple analytes directly from intact tissue sections. A recent technological advancement, trapped ion mobility spectrometry (TIMS) combined with MSI (IMS-MSI), holds the promise to provide a critical need to the imaging community by providing an orthogonal separation technique thus supplementing the lack of chromatographic separation. However, the rich, multidimensional data produced by IMS-MSI, combined with the lack of comprehensive software solutions that cover the entire workflow needed for a complete data analysis, poses a major challenge preventing its full exploitation. Here we introduce Pyxis: the first vendor-neutral software for comprehensive targeted and untargeted IMS-MSI data analysis, from raw data import to spectra and image visualization and identification.

### **Methods**

To evaluate the spectra and image analysis, and automated identification capabilities of Pyxis, data was acquired from mouse kidney tissue. The kidney from a healthy mouse was embedded in carboxymethylcellulose, snap-frozen and sectioned to about 16  $\mu\text{m}$  thickness. Sections were sublimation-coated with 2,5-DHAP matrix. TIMS-MALDI-2-MSI measurements were conducted on a timsTOF fleX MALDI-2 instrument (Bruker, DE) with pixel sizes of 20  $\mu\text{m}$  and a ramp time of 100 ms.  $1/k_0$  was measured from 0.8 to 1.8 and the  $m/z$  range was from 300 to 1500. All data analysis and identification was performed using Pyxis.

### **Preliminary data (results)**

MALDI-MSI especially with MALDI-2 post ionization often leads to highly complex datasets with numerous isobaric species. TIMS separation crucially helps to unravel these datasets by providing an orthogonal separation technique. By performing peak picking in 3D ( $m/z$ ,  $1/K_0$  and intensity) and browsing the obtained mobilogram we were able to differentiate  $[\text{PE}(38:4)+\text{Na}]^+$  and  $[\text{PE}(40:7)+\text{H}]^+$  something that is otherwise only possible with an extremely high mass resolution, often leading to greatly reduced scan rates compared to the employed method (Figure 1). These two lipid species showed vastly different distributions inside the tissue (Figure 2). The ion mobility separation also provides an additional parameter for identification in terms of the CCS value, leading to an unambiguously identification of several lipids. These advantages provide a more complete insight into the metabolic hallmarks of potentially investigated disease states and are thus highly beneficial for the research community. For this Pyxis provided a powerful platform for comprehensive IMS-MSI data analysis, greatly streamlining biochemical data interpretation. Its use will pave the way to establish a data-driven histology exploitation of high resolution IMS-MSI *omics* and to achieve unprecedented functional interpretation.

### **Please explain why your abstract is innovative for mass spectrometry?**

Pyxis is the first integrated software to-date capable of supporting comprehensive IMS-MSI data analysis and analytes identification for lipidomics and metabolomics applications.

### **Co-authors:**

*Giuseppe Arturi, Molecular Discovery Ltd, Pinner, Middlesex*

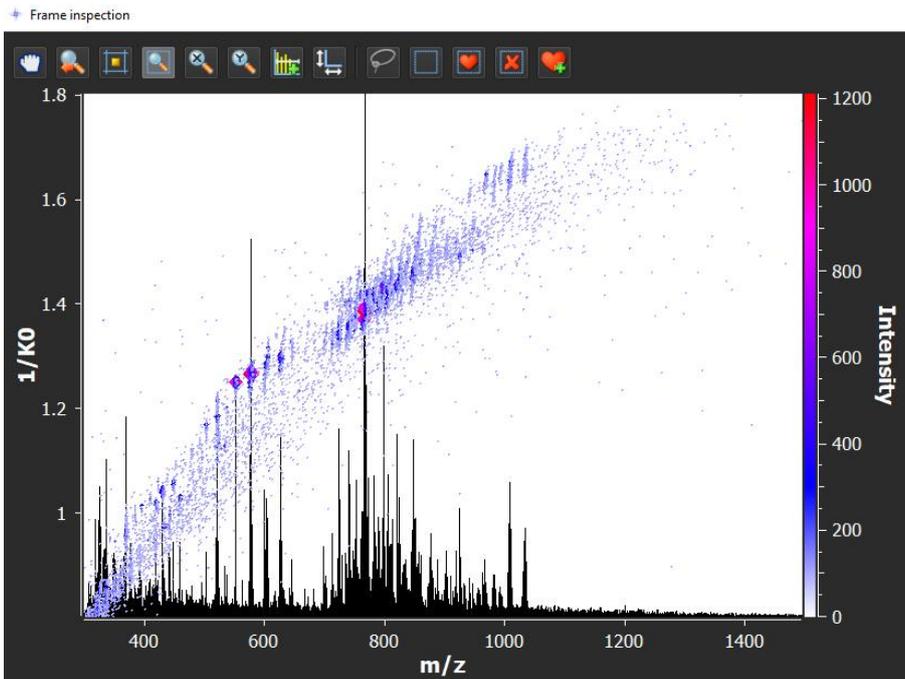
*Giulia Sorbi, Molecular Horizon srl*

*Sebastian Bessler, Institute of Hygiene, University of Münster*

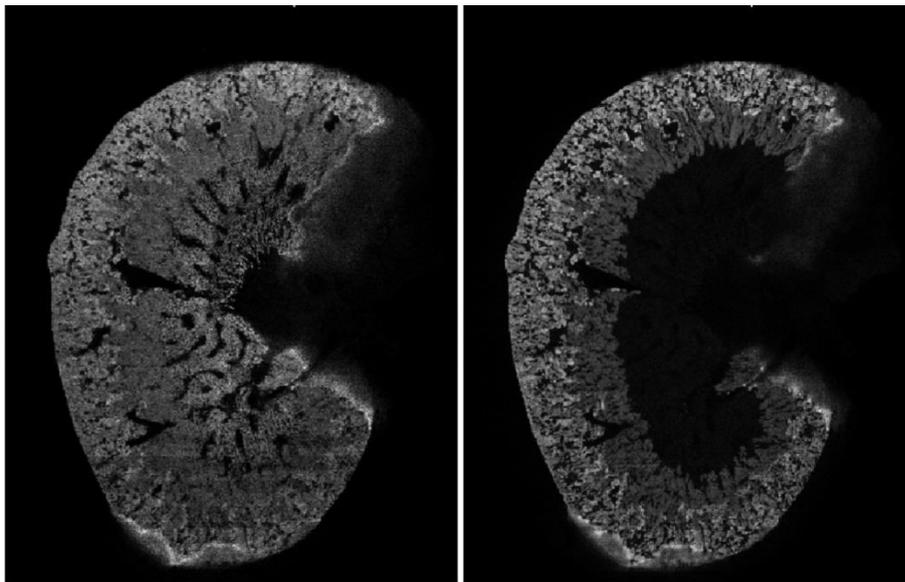
*Gabriele Cruciani, Department of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8, 06123*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



m/z against 1/K<sub>0</sub> plot in Pyxis



500 μm [PE(38:4)+Na]<sup>+</sup>  
m/z=790.53 1/k0=1.42

[PE(40:7)+H]<sup>+</sup>  
m/z=790.53 1/k0=1.38

Isobars clearly separated by TIMS-MALDI-2-MSI data interpretation

Poster number: **IM-PA-023**

## **FIDELITYCHECK™ SOFTWARE FOR IDENTIFICATION OF AMINO ACID VARIANTS IN RECOMBINANT AND PURIFIED PROTEINS**

Abstract ID: **577**

**Presenting author: Tyler Fletcher, GenNext Technologies**

### **Introduction**

Natural and recombinant proteins are heterogeneous mixtures that contain varying levels of protein variants. These variants arise from post-translational modifications or misincorporation of amino acids during protein biosynthesis. Protein variants impact the quality of biotherapeutics by altering the function, activity, folding, therapeutic efficacy, and serum half-life. Peptide variants are often missed by LC-MS analysis, for several reasons, which include low abundance of the variant prohibiting selection for MS/MS analysis, and the variant fragmenting in a manner making it difficult to identify. Quantification of these variants often necessitates a second experiment. Here, we describe a new software tool, which incorporates LC retention prediction to complement MS data, for the identification and quantification of peptide variants that are missed by current software.

### **Methods**

Recombinant human therapeutic IgGs and several purified serum albumins were processed using standard bottom-up proteomic workflows. Samples were analyzed via HILIC-MS with a QToF operating in a data-dependent acquisition mode. The FidelityCheck™ software first identifies native peptides from MS/MS data and assigns retention times to the peptides. These sequences and retention times are used to generate a retention time model that predicts retention time windows in which variant peptides are most likely to elute. Isotopic XIC alignment is calculated for native peptides not initially identified and predicted variant peptides. Peptides with sufficient userdefined alignment are reported.

### **Preliminary data (results)**

We have created a data analysis scheme (FidelityCheck™ Software) to identify and quantify protein variants. In this workflow, a protein is proteolytically digested, followed by LC-MS/MS analysis using hydrophilic interaction chromatography. The retention times of the expected peptides identified in the experiment are used to generate a retention model which is used to predict the retention of possible variant peptides. Peptides expected from each protein that were identified were used to generate a retention model predicting the elution time of every possible peptide variant. The LC-MS data is then investigated to see if LC peaks with the mass and LC retention time for each variant are found. If a peak is observed that may result from a variant peptide, the experimental isotope distribution is compared to that predicted from the sequence. For peaks meeting these criteria, the ratio of the areas for the extracted ion traces abundance for the variant to expected peptide is calculated. A report is generated for these probable peptide variants. We have evaluated this software with the analysis of adalimumab and several serum albumins. These experiments identified the sites and level of the following modifications: methionine oxidation, asparagine deamidation, and aspartic acid isomerization. Additionally, several hypothetical peptide variants due to G-U base pairing were identified. We will extend this work to other therapeutic proteins. We will also perform experiments where the extent of modification is altered in a predictable manner to test the ability of FidelityCheck™ Software to quantify peptide variants.

### **Please explain why your abstract is innovative for mass spectrometry?**

Only software to utilize both chromatographic and spectrometric data for identification of amino acid variants of recombinant proteins.

### **Co-authors:**

*Emily Chea, GenNext Technologies*

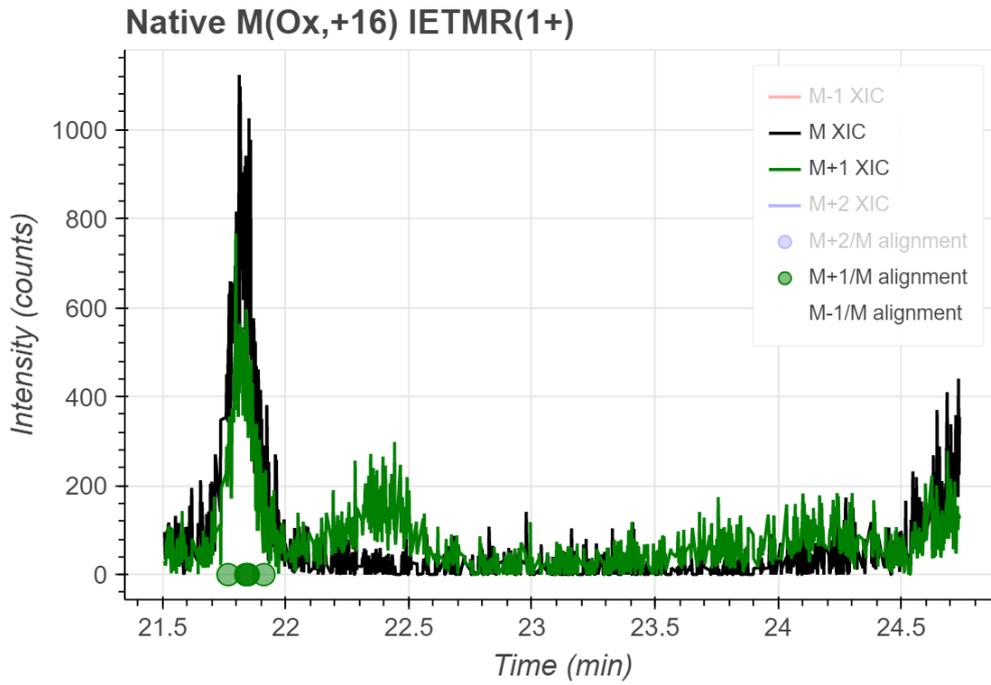
*Robert Egan, GenNext Technologies*

*Ron Orlando, GenNext Technologies, University of Georgia*

*Scot Weinberger, GenNext Technologies*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Isotopic XICs of variant peptide identified by FidelityCheck™ Software.

Poster number: **IM-PA-024**

## **SHAPLEY VALUE AS A QUALITY CONTROL FOR MASS-SPECTROMETRY DATA**

Abstract ID: **652**

**Presenting author: Denis Zavorotnyuk, The Moscow Institute of Physics and Technology, 9 Institutskiy per., Dolgoprudny, Moscow Region, Russian Federation, 141701**

### **Introduction**

Energy metabolism alteration is the well-known hallmark of cancer that leads to substantial changes in cell lipid composition. The notable change in the lipid composition of cancer tissues compared to healthy ones is of interest enabling the possibility of discriminating pathological and healthy tissues in a clinic, which is especially important in neuro-oncology. The accuracy of tumor border determination is crucial, as excessive resection of healthy brain tissue is unacceptable. Mass spectrometry identification of tumor tissues based on their lipid composition is an emerging technique among the variety of navigation techniques in neurosurgery. Mass spectrometry with ambient ionization has a number of particulars which can lead to even adjacent scans differ substantially and can affect experimental data treatment and decrease the accuracy of data classification.

### **Methods**

Human tumor brain tissues were used in this work. Analysis was conducted by means of ICE-MS. Data analysis was performed with in-laboratory created software.

### **Preliminary data (results)**

A number of techniques, for example, MALDIquant R-package and Bioconductor R-packages library, MZmine 2 and SeeMS can help to conduct an analysis manually. But these methods are not applicable for automatic data treatment. We apply ShapleyData analysis for mass spectrometry scan quality assessment. Algorithm ShapleyData is designed to obtain player contribution into overall team profit. In our case we compute the scan contribution in the classification problem. The ShapleyValue were calculated for a chosen number of scans of mass spectrometry profiles obtained from human brain tissues of patients with diagnosis glioblastoma and non-tumor pathology. Calculated Shapley-value were used for regression model construction to obtain Shapley-value for the rest part of scans.

### **Please explain why your abstract is innovative for mass spectrometry?**

The obtained results suggest the ShapleyValue can be used as a quality metric in the automatic process of data treatment which may lead to decreasing the time data analysis consumes.

### **Co-authors:**

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*Eugene Nikolaev, Skolkovo Institute of Science and Technology, Bolshoy Boulevard 30, bld. 1, Moscow, Russia, 121205*

*Igor A. Popov, The Moscow Institute of Physics and Technology, 9 Institutskiy per., Dolgoprudny, Moscow Region, Russian Federation, 141701*

Poster number: **IM-PA-025**

## **PIPELINES AND SYSTEMS FOR THRESHOLD AVOIDING QUANTIFICATION OF LC-MS/MS DATA (PASTAQ)**

Abstract ID: **725**

**Presenting author: Horvatovich Peter, University of Groningen**

### **Introduction**

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) remains one of the most popular tools for measuring organic compounds, with applications in proteomics and metabolomics. Lower intensity signals may contain relevant biological information and should not be discarded. Furthermore, the majority of existing tools rely on MS/MS identification for quantitative or pre-processing purposes, which due to the stochasticity of the precursor ion selection in data-dependent acquisition (DDA) methods leads to the quantification of a fraction of the existing biological compounds in the data. In this work, we present a single-stage LC-MS(/MS) pre-processing pipeline, which extracts all compound relevant signals irrespective of identification annotation or abundances.

### **Methods**

The Pipelines And Systems for Threshold Avoiding Quantification (PASTAQ) offers a toolset for accurate quantification and pre-processing of data-dependent acquisition (DDA) or MS1 only LC-MS(/MS) datasets. The pre-processing algorithms in PASTAQ operate on single-stage (MS1) data, with support for annotations and identifications linkage in the standard mzIdentML format such as obtained from MS-Fragger or SearchGUI/PeptideShaker.

PASTAQ is written in high-performance C++ code and offers Python bindings for the creation of tailored pipelines or integration with an existing ecosystem of mass spectrometry tools. The code is freely available under a permissive open-source license (MIT) on GitHub: <https://github.com/PASTAQ-MS/PASTAQ>.

### **Preliminary data (results)**

PASTAQ algorithms can be used in isolation or via the default DDA pipeline. This pipeline is simple to parametrize, requiring only to adjust the expected width of chromatographic peaks, type of mass spectrometry instrument used as well and its resolution at the reference m/z. PASTAQ performs isotopic peak detection, feature detection, retention time alignment and automatically generates a number of quality control figures that can be used to assess the quality of the data and preprocessing parametrization.

We showed the performance of PASTAQ with three different datasets: A proteome mixture of HeLa, yeast, and Escherichia coli (E. coli) mixed at three different ratios, a HeLa matrix spiked in with an artificial concatemer at increasing amount covering three orders of magnitude of concentration range, and a publicly available human serum dataset. We used the first two datasets to demonstrate the quantitative performance of PASTAQ compared to the state-of-the-art MaxQuant software, showing a reduction in the coefficient of variation (CV) of technical replicates and an increase in the number of quantified low intensity peptides. The biological dataset was used to detect gender related peptides and proteins by means of principal component analysis (PCA) and univariate hypothesis testing.

PASTAQ identification annotation currently is limited to proteomics application, however the MS1 capability of PASTAQ allow to process untargeted metabolomics, lipidomics, or mixed omics samples and can be used to pre-process stable-isotope labeled proteomics and metabolomics LC-MS(/MS) data.

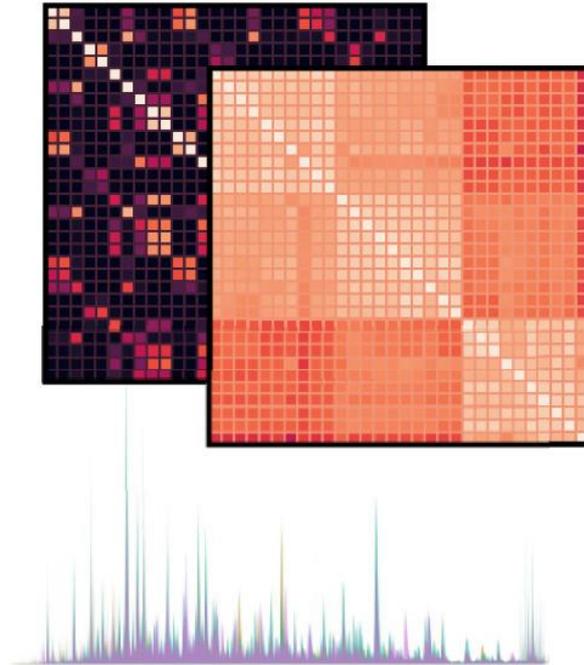
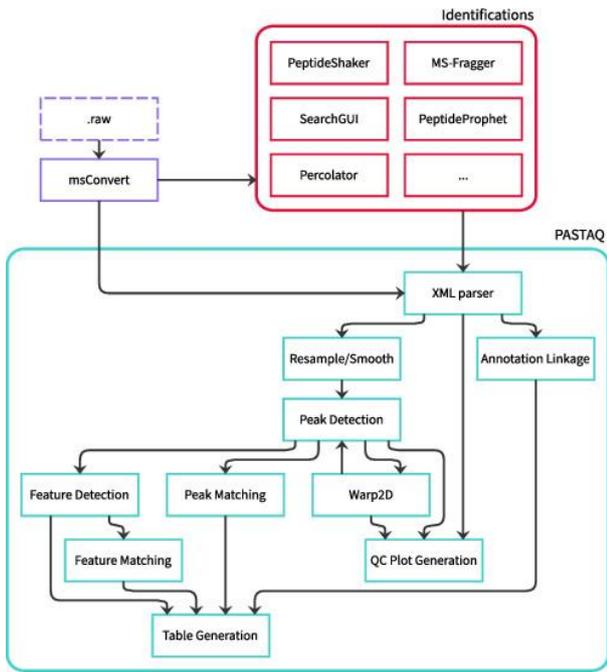
Pre-processing LC-MS/MS data acquired with data independent acquisition (DIA) is currently being implemented.

### **Please explain why your abstract is innovative for mass spectrometry?**

- threshold avoiding quantification LC-MS/MS pre-processing pipeline
- works with DDA/DIA and MS1 only proteomics and metabolomics LC-MS/MS
- accurate retention time alignment and MS1 based quantification

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



PASTAQ modules and LCMS similarity matrices before and after alignment.

Poster number: **IM-PA-026**

## **GOING BEYOND SIMPLE LIBRARY SEARCHING: INCORPORATING ACCURATE M/Z, MOLECULAR WEIGHT AND CHROMATOGRAPHIC INFORMATION FOR QUALITATIVE ANALYSIS.**

Abstract ID: 741

**Presenting author: Robert Cody, JEOL USA, Inc.**

### **Introduction**

Library searching suffers from limitations if there are no entries in the library for a given compound or if multiple compounds have indistinguishable mass spectra. For high- and low-resolving-power GC-MS analysis, software was developed that integrates all of the mass spectrometric and chromatographic information, providing greater confidence in compound assignments and facilitating the identification of unknowns. If accurate- $m/z$  information is available in the library, greater match specificity can be achieved by using the In-Source HiRes search function in the NIST MS Search Program. Examples are presented for DART mass spectra of wood and polymers.

### **Methods**

Integer- $m/z$  GC-MS data were obtained with the JEOL Q1500 single quadrupole GC-MS system. Exact- $m/z$  GC-MS data were obtained with the JEOL AccuTOF-GC-Alpha reflectron time-of-flight mass spectrometer. GC-MS data analysis was accomplished with the msFineAnalysis IQ and msFineAnalysis software programs. Indexed versions of the DART mass spectral drug and ForeST (wood) libraries were created to compare the In-Source HiRes search function with the Normal (integer) Identity search for DART data. Mass Mountaineer software using the In-Source HiRes search with a post-search exact mass filter gave better specificity for DART mass spectra of drug mixtures.

### **Preliminary data (results)**

GC-MS analysis of cannabis headspace volatiles measured by using the quadrupole mass spectrometer was dominated by terpenes with similar EI mass spectra. Chromatographic deconvolution followed by database searching resulted in ambiguous assignments for many compounds. Compound assignments for compounds with entries in the mass spectral libraries were confirmed by including retention index matching and nominal mass information. The high-resolving power, accurate- $m/z$  information from the reflectron time-of-flight mass spectrometer provided even greater specificity from the elemental compositions for molecular ions and fragments. In-Source HiRes search results for pyrolysis DART mass spectra of wood and polymer samples showed improved accuracy and higher Match Factors for all samples examined than by using the integer identity search.

### **Please explain why your abstract is innovative for mass spectrometry?**

GC-MS qualitative analysis improved by integrating all available information. DART-MS libraries match improved by using In-Source HiRes search.

### **Co-authors:**

*Masaaki Ubukata, JEOL Ltd.*

*Takao Fukudome, JEOL Ltd.*

*David Sparkman, University of the Pacific*

*Edgard Espinoza, US Fish and Wildlife Forensic Laboratory*

Poster number: **IM-PA-027**

## **COMPARISON OF GC-MS AND GCXGC-MS ANALYSIS OF VOLATILES AND PYROLYSIS PRODUCTS**

Abstract ID: **745**

**Presenting author: Scott Campbell, Spectralworks Ltd.**

### **Introduction**

Gas chromatography coupled with mass spectrometry (GC-MS) or high-resolution mass spectrometry (GCxGC-HRMS) is widely used to identify differences between similar samples. Comprehensive two-dimensional gas chromatography combined with high-resolution mass spectrometry (GCxGC-HRMS) uses two columns with different phases to achieve greater separation of complex mixtures. Each separation method has its strengths and weaknesses with respect to hardware setup and information content. Chemometric analysis of GCxGC-HRMS data provides an additional challenge because of the complexity of the data set. Here we compare the information and data analysis for identical sample sets analyzed by both GC-MS and GCxGC-MS using a high-resolution time-of-flight mass spectrometer.

### **Methods**

An Agilent 8890 gas chromatograph fitted with a Zoex ZX-2 thermal modulator was interfaced with a JMS-T2000GC (AccuTOF GC-Alpha, JEOL USA, Inc) reflectron time-of-flight mass spectrometer. An HTA HT2800T All-In-One autosampler was mounted on the gas chromatograph and used for headspace SPME measurements of Scotch whisky samples. A Frontier Labs Multi-Shot pyrolyzer was used to analyze polymer samples. Data analysis for both GC-MS and GCxGC-MS data was carried out with AnalyzerPro XD software (Spectralworks Ltd.).

### **Preliminary data (results)**

One-dimensional gas chromatography (1D GC) was capable of detecting volatile compounds that were not trapped by the cold gas jet in the GCxGC thermal modulator. Although 1D GC was not as effective at separating complex mixtures as GCxGC, chromatographic deconvolution software could separate partially coeluting peaks, leading to a simpler data analysis problem for comparing volatile samples. GCxGC excelled for more complex mixtures and mixtures having components with wide differences in boiling point and polarity. The chemometric tools in the AnalyzerPro XD software, originally developed for 1D GC-MS were expanded to handle GCxGC-HRMS data, including exact mass, high-resolution data, simplifying the task of chemometric analysis for more the complex data.

### **Please explain why your abstract is innovative for mass spectrometry?**

Compare hardware, information content, and data analysis challenges for chemometric analysis of GC-HRMS and GCxGC-HRMS data for volatiles and pyrolysis products.

### **Co-authors:**

*Robert Cody, JEOL USA, Inc.*

*John Moncur, Spectralworks Ltd.*

*John Dane, JEOL USA, Inc.*

Poster number: **IM-PA-028**

## **MACHINE LEARNING APPROACH FOR THE PREDICTION OF THE NUMBER OF SULFUR ATOMS IN PEPTIDES USING THE THEORETICAL AGGREGATED ISOTOPE DISTRIBUTION.**

Abstract ID: **806**

**Presenting author: Annelies Agten, Data Science Institute, Hasselt University**

### **Introduction**

In shotgun proteomics, the observed isotope pattern has been proven a useful feature to pre-process mass spectrometry data. The isotope distribution is completely defined by the elemental composition of the biomolecule and the elemental isotope definition. Sulfur atoms have a very distinctive isotope distribution and therefore they have a major impact on the course of the isotope distribution of peptides. Consequently, knowledge on the number of sulfur atoms can improve identification of peptides and can act as quality control in mass spectral experiments. We propose a machine learning approach to predict the number of sulfurs in a peptide using features constructed from the masses and isotope abundances from the theoretical aggregated isotopic variants.

### **Methods**

We use all tryptic peptides containing up to 6 sulfur atoms from the human UNIPROT database. Peptides with a redundant amino acid sequence and non-unique composition are omitted. The theoretical aggregated isotope distribution for each peptide is computed by the BRAIN. For each peptide a set of features is created, including the mass difference and relative abundance of consecutive isotope variants, and the mass and the fractional part of the mass of the first  $k$  isotopic peaks. We apply a gradient boosting algorithm for the prediction of the number of sulfurs. Hyperparameter tuning is performed using 5-fold cross validation.

### **Preliminary data (results)**

A gradient boosting algorithm is applied to a training dataset including features constructed using the first three, five and eight isotopic variants. The performance of the algorithm is evaluated on an independent test set. We can conclude that the algorithm performs well as prediction accuracy of the number of sulfurs is at least 99% in all cases. Moreover, it is demonstrated that increasing the number of isotopic variants used for prediction does not highly affect prediction accuracy.

We assessed variable importance by repeating the process 100 times on a different training dataset using stratified sampling. For each model the five highest ranked variables in importance are recorded. It can be concluded that the relative isotope abundance ratios of isotopic variants are the most important factors in estimating the peptide sulfur content. Moreover, our results show that the highest relative abundance ratios, i.e. abundance ratio of the third and second peak, abundance ratio of the fifth and fourth peak and abundance ratio of the eighth and seventh peak respectively, are consistently ranked as the most important variable. The highest mass differences, i.e. the mass difference between the eighth, fifth or third isotopic variant and the monoisotopic peak as well as the monoisotopic mass itself are also proven to be essential in the sulfur prediction model.

The mass and isotope abundance features we used to predict the number of sulfur atoms in peptides are shown to be highly correlated. How this affects the gradient boosting algorithm remains a subject for further research.

### **Please explain why your abstract is innovative for mass spectrometry?**

A complete theoretical study on the prediction of the number of sulfur atoms in peptides.

### **Co-authors:**

*Dirk Valkenburg, Data Science Institute, Hasselt University*

*Jurgen Claesen, Data Science Institute, Hasselt University*

*Tomasz Burzykowski, Data Science Institute, Hasselt University*

## Session: High resolution mass spectrometry

Poster number: **IM-PA-029**

### **ACHIEVING PPM-MASS ACCURACY FOR IONS UP TO M/Z 17,000 WITH MATRIX-ASSISTED LASER DESORPTION/IONIZATION ON A TRAPPED ION MOBILITY-QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETER**

Abstract ID: **26**

**Presenting author: Jürgen Gross, Institute of Organic Chemistry, Heidelberg University**

#### **Introduction**

Trapped ion mobility-quadrupole-time-of-flight (TIMS-Q-TOF) mass spectrometers are designed with high-throughput omics and molecules up to 4000 u in mind. Based on their orthogonal-acceleration type TOF analyzer, they deliver mass resolving power of about 40,000. Recent models can include a combination ion source enabling matrix-assisted laser desorption/ionization (MALDI), thereby creating a demand for the analysis of high-mass analytes, in particular, as isotopic separation is provided. In our laboratory, giant cage molecules and catenated cages drive the exploration of this field of application. This study investigates the mass accuracy of a Bruker timsTOFfleX instrument (Bruker Daltonics, Bremen, Germany) in MALDI mode for analytes up to  $m/z$  20,000. This instrument has proven 1–2 ppm mass accuracy in the range up to  $m/z$  4000.

#### **Methods**

To access a higher  $m/z$  range, the ion source housing is operated at 1.7 mbar as opposed to 2.7 mbar in standard  $m/z$  4000 range. Mass calibration across the entire range can be performed based on cluster ions created by laser desorption/ionization (LDI) of red phosphorous. Opposed to high vacuum LDI, the fine vacuum of the present source achieves sufficient thermalization to stabilize cluster ions up to  $m/z$  20,000. As phosphorous cluster ions are monoisotopic, there is no risk of erroneous peak assignment that can happen when reference peaks need to be identified within isotopic patterns.

#### **Preliminary data (results)**

Following external mass calibration by positive-ion LDI of red phosphorous, the analytes were examined. The first group was a set of dendrimers, commercially available as mass calibrants for MALDI-MS (SpheriCal, Polymer Factory, Stockholm, Sweden). They were applied in DCTB matrix doped with CsI<sub>3</sub> to foster cesium adduct ion formation. Per set, the  $m/z$  values of SpheriCals in the range of  $m/z$  4930 to 15,065 were determined. Second, [M+H]<sup>+</sup> ions of small proteins in sinapinic acid (SA) matrix (bovine insulin, ubiquitin, cytochrome C) were analyzed. Cytochrome C also formed [M+2H]<sup>2+</sup> ions that were included in this study. Each set was studied in five-fold replicate and repeated on different days. Mass accuracies and standard deviations were determined using Microsoft Excel.

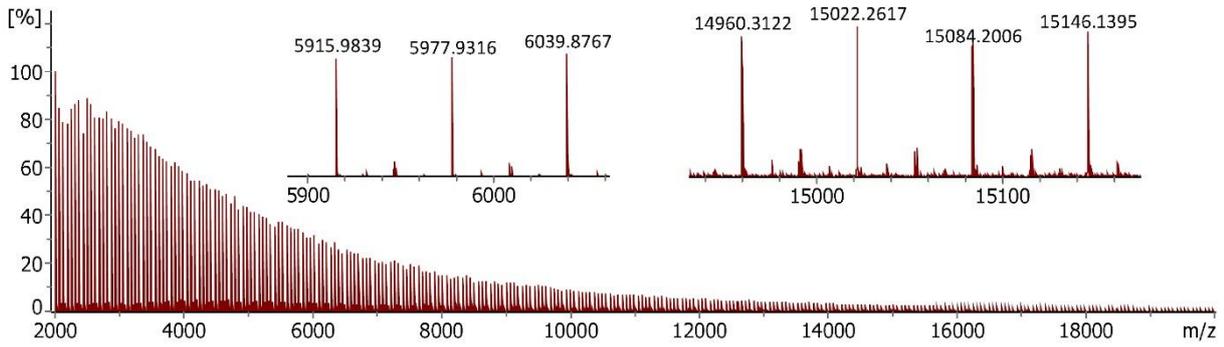
The instrument was not only able to well resolve the isotopic patterns of all analytes, it also delivered an impressive 1–2 ppm mass accuracy even for the largest dendrimer at just 1% relative intensity of the smallest. Notably, the mass accuracy was not affected by switching from LDI to MALDI using either DCTB or SA matrix and it could be achieved whenever a reasonable signal-to-noise ratio of the signal was obtained. The outstanding mass accuracy of the Bruker timsTOFfleX instrument supposedly is a) based on the oaTOF design decoupling initial ion kinetic energy spread introduced during LDI or MALDI from ion kinetic energy applied for TOF analysis and b) in part is due to the stabilized temperature of the TOF analyzer.

#### **Please explain why your abstract is innovative for mass spectrometry?**

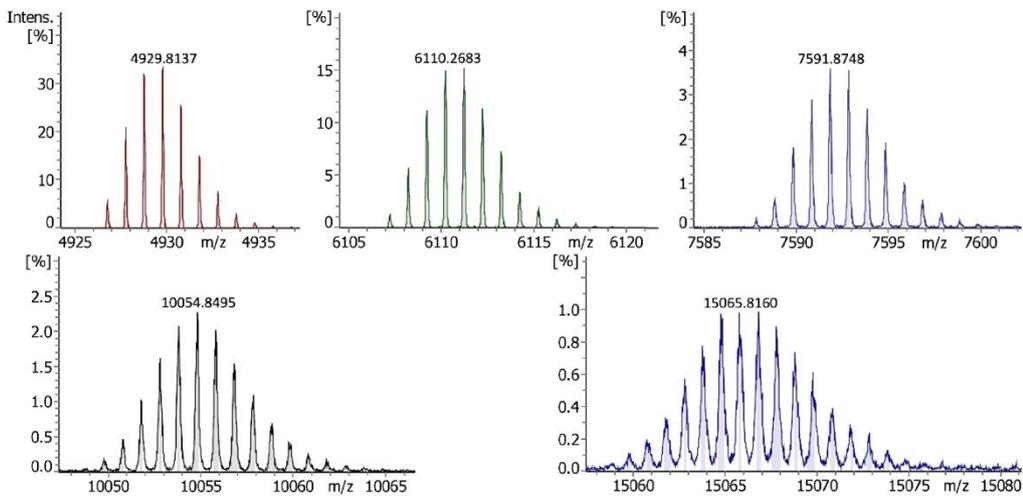
Technique for reliable ppm-mass accuracy in matrix-assisted laser desorption/ionization using a trapped ion mobility-quadrupole-time-of-flight mass spectrometer operated to access the high-mass range up to  $m/z$  20,000.

POSTER SESSION A

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Positive-ion LDI spectrum of red phosphorous reaching  $m/z$  20000



MALDI spectrum sections of Spherical dendrimers to  $m/z$  15065

Poster number: **IM-PA-030**

## **COMBINING THE DATA-DRIVEN AND HYPOTHESIS-DRIVEN APPROACHES IN ONE GO VIA A NOVEL INTELLIGENT DATA ACQUISITION HYBRID-DIA MASS SPECTROMETRY STRATEGY**

Abstract ID: 38

**Presenting author: Yue Xuan, Thermo Fisher Scientific (Bremen) GmbH**

### **Introduction**

Translational scientists face the dilemma to choose between comprehensive profiling and sensitive targeted quantitation. Proteomic profiling is commonly used to discover biomarkers, having a great potential for prognostic and predictive biomarkers; however, it still misses the sensitivity to quantify all the markers of interests. Therefore, targeted quantitation experiments of the potential markers are analyzed in the validation phase. This leads to high cost, time losses and more sample consumption. To address these challenges, we develop a novel intelligent data acquisition “Hybrid-DIA” MS strategy that enables comprehensive proteome profiling via high resolution MS1-based data-independent-acquisition (HRMS1-DIA)<sup>1</sup> MS and on-the-fly intelligently switching the acquisition mode to parallel reaction monitoring (PRM) for sensitive and absolute quantification of the markers, substantially increasing throughput and reducing sample consumption.

### **Methods**

Experiments comparing standard DIA methods to the novel Hybrid-DIA methods were performed using a nanoLC system coupled to the Orbitrap Exploris 480 MS. The hybrid-DIA method is programmed in C#, utilizing the Exploris API.

To evaluate the profiling and quantitation performance of hybrid-DIA method, a HELA digest sample spiked with a peptide reference mix, as well as depleted plasma sample spiked with a mixture of stable isotope labelled plasma reference peptides representing relevant targets for biomarker screening are analyzed at different LC gradients, benchmarking against the standard DIA methods. The raw data files were analyzed by Spectronaut, SpectroDive, and Skyline

### **Preliminary data (results)**

The hybrid-DIA strategy consists of a standard DIA scan cycle, where MS scan is followed by DIA MS/MS scans. Fast (multiplexed) PRM MS/MS scans are triggered from MS data based on isotope labelled peptides' signals and are used as a second layer of confirmation for isotope labelled peptides. Successful isotope labelled peptide detection triggers the high-quality measurement of corresponding endogenous peptides multiplexed (msx) with the isotope labeled peptides through msxPRM MS/MS scans acquired with narrower isolation window width and maximizing ion injection time for each species. This data acquisition scheme maximizes instrument productivity and, in turn, resulting in only minor decreases in DIA acquisition time.

The global profiling and quantitation performance of Hybrid-DIA MS are investigated and benchmarked against the standard DIA MS methods by analyzing a mixture of stable isotope labelled peptides with a concentration range of 4 order of magnitude spiked in a HELA digest on the same LC system coupled to the Orbitrap Exploris 480 MS. Similar number of proteins are identified with 1% FDR and quantified with CV<20% by Hybrid-DIA and DIA experiments; while, the LOD and LOQ of the targeted quantitation of spiked in peptides reach the 20-50 attomole range with the hybrid-DIA method. Depleted plasma sample spiked with a mix of isotope labelled plasma reference peptides representing relevant targets for biomarker screening are also analyzed. Hybrid-DIA method can simultaneously quantify a promising number of endogenous biomarkers with high precision and reproducibility, while simultaneously comprehensive profiling plasma proteomes in one single experiment.

### **Please explain why your abstract is innovative for mass spectrometry?**

A novel intelligent data acquisition hybrid-DIA MS to sensitively quantify the biomarkers, while simultaneously unbiased DIA profiling the sample.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### **Co-authors:**

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*Tao Bo, Thermo Fisher Scientific (China) Co. Ltd*

*Kyle Fort, Thermo Fisher Scientific (Bremen) GmbH*

*Jesse D. Canterbury, Thermo Fisher Scientific*

*Daniel Lopez Ferrer, Thermo Fisher Scientific*

*Andreas F. Huhmer, Thermo Fisher Scientific*

*Alexander Makarov, Thermo Fisher Scientific (Bremen) GmbH*

Poster number: **IM-PA-031**

## **ACCURATE MASS LIBRARY FOR NATURAL PRODUCTS BASED ON COMPOUNDS IDENTIFIED IN HEMP OIL**

Abstract ID: **52**

**Presenting author: Remko Van-Loon, Agilent Technologies, Inc.**

### **Introduction**

The use of hemp and CBD oils has become increasingly popular in many parts of the world as a direct use product and incorporating into another product. One of the important directions of the chemical analysis of these goods includes a broad overview of the chemical composition for different strains of CBD products and hemp to identify compounds with bioactive properties. Concentrated hemp oils typically produce between 350 - 560 chromatographic peaks under a simple 1D configuration. Here we describe the development of the Retention Index based accurate mass library for these types of samples with the idea that it would reduce the overall data analysis and allow for a focus on the unique components of the individual sample.

### **Methods**

Five different hemp oil samples were analyzed using a high-resolution GC/Q-TOF in either 1D or comprehensive GCxGC configuration using the ZOEX ZX2 thermal modulator. A 5% phenyl, 30m column was used for the 1D data while the GCxGC configuration was a 5% phenyl 30m column coupled to a 2.8m DB-HeavyWAX. The data were acquired at 70eV. The retention indices were calculated based on the alkane ladder to assist compound identification and library curation. The GC/Q-TOF data were processed using the Unknown Analysis tool of MassHunter Quantitative Analysis Software, MassHunter Qualitative Analysis Software and GC Image.

### **Preliminary data (results)**

The goal of the present study is to create a comprehensive accurate mass PCDL (Personal Compound Database and Library) based on hemp oil samples for higher confidence quick screening in 1D GC configuration. In order to achieve adequate chromatographic separation of these complex samples, the data were collected using GCxGC configuration. The GCxGC data were visualized using GC Image software and compounds were identified using NIST17 and NIST20 libraries. Compound spectra identifications were supplemented using accurate mass, accurate isotopes, as well as retention indices (RI). The spectra of the identified compounds were annotated with fragment formulas using MassHunter Qualitative Analysis Software and exported to the PCDL after curation and automatic conversion of the measured m/z values to the theoretical values from the elemental compositions of the individual ions. Whenever a precise identification of an isomer was impossible, a compound would be assigned an indexed molecular formula instead of a name. The current PCDL contains approximately 350 compounds. The PCDL has been validated using the cannabis extracts and hemp oils data acquired using 1D GC and compound metadata has been crosschecked for accuracy. Despite the complexity of the samples, the majority of the PCDL hits generated the library match scores of over 80.

### **Please explain why your abstract is innovative for mass spectrometry?**

An instrumental method to build and utilize a new accurate mass library for cannabis and other natural products

### **Co-authors:**

*Sofia Nieto, Agilent Technologies, Inc.*

Poster number: **IM-PA-032**

## **HIGH THROUGHPUT SEQUENCE CONFIRMATION AND IMPURITY LOCALISATION OF SYNTHETIC OLIGONUCLEOTIDES USING MASS SPECTROMETRY (MS/MS AND MSe)**

Abstract ID: 147

**Presenting author: Chris Knowles, Waters**

### **Introduction**

Oligonucleotides are a rapidly expanding category of therapeutics that have the potential to revolutionise modern medicine. A critical step in the development of successful oligonucleotide therapies is the incorporation of synthetic nucleotide analogues that allow for enhanced target specificity, binding efficiency, and stability. The nature of these chemical modifications interferes with enzyme-based sequencing technologies, and therefore the industry is turning towards mass spectrometry to identify and characterize the oligonucleotide sequence and structure. Here we demonstrate a new software driven approach that uses both MS/MS and MSe data for sequence confirmation of various chemically modified oligonucleotides. Predicted fragment ions are automatically calculated from libraries of custom nucleotides, modifications and sequences; then matched to raw data using a bespoke targeted isotope clustering algorithm.

### **Methods**

Oligonucleotide sequences include analogues to various commercial therapeutics, with several common chemical modifications (Phosphorothioate, 2'OMe, 5'Me, and LNA).

IP-RP LC-MS assays were used for the separation of synthetic oligonucleotides and their corresponding impurities with a mobile phase containing 40 mM HFIP (hexafluoroisopropanol) and 7 mM TEA (triethylamine). UHPLC was performed using a 2.1 x 100 mm column packed with 1.7  $\mu$ m C18 particles. UV chromatograms were acquired at a wavelength of 260 nm. High resolution (> 20,000) ESI-MS, MS/MS and MSe spectra were acquired in negative ion mode on a QTOF instrument.

### **Preliminary data (results)**

Using both MS/MS and MSe approaches, we demonstrate the optimal CID conditions to confirm the sequence of a various oligonucleotides. Data presented here highlights the ease in which pre acquired high energy data can be processed in high throughput for sequence confirmation, and the ability to locate a tentatively assigned impurity modification using data analysis of predicted sequence variants.

The software used employs a bespoke targeted clustering algorithm that matches raw data against a predicted set of fragment ions and provides matching information in both graphical (via m/z traces) and table format. Sequence coverage is also presented in 'dot map' figures which allows for rapid assessment of multiple sequences against large datasets. Information on the coverage of ions in the raw data is also shown.

Statistical analysis is provided via scoring of the isotope fit, mass error, and the distribution of mass error across an isotope profile. Data analysis is performed directly on the raw data which circumvents the need for deconvolution and allows for rapid processing times.

In summary we show a fully compliant workflow for batch processing of pre acquired MS/MS or MSe data for sequence confirmation, impurity location and experimental optimisation.

### **Please explain why your abstract is innovative for mass spectrometry?**

High throughput, fully compliant and automated processing of pre acquired MS/MS or MSe data for sequence confirmation and impurity location of oligonucleotides

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

**Co-authors:**

*Emma Harry, Waters*  
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*Rory Mullins, Waters*  
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*Paul Henesey, Waters*  
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*Joe Fredette, Waters*  
*Ying Qing Yu, Waters*

Poster number: **IM-PA-033**

## **RHAMNOLIPID IDENTIFICATION AND STRUCTURAL CHARACTERIZATION IN COMPLEX BIOTECHNOLOGICAL SAMPLES BY SFC-HRMS/MS AND KENDRICK MASS DEFECT PLOTS**

Abstract ID: **216**

**Presenting author: Anna Lipphardt, Institute of Inorganic and Analytical Chemistry, University of Muenster**

### **Introduction**

Rhamnolipids are among the most promising biosurfactants representing a bio-based alternative to synthetic surfactants and are currently being intensively researched. Those glycolipids consist of one or two L-rhamnose moieties and up to three  $\beta$ -hydroxy fatty acids. In addition to their use as surfactants, rhamnolipids can dissolve biofilms and have antimicrobial activity, opening up exciting applications in biomedicine. Microbial production is based on more than 95% renewable raw materials or industrial waste and can be carried out by various microorganisms, especially *Pseudomonas aeruginosa*. The different fields of application show great potential, but the investigation of the biodiversity of rhamnolipid producers is still an analytical challenge, as so far established methods do not cover the whole spectrum of rhamnolipids produced in a simple and straightforward way.

### **Methods**

In this work, analytical methods were developed for the study of rhamnolipids covering the entire biosynthetic pathway from free  $\beta$ -hydroxy fatty acids and 3-(3-hydroxyalkanooyloxy)alkanoic acids (HAA) to di-rhamno-di-lipids. We hereby present an SFC-MS hyphenation for the rapid and comprehensive identification and structural characterization of rhamnolipids. Identification at sum formula level is achieved through high-resolution MS based on accurate mass. Furthermore, data-dependent MS/MS fragmentation experiments enable the assignment of the bound  $\beta$ -hydroxy fatty acids and their positions.

### **Preliminary data (results)**

In particular, SFC allows rapid separation of rhamnolipid subclasses differing in the number of L-rhamnose moieties and linked  $\beta$ -hydroxy fatty acids, as well as differentiation of rhamnolipids from their precursors and degradation products. Using this method, 33 rhamnolipid species were identified in a fermentation supernatant of a biotechnologically relevant strain, i.e. *Pseudomonas putida* KT2440, with species with one or two  $\beta$ -hydroxydecanoic acid chains dominating. Software-assisted data processing in MZmine 3, an open-source software for MS data processing, was introduced to facilitate the handling of large datasets in bacterial screening. Furthermore, Kendrick mass scale was used to visualize homologous series. In this way, the fermentation supernatants of different gene-modified rhamnolipid producers can be compared graphically by means of their Kendrick mass defect plots. At a glance, remarkable differences can be seen both in terms of the congeners contained and the complexity of the mixtures.

### **Please explain why your abstract is innovative for mass spectrometry?**

A rapid rhamnolipid screening method for addressing the production diversity of bacterial strains with the use of rhamnolipid annotation in MZmine 3 and Kendrick mass defect plots is presented.

### **Co-authors:**

*Heiko Hayen, Institute of Inorganic and Analytical Chemistry, University of Muenster*

Poster number: **IM-PA-034**

## **MS/MS BASED DISTINCTION OF GLYCOPEPTIDE ISOMERS**

Abstract ID: **217**

**Presenting author: Joshua C.L. Maliepaard, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Utrecht, the Netherlands, Netherlands Proteomics Center, Utrecht, The Netherlands**

### **Introduction**

Glycoproteins are involved in a wide range of physiological processes and their impact on human health can hardly be overstated. Critically, it is not only the composition but also the structural isomerism of glycans that determines glycoprotein function. A prominent example of this is the difference between  $\alpha$ 2,3- and  $\alpha$ 2,6-linked sialylation: immunoglobulin G (IgG) with  $\alpha$ 2,6-linked sialylated glycans on the fragment crystallisable (Fc-) domain has a significantly higher anti-inflammatory activity than IgG with  $\alpha$ 2,3-linked sialylated glycans (Anthony, *et al.*, 2008). On top of the standard glycoproteomics challenges, distinguishing glycopeptide structural isomers adds another layer of complexity since these typically have very similar chemical properties. As such, current liquid chromatography mass spectrometry (LC-MS) methods are not generally applicable for distinction of glycopeptide isomers.

### **Methods**

Starting from isomerically defined glycopeptide standards, facilitated by methods for chemoenzymatic synthesis (Liu, *et al.*, 2019), we have developed an MS-based approach that uses product ion intensities to differentiate between isomers. The influence of peptide length on our approach's ability to differentiate between isomers was tested by LC-MS/MS analysis of sialylglycopeptide (SGP) and trastuzumab.

### **Preliminary data (results)**

With our approach, we have thus far been able to distinguish  $\alpha$ 2,3- and  $\alpha$ 2,6-linked sialic acids on *N*-glycopeptides as well as various isomers of monogalactosylated glycans, with more isomeric features under consideration. The approach for differentiation appears largely independent of the peptide to which the glycan is connected and therefore has the potential to be generalized to a wide range of glycoproteins. If successful, MS-based differentiation between structural glycan isomers would not only revolutionize the field of glycoproteomics, but also open up new insights into the mechanisms behind diseases, and enable better therapeutical antibody design and quality control.

### **Please explain why your abstract is innovative for mass spectrometry?**

Innovations include: 1) the use of MS/MS to differentiate structural glycopeptide isomers, 2) glycan linkage information in proteomics data, 3) new insights into glycan structure without necessitating glycan release.

### **Co-authors:**

*Geert-Jan Boons, Department of Chemical Biology and Drug Discovery, Utrecht University, Utrecht, the Netherlands*  
*Karli R. Reiding, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Utrecht, the Netherlands, Netherlands Proteomics Center, Utrecht, The Netherlands*

Poster number: **IM-PA-035**

## HALOGENATED POPS NON-TARGETED SCREENING BY GC-APCI-HRMS AND ION MOBILITY SPECTROMETRY

Abstract ID: 274

**Presenting author: Fan Yang, The Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM)**

### Introduction

Halogenated legacy organic contaminants (OCs) and related contaminants of emerging concern (CECs) threaten the environment and human health due to their persistence and long-range transport in the food chain. Ion mobility spectrometry (IMS) adds an orthogonal separation dimension, which enhances co-eluting compound separation and yields a more specific and accurate HRMS spectrum and a cleaner isotopic pattern. The Collision Cross Section (CCS) offers an additional filter, which differentiates ions by their size, structure, and charge under a given set of conditions like the type of gas, pressure, temperature. In our method, APCI was employed producing molecular ions. However, lack of an empirical APCI library, it is challenging to identify unknowns. In this study, we investigate the potential of IMS and CCS in NTS identification.

### Methods

POP standard and  $^{13}\text{C}$ -labeled standard kit (Wellington Laboratories, Guelph, Ontario) were separated on a Bruker GC SCION device equipped with a Rxi5SilMS high-resolution column (40m x 0.18mm x 0.18 $\mu\text{m}$ , Restek, France). MS data were acquired on a Bruker timsTOF instrument enhanced in APCI positive full scan mode. IMS was set under  $\text{N}_2$  at 25°C. Raw Data was processed by Data Analysis software (Bruker Daltonics). Since limited spectrum libraries exist in GC-APCI-MS, an in-house database with CCS was made in the new TASQ® 2021 (Bruker Daltonics) for further analysis.

### Preliminary data (results)

In this study, over 160 standards corresponding to PAHs, PCDD/Fs, PCBs, OCPs, PBDEs, and OH-PBDEs were characterized by IMS-HRMS with retention time (RT), exact mass, isotope, and CCS value. In the acquisition of PBDEs, both  $[\text{M}]^+$  and  $[\text{M}+\text{H}]^+$  were both produced in APCI, an in-source fragment of  $[\text{M}-\text{Br}]^+$  occurs with a low peak intensity. As shown in figure 1,

the M-Br fragment from Tetra-BDE ( $\text{C}_{12}\text{H}_6\text{Br}_4\text{O}$ ) has the same chemical formula as Tri-BDE ( $\text{C}_{12}\text{H}_7\text{Br}_3\text{O}$ ).  $\text{C}_{12}\text{H}_6\text{Br}_4\text{O} \rightarrow \text{C}_{12}\text{H}_7\text{Br}_3\text{O}^+ + (\text{Br})^-$ . As for NTS, fragments of unknowns could be an isobar of others, like the example presented before. It may result in a false positive detection. However, if ions are grouped by CCS value, due to the difference of CCS value, fragments could be easily associated with the molecular ion. CCS is also beneficial to clean up the mass spectra in NTS shown in figure 2.

One of the main challenges in NTS is the massive work in data treatment, IMS with CCS provides a novel aspect in data treatment and data minimization. The CCS-mass trendline can estimate the unknown into different chemical families, CCS can also combine with mass defect to elucidate the unknown's formula. GC-IMS-HRMS offers a novel aspect in NTS.

### Please explain why your abstract is innovative for mass spectrometry?

CCS could be an alternative criterion to  $\text{MS}_n$  in NTS identification. APCI combined with IMS can provide structural information without fragment information.

### Co-authors:

Yann AMINOT, French Research Institute for Exploitation of the Sea (IFREMER)

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Hugues PREUD'HOMME, The Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM)

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

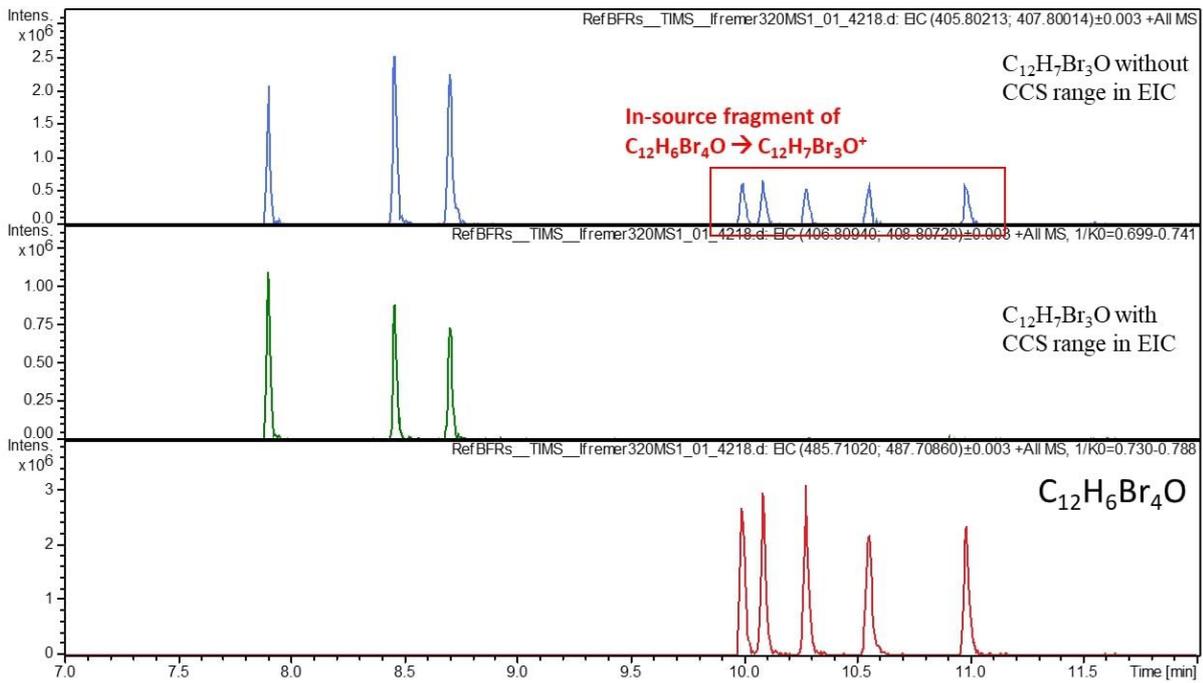


Figure 1 Chromatogram of Tetra-BDE (C<sub>12</sub>H<sub>6</sub>Br<sub>4</sub>O) and Tri-BDE (C<sub>12</sub>H<sub>7</sub>Br<sub>3</sub>O)

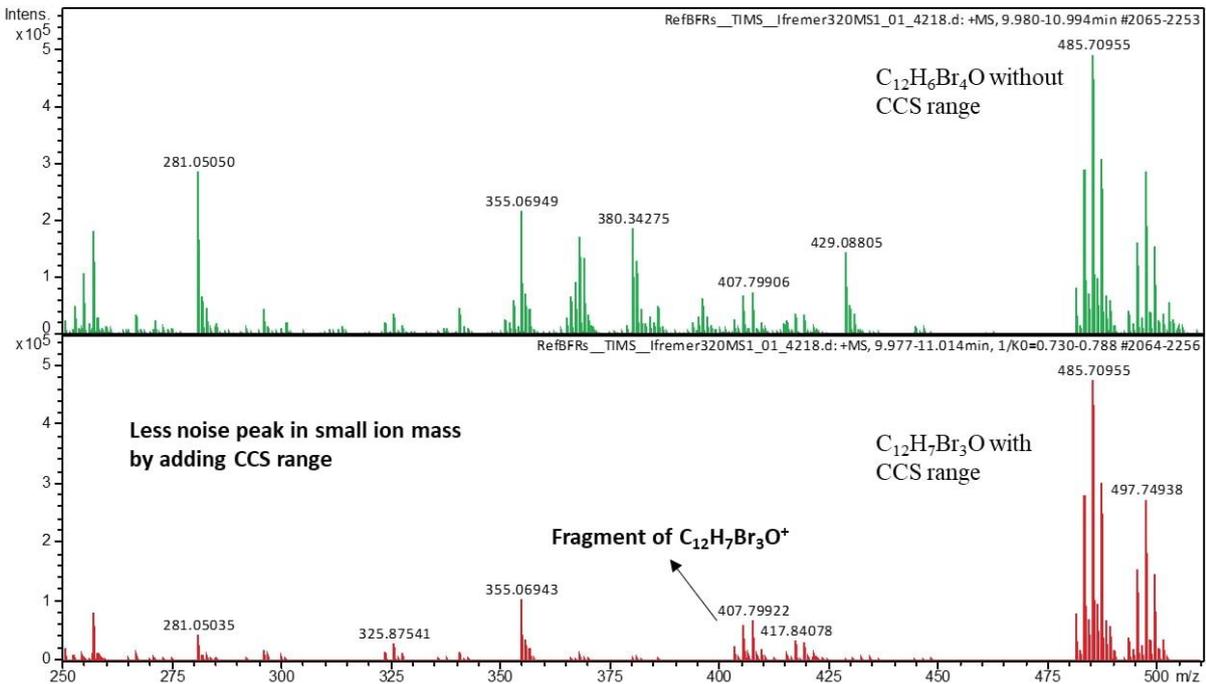


Figure 2 comparison of MS with CCS filter range

Poster number: **IM-PA-036**

## **DEEPER AND HIGHER CONFIDENT ANNOTATION OF COMPLEX METABOLOMICS DATA BY COMPLEMENTARY LARGE-SCALE SPECTRAL LIBRARIES**

Abstract ID: **275**

**Presenting author: Mohamed Gamaleldin Elsadig, Bruker Daltonics**

### **Introduction**

In recent years the number of spectral libraries available for metabolite annotation constantly increased. Especially larger libraries such as NIST (<https://chemdata.nist.gov/>) and Metlin (<https://metlin.scripps.edu>) raise the question how complementary and comparable these libraries are. Different data acquisition parameters and missing availability of comparable meta information complicate a direct comparison. In this study we investigated two large and commonly used libraries (namely Bruker NIST 2020 MS/MS library and Bruker MetaboBASE 3.0 Personal library (based on Metlin) containing  $2.8 \times 10^4$  and  $1 \times 10^5$  compounds, respectively) in respect of complementarity and suitability for annotating common metabolomics sample types such as urine.

### **Methods**

A human urine sample and naphthalenes standards were analyzed by RP-LC-TIMS-MS/MS on a timsTOF Pro 2 (Bruker, Germany) in PASEF mode. MetaboScape 2021b (Bruker, Germany) was used for data processing and spectral library search. For comparing spectral similarity cosine scores were calculated in both libraries for compounds assignment. A cosine score of  $>0.8$  was considered a high similarity and score between 0.4 and 0.8 as medium. The chemical similarity was assessed by the Tanimoto coefficient. Calculation was performed in Python using RDKit with a similarity cutoff of 0.7. This allowed the construction of a chemical similarity network using CytoScape.

### **Preliminary data (results)**

MS/MS similarities were calculated for comparing the NIST 2020 MS/MS library and MetaboBASE 3.0 Personal library (Fig. 1). The spectral matching of compounds revealed that these spectral libraries contain many unique compounds (45% and 78%, respectively). From these values we expected that the two libraries applied to real world samples, would result in mostly orthogonal and higher total number of annotations. The hypothesis was substantiated using urine samples, where the number of annotated features increased to  $>120\%$  when using both instead of only on spectral library.

The chemical space covered by the two libraries was assessed by determining the chemical similarity. For this purpose, the Tanimoto coefficient between library compounds was calculated. Using this approach, a network was generated with chemical classes clustered that indicated complementarity of chemical spaces covered by the two libraries (Fig. 2). Data acquired for several naphthalenes spiked into a urine sample supported this hypothesis as some compounds were annotated by both libraries but others only by one: Daunorubicin was found in both spectral libraries, Valrubicin only in MetaboBASE 3.0 and Daunorubicin only in NIST 2020.

### **Please explain why your abstract is innovative for mass spectrometry?**

Comparison of large spectral libraries showed their complementarity and suitability for annotating common metabolomics sample types such as urine.

### **Co-authors:**

*Nikolas Nikolas Kessler<sup>1</sup>, Bruker Daltonics*

*Matthias Szesny, Bruker Daltonics*

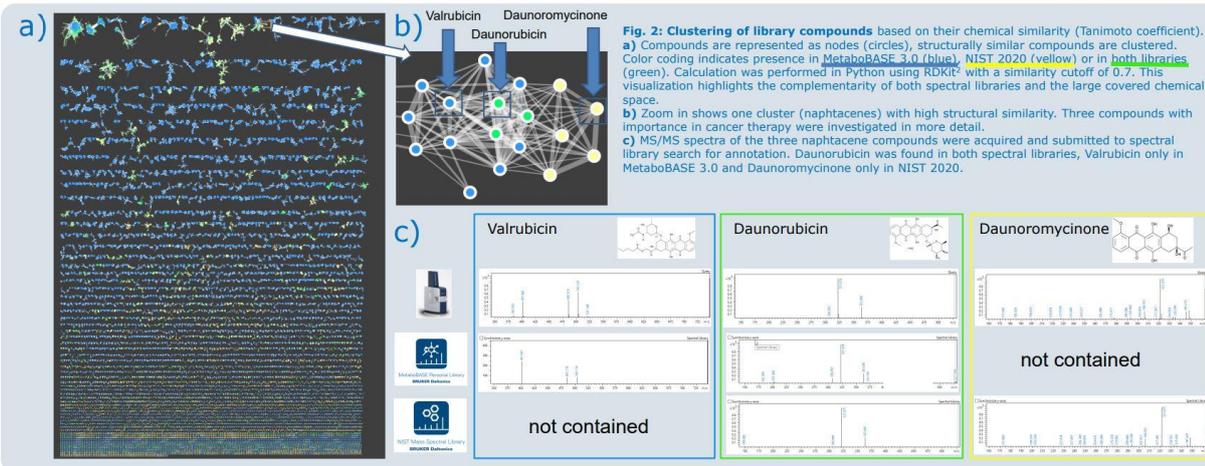
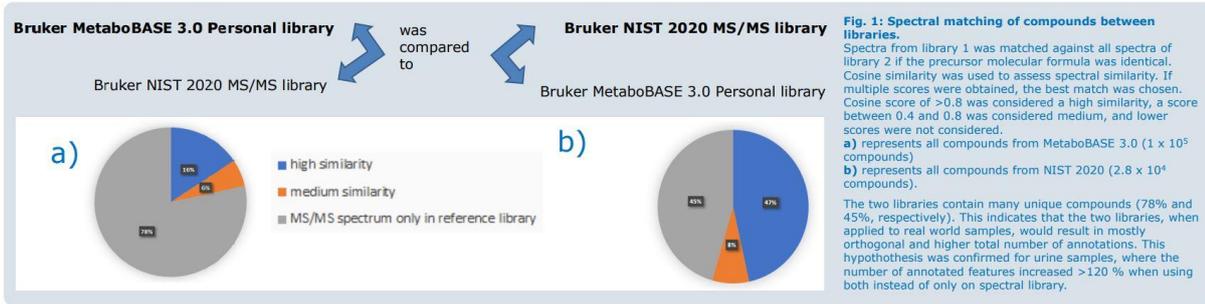
*Aiko Barsch, Bruker Daltonics*

*Ulrike Ulrike Schweiger-Hufnagel, Bruker Daltonics*

*Lucy Woods, Bruker Daltonics*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Poster number: **IM-PA-037**

## TEMPERATURE-CONTROLLED CYCLIC ION MOBILITY MASS SPECTROMETRY – A NOVEL ANALYTICAL STRATEGY FOR THE CHARACTERIZATION OF BIOPHARMACEUTICALS

Abstract ID: 299

**Presenting author: Philipp Bittner, Department of Chemistry and Applied Biosciences, ETH Zurich, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland**

### Introduction

Over the last few decades, electrospray ionization mass spectrometry (ESI-MS) has evolved into the gold standard method for the analysis of biomolecules in drug discovery and development. Despite the availability of very high-resolution MS instruments, the analysis of isomeric compounds such as peptides or peptide complexes, glycosylated proteins or micro-heterogeneities in antibody formulations remains challenging and time-consuming. With the recent development of high-resolution ion mobility (IM) devices, the experimental possibilities have been brought to another level. Here we combine this technology with our home-built temperature-controlled nano-ESI (TCnESI) source to develop novel analytical strategies for the above-mentioned challenges.

### Methods

The combination of our TCnESI source with a cyclic IM-MS instrument (Waters) gives unique access to thermodynamic and kinetic information of complex mixtures, such as the co-formation or stability of isomeric/isobaric peptide complexes, which is currently not possible by any other technique. The implementation of multiple pre- and post-cIM fragmentation methods such as surface-induced dissociation (SID) or electron capture dissociation (ECD) further allows one to obtain stoichiometric and structural information of a variety of samples, for example to analyse various proteoforms or aggregates of therapeutic monoclonal antibodies.

### Preliminary data (results)

In an initial approach, we used our method for the label-free analysis of isomeric collagen model peptides (CMPs). Preliminary results (Attachment 1) show that cIM allows the separation of three isomeric CMPs with the same ionization profile, differing only in the position of an aspartic acid. Applying more passes in the cIM of is expected to give clear separation of these monomers and also allow analyses of other functionalized isomeric peptides in same m/z ranges. We plan to apply this method to fully characterize the specificity of heterotrimer formation in complex mixtures of three or even more label-free peptides. The expansion of this CMP toolbox and characterization by IM-MS is expected to afford a much deeper understanding of the controlled formation of collagen triple helices.

### Please explain why your abstract is innovative for mass spectrometry?

A combined setup of a temperature-controlled nanoESI source with cyclic ion mobility separation gives novel access to thermodynamic and kinetic information of complex mixtures of isomeric/isobaric biological samples.

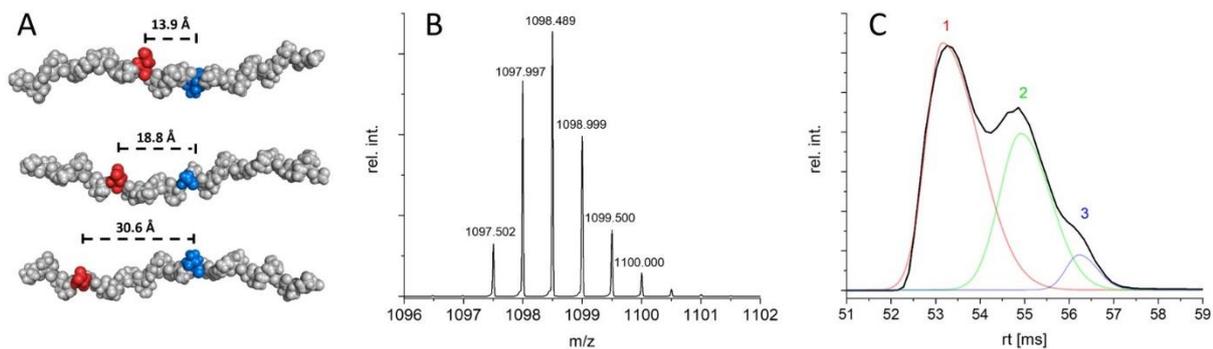
### Co-authors:

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*Renato Zenobi, Department of Chemistry and Applied Biosciences, ETH Zurich, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Cyclic ion mobility separation of three isomeric collagen model peptides.

Poster number: **IM-PA-038**

## HIGHLY EXOTHERMIC PROTON TRANSFER REACTIONS FROM PROTONATED ARGON TO METHANOL AND ACETONE

Abstract ID: **303**

**Presenting author: Maximilian G. Müntz, Universität Innsbruck**

### Introduction

Proton transfer reactions (PTR) are widely used for monitoring atmospheric compounds, e.g. PTR from  $\text{H}_3\text{O}^+$ , which is used to trace volatile organic compounds.<sup>[1]</sup> In an effort to expand the usage of chemical ionization to high purity gas analysis, we investigate PTR from protonated argon. Argon has a comparably low proton affinity, which affords protonation of compounds like CO,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , as well as typical hydrocarbons including methane.

[1] W. Lindinger, A. Jordan, *Chem. Soc. Rev.* **1998**, 27, 347.

### Methods

The experiments were performed on a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS).  $\text{ArH}^+$  primary ions are generated by laser vaporization of a solid magnesium disk, followed by supersonic expansion of the plasma in argon seeded with hydrogen. While measuring the rates of proton transfer reactions to molecules with potential relevance in gas analysis, unexpectedly complex reaction profiles for  $\text{ArH}^+$  with methanol and acetone were encountered.

### Preliminary data (results)

PTR from  $\text{ArH}^+$  to acetone leads to the formation of  $\text{C}_2\text{H}_3\text{O}^+$ ,  $\text{C}_3\text{H}_5^+$ , and protonated formaldehyde, in addition to the main product viz. protonated acetone. Proton transfer to methanol forms protonated formaldehyde, in addition to protonated methanol. In a secondary reaction step, methanol and protonated formaldehyde forms protonated dimethyl ether. Quantum chemical calculations reveal the most probable reaction paths.

### Please explain why your abstract is innovative for mass spectrometry?

Chemical ionization via highly exothermic proton transfer

### Co-authors:

*Erik Barwa, Universität Innsbruck*

*Martin K. Beyer, Universität Innsbruck*

Poster number: **IM-PA-039**

## DEEP METAPROTEOME ANALYSIS USING A NEW AUTOMATED SAMPLE PREPARATION PLATFORM AND A VANQUISH NEO UHPLC SYSTEM COUPLED TO AN ORBITRAP ECLIPSE TRIBRID MASS SPECTROMETER WITH FAIMS PRO INTERFACE

Abstract ID: **318****Presenting author: Amirmansoor Hakimi, Thermofisher Scientific**

### Introduction

LCMS-based proteomics is a powerful tool for deep profiling of peptides and proteins in complex biological samples but when it comes to high throughput applications manual sample preparation workflow remains a major challenge in the field. Here we introduce a fully automated sample preparation platform for proteomics samples that enables standardized, hands-off operation and provides robust workflows for label-free proteomics applications. The platform was tested with metaproteomics samples that are very difficult to process. Coupling it to a newly developed nLC column extensive peak capacity hyphenated to a High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS™) on a Thermo Scientific™ Orbitrap Eclipse™ Tribid™ mass spectrometer, reduces the sample preparation time, increases the reproducibility significantly and maximizes the proteome coverage of metaproteome samples with high protein diversity.

### Methods

Metaproteome standards were purchased from ZymoBIOMICS and prepared using a fully automated sample preparation platform. Proteins were lysed, reduced, alkylated, and digested to peptides in the automated platform. 1 µg of peptides were separated using an Easy-Spray™ PepMap™ Neo column on a Vanquish Neo UHPLC system coupled to an Orbitrap Eclipse Tribid MS with/without FAIMS. The final FAIMS MS method was set to switch between different CVs with a top-speed method in a 3 second cycle time over a 140 minutes gradient. The raw files were searched using Proteome Discoverer 3.0 utilizing, Sequest HT, and INFERYS re-scoring algorithm.

### Preliminary data (results)

The automated sample preparation platform is an intuitive turnkey system (instrument, software, reagents) to provide robust and reproducible sample preparation for MS proteomics analysis associated with easy to use and limited user involvement and without any compromise in performance compared to traditional methods. We performed gas-phase fractionation using the FAIMS to gain dynamic range and improve proteome coverage. Preliminary results quantified over 12,000 proteins in the ZymoBIOMICS Microbial Community standard. Similar proteome coverage was observed in the ZymoBIOMIC Gut Microbiome standard dataset. Importantly, the system exhibited an extremely high digestion efficiency even for challenging samples. Zero peptide missed cleavages of 95% could be achieved for plasma samples with 1-hour digestion. The sample preparation process is robust and reproducible and requires less than 20 min setup.

### Please explain why your abstract is innovative for mass spectrometry?

Deep Proteome coverage using a fully automated sample preparation platform

### Co-authors:

*Kevin Schauer, Thermofisher Scientific*  
*Runsheng Zheng, Thermofisher Scientific Germany*  
*Alexander Boychenko, Thermofisher Scientific Germany*  
*David Horn, Thermofisher Scientific*  
*Steven Reeber, Thermofisher Scientific*  
*Kristan Bahten, Thermofisher Scientific*  
*Woong Kim, Thermofisher Scientific*  
*Daniel Lopez Ferrer, Thermofisher Scientific*

Poster number: **IM-PA-040**

## **FROM UNKNOWN TO KNOWN: UTILITY OF HIGH QUALITY CURATED HRAM MSN SPECTRAL LIBRARIES AND REAL TIME LIBRARY SEARCH FOR HIGH CONFIDENCE ANNOTATION OF METABOLITES**

Abstract ID: **320**

**Presenting author: Rahul Deshpande, Thermo Fisher Scientific**

### **Introduction**

Untargeted metabolomics aims to annotate and quantify the complete set of metabolites within a biological system. Confident compound annotation is essential for translating metabolomics data into meaningful biological information. Local mass spectral libraries derived from authentic standards provide the opportunity to confidently annotate and analyze large amounts of MS<sup>n</sup> data. This can then lead to the transfer of knowledge from research into more routine, quantitative applications. For high confident compound identification, the libraries must contain high-quality and accurate data. Here we describe the process of creating a high-quality spectral library of flavanoid authentic standards mixture using the high-resolution accurate mass (HRAM) capability of Thermo Scientific™ Orbitrap Mass Spectrometer and its utility for confident annotation of metabolites using Real Time Library Search (RTLS).

### **Methods**

A Thermo Scientific™ Orbitrap IQ-X Tribrid mass spectrometer with a Thermo Scientific™ Vanquish™ UHPLC system was used for data acquisition. Extracts from different varieties of tea were separated on a Thermo Scientific™ Hypersil™ Gold C18 column (2.1x150mm, 1.9µm). The library compounds were acquired using Thermo Scientific™ Tune 3.5 software, which includes library builder templates for infusion and LC/MS. Automated curation and structural annotations were performed using Thermo Scientific™ Mass Frontier™ 8.0 structure identification software. The created flavanoid library is utilized for RTLS detection of unknown flavonoids in tea.

### **Preliminary data (results)**

The in-built library template automatically acquires multiple collision energies, multiple spectra for each MS<sup>n</sup> (up to MS<sup>4</sup>) stage in both HCD and CID. The purpose is to capture comprehensive fragmentation data at multiple collision energies and fragmentation modes for each compound in the library. This allows for confident matching with LC/MS fragmentation data from biological samples. The created MS<sup>n</sup> spectral library with structure annotations enables library searching to quickly identify unknown compounds. It also enables substructure matching for unknown compounds not in the library. The Thermo Scientific™ Orbitrap IQ-X Tribrid mass spectrometer has an in-built RTLS functionality, which generates familiar measures of similarity and identification confidence during data acquisition. This RTLS is used to detect structurally similar components to annotate unknown flavonoids in tea.

### **Please explain why your abstract is innovative for mass spectrometry?**

Here we show the creation of a high quality HRAM MS<sup>n</sup> spectral library, which is then utilized for confident annotation of flavonoids using RTLS.

### **Co-authors:**

*Bashar Amer, Thermo Fisher Scientific*

*Reza Jafari, MetaSci Inc*

*Andreas Hühmer, Thermo Fisher Scientific*

Poster number: **IM-PA-041**

## **INTELLIGENT DATA ACQUISITION WORKFLOW TO OPTIMIZE QUANTITATION OF LOW ABUNDANT METABOLITES IN HIGH QUANTITATIVE DYNAMIC RANGE SAMPLES**

Abstract ID: **323**

**Presenting author: Bashar Amer, Chromatography and Mass Spectrometry Division, Thermo Fisher Scientific, San Jose, California, United States**

### **Introduction**

Biological matrices complexity with differences in metabolites abundance and extensive variety of their physicochemical properties continue to challenge metabolomics platforms. Efforts are continuously made to improve analytical workflow in term of sensitivity, mass accuracy, and linear dynamic range. Thermo Scientific™ developed an intelligent data acquisition workflow called AcquireX for efficient and comprehensive sample, and study characterization. This approach enhances real-time spectral analysis with selective LC-MS<sup>n</sup> acquisition, thereby experimentally connecting and integrating information from otherwise independent experiments. Through automated creation of iterative background exclusion and inclusion lists, AcquireX enables MS<sup>n</sup> acquisition for low abundant features, which would have not been possible by conventional data dependent acquisition methods. This improves the MS<sup>n</sup> coverage of low abundant compounds, which might be of biological interest in complex samples.

### **Methods**

NIST SRM 1950 plasma and chemical standards solutions were diluted to several magnitudes. Spiked plasma with isotopically labeled metabolites at various concentrations were also used. Extracted metabolites separated on a Hypersil GOLD™ column connected to a Vanquish™ system. Data were acquired on an Orbitrap Exploris™ 240 using an AcquireX acquisition workflow. Deep scan was used to automate the creation of exclusion (MS<sup>1</sup> of background ions) and inclusion (MS<sup>1</sup> of metabolites of interest for MS<sup>2</sup> acquisition) lists with dynamic modification in between each injection. Compound Discoverer™ and TraceFinder™ were used for metabolites annotation and quantitation.

### **Preliminary data (results)**

The described workflow helps in confident annotation of low abundant features. This study also shows a potential potency of AcquireX for accurate quantitation of low abundance metabolites in high dynamic range biological samples such as plasma.

### **Please explain why your abstract is innovative for mass spectrometry?**

Here we extend the AcquireX workflow to assess potential improvement on the quantitation of low abundance metabolites in high dynamic range samples.

### **Co-authors:**

*Rahul Ravi Deshpande, Chromatography and Mass Spectrometry Division, Thermo Fisher Scientific, San Jose, California, United States*

*Andreas Hühmer, Chromatography and Mass Spectrometry Division, Thermo Fisher Scientific, San Jose, California, United States*

Poster number: **IM-PA-042**

## **AN AUTOMATED SAMPLE PREPARATION PLATFORM FOR ROBUST MICRO-FLOW LC-MS PROTEOMICS ANALYSIS IN MAMMALIAN PERIPHERAL BLOOD MONONUCLEAR CELLS**

Abstract ID: **325****Presenting author: Yang Liu, Thermo Fisher Scientific**

### **Introduction**

Peripheral blood mononuclear cells (PBMCs) are easily collected and act as sentinels monitoring physiological perturbations after certain biological inputs in the organism. Therefore, understanding the differences in proteomes of these cells are an attractive platform to carry out pharmacogenomics, molecular and immunologic studies. Here, we use liquid chromatography-mass spectrometry (LC-MS) to study protein expression differences among PBMCs harvest from clinically relevant animals. We introduce an automated sample preparation platform that allows robust proteomics analysis in mammalian PBMCs. This platform provides standardized sample preparation processes, with high ease-of-use experience and limited user involvement. Together with a robust micro-flow LC-MS workflow, we demonstrate a high quality, deep analysis of the proteomes of human, non-human primate, mouse, rat, canine, and minipig PBMCs.

### **Methods**

PBMCs were purchased from BioIVT (human, non-human primate, mouse, rat, canine, and minipig) and were prepared by the automated platform for hands off processing. 1 µg of digested peptides were loaded onto a Thermo Scientific™ Vanquish Neo UHPLC system coupled to a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer at a throughput of 30 samples/day. A Thermo Scientific™ High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) Pro Duo interface provided additional separations at different compensation voltages (CVs). Data was analyzed on Thermo Scientific™ Proteome Discoverer™ 3.0 software (PD) using CHIMERYS. Protein and peptide groups were filtered at 1% FDR rate.

### **Preliminary data (results)**

The novel automated platform executes the standardized procedures for proteomics sample preparation, such as protein lysis, DNA removal, protein reduction and alkylation, digestion, clean-up and peptide concentration measurement. We evaluated different aspects of the performance of the automated sample preparation platform, such as digestion efficiency, alkylation efficiency and artificial modification. These metrics indicate the performance of this platforms in each critical step during the automated procedure. For all six proteomes, digestion efficiency was 98% and the alkylation rates were over 99%. Methionine oxidation was identified in 5% of the peptides in each sample. After being prepared on the platform, samples were dried, reconstituted and injected to LC-MS analysis. The FAIMS Pro Duo interface was coupled to micro-flow LC to offer further separations in gas phase. Each sample was fractionated by 6 CVs (-30V to -55V) and acquired in triplicate. GPF provides deep proteome profiling without the need for off-line RPLC fractionation, which reduced the overall experiment time. The additional separation also enabled less background noise in the MS spectra. Data was searched against the Uniprot mammalian database (98280 entries) in PD 3.0 using CHIMERYS. Percolator FDR calculation was used to only report spectra within 1% FDR rate. For each animal species, the combination of 6 gas fractions increased the number of proteins by 25% - 30%, compared to the maximum identification from a single CV. These six mammalian animal species share homology proteins. Proteins discovered in every sample in common were quantified to demonstrate the differential expression in each animal species.

### **Please explain why your abstract is innovative for mass spectrometry?**

This novel end-to-end automated sample preparation platform coupled with the robust micro-flow LC-FAIMS-MS workflow can significantly increase the experiment throughput while maintaining reproducibility, robustness and performance in LFQ proteomics analysis.

### **Co-authors:**

*Amirmansoor Hakimi, Thermo Fisher Scientific**Steven L. Reeber, Thermo Fisher Scientific**David M Horn, Thermo Fisher Scientific*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Kristan Bahten, Thermo Fisher Scientific*  
*Runsheng Zheng, Thermo Fisher Scientific*  
*Oleksandr Boychenko, Thermo Fisher Scientific*  
*Woong Kim, Thermo Fisher Scientific*  
*Daniel Lopez-Ferrer, Thermo Fisher Scientific*

Poster number: **IM-PA-043**

## DECONVOLUTION OF HIGH RESOLUTION SPECTRA FOR TOP-DOWN PROTEOME

Abstract ID: **378**

**Presenting author: Anatoly N Verenchikov, MSC-CG Ltd**

### Introduction

Applying LCMS with Electrospray ionization for Top-down proteome is limited by complexity and by a low dynamic range of protein mass spectra. Typically, MS spectrum of mid-size protein (20-30kDa) is presented by ~1000 isotopic peaks, and MSMS spectra - by ~10,000 isotopic peaks at various charge states. Using high resolution FTMS ICR and Orbitraps sets the limit onto a total charge content of mass spectra up to  $1E+6$  charges and the detection limit is ~10-100 charges, thus limiting overall dynamic range under  $1e+5$ . MR-TOF technology improves total dynamic range by factor of 100 while resolving protein isotopes. Multiplicity of mass peaks is wrapped with a deconvolution software and MSMS spectra are interpreted with MSMS fit for proteins.

### Methods

A home-built prototype of multi-reflecting TOF at resolution  $R \sim 200K$  allows resolving isotopic peaks of mid size proteins at  $\sim 1e-7$  total abundance per total ion content. An in-house developed deconvolution software wraps multiple MS peaks into mono-isotopic molecular peaks of proteins, thus, greatly simplifying interpretation of protein mixtures and of fragment protein spectra. The algorithm accounts for an average isotopic distribution and selects isotopic peaks based on their intensity and accurately measured mass. An in-house developed MSMS-fit algorithm has been designed to interrogate protein MSMS spectra against the reduced number of sequence hypotheses and mutations.

### Preliminary data (results)

**Figure 1** presents an example of resolved isotopes of 80kDa protein Apotransferin. At first glance, MS spectra of protein mixtures or protein MSMS spectra look like a heavy mess, further complicated by a limited statistics of individual peaks. Deconvolution procedure accumulates ion statistics from a huge peaks multiplicity, which improves the mass accuracy greatly. **Figure 2** presents an example of deconvolved molecular weight spectrum for mixture of 6 proteins. In addition to annotated proteins, MW spectrum contains minor protein fragments in  $\sim 1000$  fold intensity span. The obtained 300ppb mass accuracy substantially reduces the number of sequence candidates. MSMS-fit algorithm allows interrogating protein MSMS spectra against sequence hypotheses. The developed prototype instrument and the software approach would be most efficient for peptidome and mid-down proteome.

### Please explain why your abstract is innovative for mass spectrometry?

MR-TOF improves dynamic range for protein analysis. Resolution of 200K in combination with charge/isotope deconvolution and MSMS-fit are efficient for peptidome and mid-down proteome.

### Co-authors:

*Vasily V Makarov, MSC-CG Ltd*

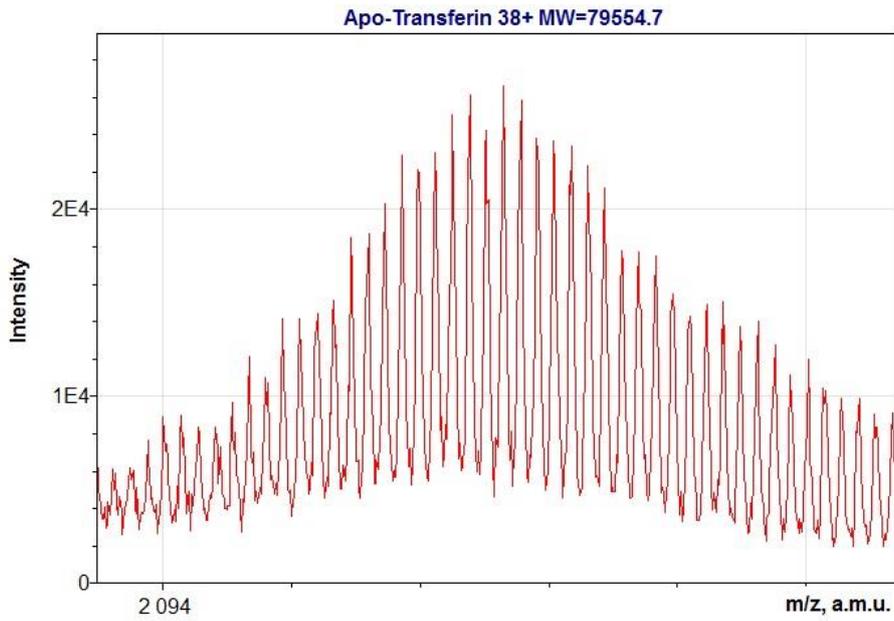


Fig.1. Isotopes of 80kDa Apotransferin protein at Z=38 charge

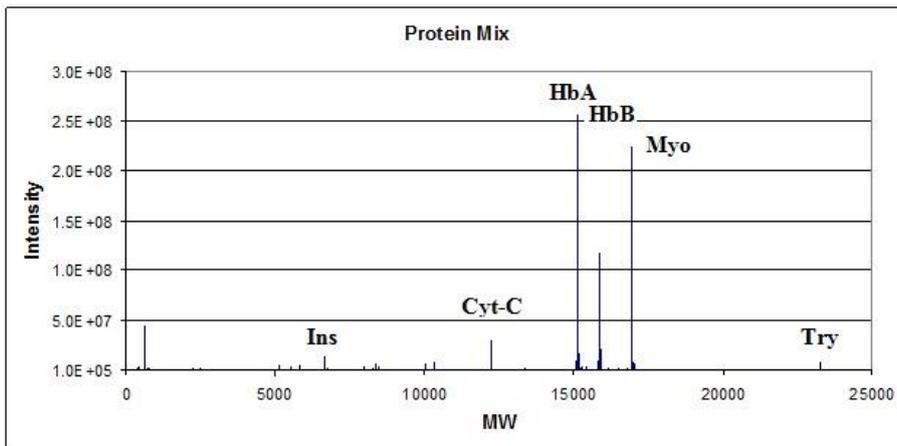


Fig.2. Mixture of 6 proteins after charge/isotope deconvolution

Poster number: **IM-PA-044**

## **AN AUTOMATED END TO END SAMPLE PREPARATION PLATFORM FOR HUMAN PLASMA PROTEOMICS ANALYSIS**

Abstract ID: **389**

**Presenting author: Woong Kim, Thermo Fisher Scientific**

### **Introduction**

Human plasma continues to be one of the major biological fluid used for diagnostics and prognosis of diseases but also expanding quickly into being used for continuous health monitoring of various physiological aspect of human health such as aging research and therapy. Mass spectrometry based proteomics has been one of the most effective tool to identify, quantify, and monitor proteins in plasma. As the throughput of data analysis is increasing a comprehensive hands off solution for sample preparation of human plasma is becoming essential to gain deeper insight into the biological information contained in the human plasma. Here we describe an automated end to end and hands-off workflow reducing the human error while increasing the reproducibility and quality of plasma processing for mass spectrometry analysis.

### **Methods**

Plasma from one source was used to focus evaluation on the variation introduced through the liquid handler, new chemistry, and the automated processing steps. 1.5ul (~96ug) of human plasma was manually aliquoted into 36 wells containing lysis buffer. The plate containing plasma was placed into the liquid handler for an hands-off automated processing of the plasma samples. Custom reagents and formats were implemented as a ready-for-use format including all required reagents. The processed samples were analyzed in Orbitrap Exploris™ 240 Mass Spectrometer with an online Vanquish Neo. Proteome Discoverer™ software was used for data analysis.

### **Preliminary data (results)**

In order to enable an automated hands-off end to end solution for plasma sample preparation for bottom up mass spectrometry analysis, custom chemistry, custom liquid handling, and custom reagents were developed. The automated hands-off end to end platform was evaluated using several aspects highlighting different steps in preparation of human plasma workflow. The characteristics evaluated included digestion efficiency, alkylation efficiency, in vitro artifactual modification introduced during the sample processing, peptide concentration, peak characteristics of PRTC peptides. Additionally, the ease of use and the user experience were also evaluated to evaluate the value of automating sample preparation. Using a data depend acquisition (DDA) method with 60min gradient, approximately 500 proteins were quantified across the 36 samples. Digestion efficiency was >90%, reduction/alkylation efficiency was >90%. Manual time the user had to spend were <20min (aliquoting samples into the sample input plate and resuspension of the clean peptides). More importantly the CV between the 36 sample were below 10% between the 36 samples. This variation encompasses manual aliquoting of the sample into the sample input plate and resuspension of the clean peptide indicating that the automated hands-off end to end platform developed is even lower than what is measured in this experiment. This platform offers an exciting opportunity to increase the throughput and reproducibility, while decreasing the variability, and simultaneously monitor the quality of the mass spectrometry analysis for each run. The platform also offers expandability into other more complex sample preparation for plasma analysis such as protein-protein interaction and PTM analysis.

### **Please explain why your abstract is innovative for mass spectrometry?**

The study highlights a platform to automate sample preparation for bottom up proteomics towards eliminating sample preparation as a bottleneck in proteomics research.

### **Co-authors:**

*Amirmansoor Hakimi, Thermo Fisher Scientific*  
*Steven Reeber, Thermo Fisher Scientific*  
*Kristan Bahten, Thermo Fisher Scientific*  
*Runsheng Zheng, Thermo Fisher Scientific*  
*Oleksandr Boychenko, Thermo Fisher Scientific*  
*David Horn, Thermo Fisher Scientific*  
*Daniel Lopez Ferrer, Thermo Fisher Scientific*

Poster number: **IM-PA-045**

## PHOSPHOPEPTIDES: FLOW PEPTIDE SYNTHESIS AND STRUCTURAL CHARACTERIZATION BY CYCLIC IMS-MS

Abstract ID: **396**

**Presenting author: Adina Noémi Borbély, MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group, Department of Analytical Chemistry, ELTE Eötvös Loránd University**

### Introduction

Synthetic phosphopeptides are ideal model compounds in the mass spectrometry (MS) based identification of protein phosphorylation. However, synthesis, characterization and identification of phosphopeptides is still a challenge, especially in the case of multiphosphorylated peptides.

### Methods

We recently developed a fast, automated flow peptide synthesis method<sup>[1]</sup> to improve the speed and efficiency of solid phase peptide synthesis. In this project, we have successfully adapted and improved the automated flow peptide synthesis method to prepare peptides containing phosphorylated serine and threonine residues with high yield and purity.

### Preliminary data (results)

Various Fmoc-deprotection procedures were investigated to preclude the main side reaction, the base-mediated  $\beta$ -elimination of the phosphate group during the synthesis of phosphopeptides. Improved deprotection protocol resulted in phosphorylated peptides with significantly improved yields.

Peptides of different length from the N-terminal tail of human H3 histone, as well as singly, doubly, and triply phosphorylated histone peptide derivatives have been analyzed using cyclic ion mobility-mass spectrometry (cIMS-MS). The phosphopeptide isomers could be differentiated based on their collision cross section (CCS) values and identified also from mixtures. Optimized tandem mass spectrometric methods provided reliable structural identification to confidently localize the site of phosphorylation.

[1] V. Farkas, K. Ferentzi, K. Horváti, A. Perczel, *Org. Process Res. Dev.* **2021**, 25, 182–191.

The research was supported by the Lendület (Momentum) Program of the Hungarian Academy of Sciences (HAS, MTA). Project no. 2018-1.2.1-NKP-2018-00005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2018-1.2.1-NKP funding scheme. The framework of ELTE Thematic Excellence Program (Synthesis+) was supported by the Hungarian Ministry for Innovation and Technology.

### Please explain why your abstract is innovative for mass spectrometry?

Optimized tandem mass spectrometric methods provided reliable structural identification to confidently localize the site of phosphorylation

### Co-authors:

*Eszter Szombati, MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group*

*Kristóf Ferentzi, MTA-ELTE Protein Modeling Research Group*

*Viktor Farkas, MTA-ELTE Protein Modeling Research Group*

*Gitta Schlosser, MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group, Department of Analytical Chemistry, ELTE Eötvös Loránd University*

Poster number: **IM-PA-046**

## **TWO APPROACHES TO IDENTIFY DIFFERENTIAL COMPONENTS BETWEEN TWO POLYMER SAMPLES BY USING INTEGRATED QUALITATIVE ANALYSIS WITH PYROLYSIS-GAS CHROMATOGRAPH MASS SPECTROMETRY AND DIRECT MASS ANALYSIS WITH FIELD DESORPTION MASS SPECTROMETRY**

Abstract ID: **466**

**Presenting author: Yoshihisa Ueda, JEOL Ltd.**

### **Introduction**

The pyrolysis gas chromatograph mass spectrometry (Py-GC-MS) requires time and advanced knowledge to obtain qualitative information for polymer samples because of the large number of components detected and almost all them do not register in the electron ionization (EI) mass spectrum database. When this happens, soft ionization is very helpful for obtaining and identifying the molecular ions. Recently we have developed an integrated qualitative analysis workflow to combine and interpret the information from EI and soft ionization data for GC-MS. In this work, we have applied this workflow to polymer samples to identify the differential components between two polymer samples. And we have also applied field desorption (FD) analysis of additives such as flame retardants, which are difficult to analyze by Py-GC-MS.

### **Methods**

Commercially-available polypropylene and polyethylene (PP/PE) copolymers and PP products were used as a test samples in this study. The PP/PE samples heated at 600 °C in the PY-3030D pyrolyzer (Frontier Lab, Fukushima, Japan) for GC/EI and GC/Field Ionization (FI) measurements and PP samples were dissolved in Acetone for FD measurement. The sample measurements were carried out using a JMS-T2000GC (JEOL, Tokyo, Japan), GC-HRTOFMS system and a combination EI/FI/FD ion source. The qualitative data processing were performed with msFineAnalysis (JEOL, Tokyo, Japan) and Mass Mountaineer (RBC Software, MA, USA).

### **Preliminary data (results)**

At the beginning, defective and non-defective PP/PE products were measured by GC/EI method (n=5, each) for the variance component analysis to extract the differential components, then GC/FI method (n=1, each) for the integrated qualitative analysis to identify the unregistered compounds in the database. In the variance component analysis, identity determination was performed based on the retention time, chromatogram peak shape, and mass spectral similarity. Differences determination was performed to be components that differed by more than twofold in intensity at a 5% level of significance. After the variance component analysis, both common components and difference components were identified by using integrated qualitative analysis with accurate mass spectrum for EI and FI. Both the variance components analysis and the integrated qualitative analysis were performed automatically on the msFineAnalysis software. In top 50 intensity components, five components characteristic of defective products were extracted. Two of the five components were database-registered compounds and were identified as Styrene and Acrylonitrile, respectively. The other three components were unregistered compounds, but integrated qualitative analysis suggested that they were aromatic compounds with molecular formula with  $C_{11}H_{11}N$ ,  $C_{14}H_{14}N_2$ , and  $C_{19}H_{19}N$ , respectively. As a result of the these analyzes, these five components indicated the presence of AS resin in the defective product.

Next, we have compared two types of the PP product A and B by FD measurements. While the Py-GC-MS method did not reveal any differences between these products, the FD mass spectra showed differences the flame-retardant additive from Product B.

### **Please explain why your abstract is innovative for mass spectrometry?**

The combination of the integrated analysis using Py-GC-MS data and the direct mass analysis using the FD method is useful as a detailed polymer analysis.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

**Co-authors:**

*Takao Fukudome, JEOL Ltd.*  
*Azusa Kubota, JEOL Ltd.*  
*Masaaki Ubukata, JEOL Ltd.*

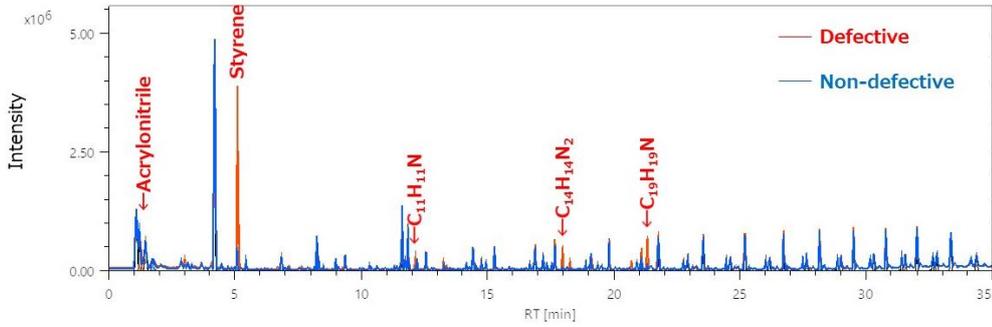


Figure1 Py-GC-EI Total ion current chromatograms for defective and non-defective

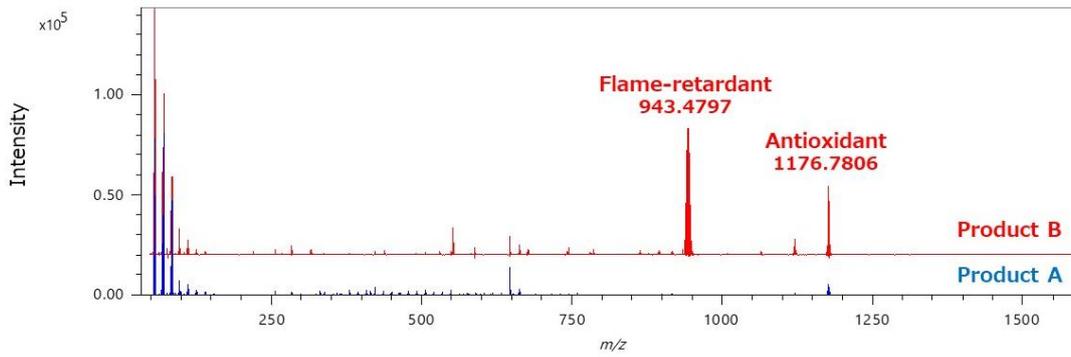


Figure2 FD mass spectra for PP products A and B

Poster number: **IM-PA-047**

## INVESTIGATING STRUCTURAL INTERCONVERSION OF GAS PHASE ISOMERS OF OLIGONUCLEOTIDES WITH TANDEM ION MOBILITY MASS SPECTROMETRY

Abstract ID: **537**

**Presenting author: Alexey Barkhanskiy, Manchester Institute of Biotechnology**

### Introduction

Phosphorothioate oligonucleotides are biopharmaceutical molecules of high interest and present a manufacturing challenge owing to a large number of stereoisomers produced in the process. Traditionally, structural characterisation of ssDNA oligos is performed with LC-MS using ion pairing reagents, and the use of standards to assign stereochemistry. The increased interest in oligos for therapeutic use and the need to characterise products from bulk synthetic material calls for the development of alternative higher throughput methods for structural characterisation. In this work, we use direct infusion tandem ion mobility mass spectrometry as an alternative to LC-MS to characterise diastereoisomers of phosphorothioate oligonucleotides. We also demonstrate how we can determine the rate constants for gas phase interconversion processes in these oligos via tandem ion mobility experiments.

### Methods

Oligonucleotide sequences (6mers and 7mers) were purchased from Integrated DNA Technologies as 100  $\mu\text{M}$  solutions in nuclease-free water. Aqueous stock solutions were diluted to 1  $\mu\text{M}$  in 50 mM  $\text{NH}_4\text{HCO}_3$  pH 8 and loaded into borosilicate glass capillaries (World Precision Instruments) pulled on a P-2000 micropipette puller (Sutter Instrument Company). A Waters Select Series Cyclic IMS mass spectrometer equipped with a nanoESI source was used for tandem ion mobility experiments, with 1 ms ion slices subjected to varying trapping time in the array region prior to separation.

### Preliminary data (results)

Ion mobility ATDs of a  $[\text{M}-2\text{H}]^{2-}$  ion of the TC\*GTCA ssDNA sequence, which contains a single phosphorothioate linkage (pS, location denoted by an asterisk) resulting in a mixture of two diastereoisomers, and the ATD of the identical sequence ssDNA ion with a regular phosphodiester linkage is shown in Figure 1. Both ATDs display multiple features which may be due to the stereochemistry and the different available sites for deprotonation giving rise to different configurations in the gas phase. Introduction of an additional chiral centre into the molecule results in a different ATD which shows the sensitivity of this method to discern stereoisomers.

Utilising the IMS-IMS capability of instrument we selectively subjected each ATD feature from a given phosphorothioate oligo to further scrutiny. Remarkably we find that for TC\*GTCA there are four mobility separated features, the first two and the last two will interconvert, but the entire ATD does not regenerate, allowing a sub classification of the mobility separated species. For example, following ion mobility isolation and reinjection into the separation region, species IV regenerates species III, indicating rapid interconversion, as demonstrated in Figure 2. By trapping ions for a varying time prior to final separation, we were able to detect changes in relative intensity, allowing us to extract structural and kinetic information. This investigation offers the potential for developing an IM-MS method with wider application to analyse oligonucleotides.

### Please explain why your abstract is innovative for mass spectrometry?

Tandem ion mobility experiments uncover information for gas phase structural interconversion for oligonucleotide ions and demonstrate potential for a general method for separation of diastereoisomers.

### Co-authors:

*Depanjan Sarkar, Manchester Institute of Biotechnology*

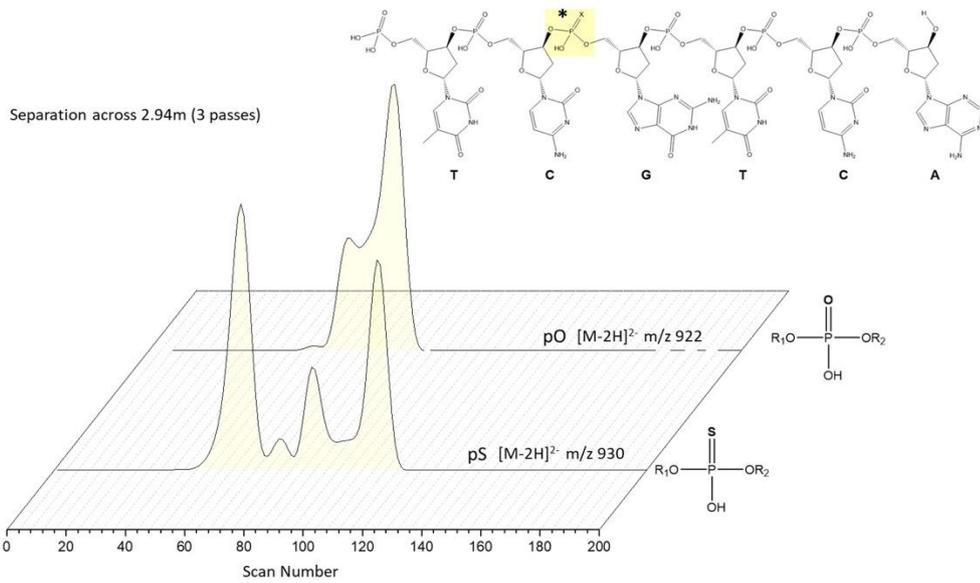
*Jakub Ujma, Waters Corporation*

*Sarah Lovelock, Manchester Institute of Biotechnology*

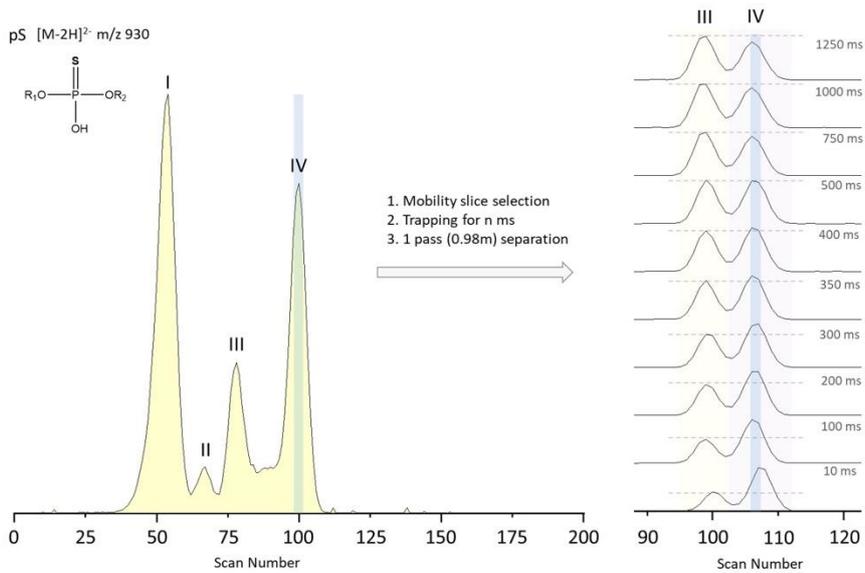
*Perdita Barran, Manchester Institute of Biotechnology*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Structure and ATDs for phosphorothioate and phosphodiester oligonucleotide ions



Interconversion of population IV of the phosphorothioate oligonucleotide ion

Poster number: **IM-PA-048**

## TOTAL CORRELATION MASS SPECTROMETRY (TOC-MS) ON A LINEAR ION TRAP

Abstract ID: **555**

**Presenting author: Nathan Cassidy, Verdel Instruments**

### Introduction

2DMS is a tandem mass spectrometry (MS/MS) method which allows the user to fragment all precursor ions in a mixture simultaneously, generating chimeric fragment spectra, and correlate which fragments derive from which precursor by modulating ions through the fragmentation zone.

Derived from the 2-dimensional mass spectrometry technique on FTICR mass spectrometers, Verdel Instruments is developing ToC-MS to provide the same tools and capabilities using smaller, and more commonly available Q-TOF mass spectrometers.

The comprehensive fragmentation patterns for all ions suggested the new acronym of ToC-MS, for "Total Correlation Mass Spectrometry". A key requirement of ToC-MS is radially dependent fragmentation of precursor ions, so that there is correlation between excitation of precursor and intensity of fragment ions.

### Methods

Starting with a MaXis HD II ETD QTOF, the quadrupole has been modified to create a linear ion trap (LIT). A 213 nm laser aligned along the z-axis of the quadrupole allows for radially dependent ultra-violet photo-dissociation (UVPD) fragmentation of ions.

The excitation of all precursor ions simultaneously is achieved through the combination of a range of frequencies in the form of a SWIM (stored-waveform ion radius modulation) waveform. A sequence of SWIM pulses encodes the intensity in the time domain for each precursor ion, with a frequency which is correlated with the m/z for each fragment ion.

### Preliminary data (results)

The new ToC-MS instrument is complete and the instrument design, performance specifications achieved, and demonstration of the simultaneous MS/MS analysis of complex mixtures will be shown.

For the ToC-MS results, during the trapping time the laser is pulsed immediately after the SWIM pulse. The UV pulse energy is selected to result in at least a 50% drop in precursor intensity. The SWIM pulses excite the precursor ions out of the UV fragmentation zone, but also result in an increased collision induced dissociation (CID) probability. The gain of the SWIM pulses is set for maximum excitation without ejection.

Sampling Substance P, applying 2048 SWIM pulses combined with UVPD, the ToC-MS technique has been used to understand its structure. The modulation of the  $[M+3H]^{3+}$  precursor is correlated with the  $B_{10}^{2+}$ ,  $B_{10}^{3+}$  and  $B_9^{2+}$  fragment intensities. The ToC-MS technique has also been used to analyse Polymyxin B, Melittin, BSA and GluFIB.

The ToC-MS technique has been used to sample influent waste water and has aided the identification of common biomarkers including acetaminophen, atenolol, caffeine, carbamazepine and codeine. The ToC-MS technique has aided the identification of these biomarkers through the correlation of UVPD fragments. For example, acetaminophen produces four fragments that were confirmed structurally: M-CH<sub>2</sub>, M-H<sub>2</sub>O, C<sub>6</sub>H<sub>5</sub>O and C<sub>4</sub>H<sub>4</sub>O.

Thus, analysis of complex mixtures through correlation between fragments and precursors is achieved without prior ion isolation or chromatographic separation.

### Please explain why your abstract is innovative for mass spectrometry?

2DMS on a Q-TOF mass spectrometer using the quadrupole as a linear ion trap (LIT).

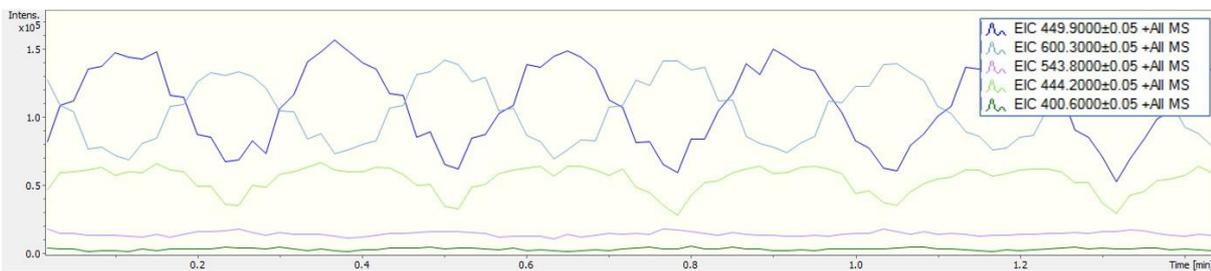
**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

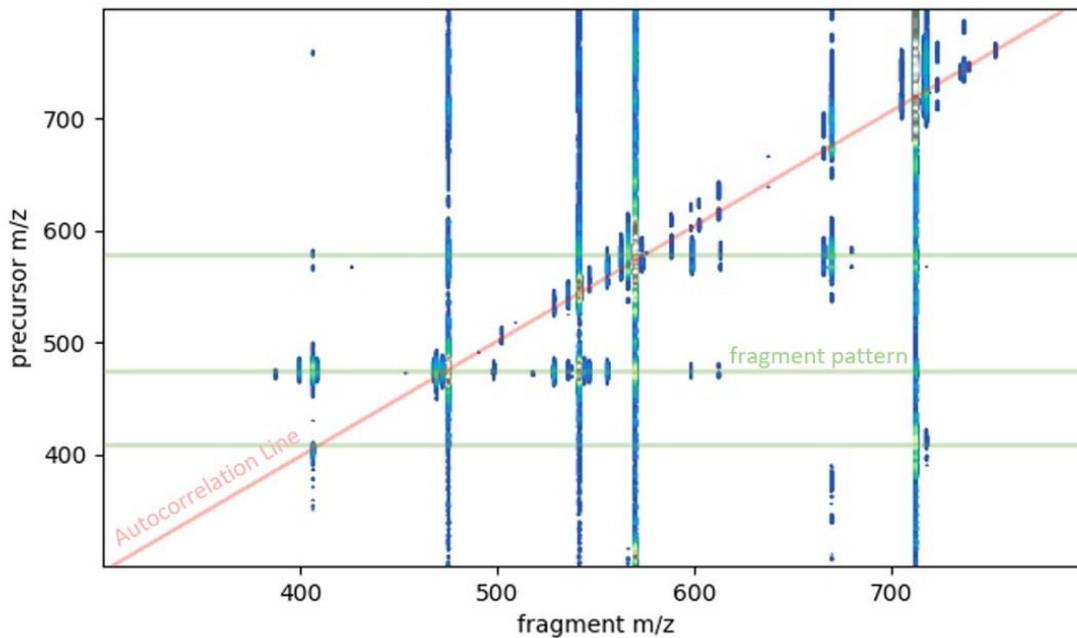
UVPD fragmentation of peptides and pharmaceuticals.

**Co-authors:**

*Robert Burch, Verdel Instruments*  
*Nik Bowdler, Verdel Instruments*  
*Alex Colburn, University of Warwick*  
*James Hobby, Verdel Instruments*  
*David Knight, Verdel Instruments*  
*Fintan Moloney, Verdel Instruments*  
*Jack Rice, Verdel Instruments*  
*Tony Seal, Verdel Instruments*  
*Steven Wright, Verdel Instruments*  
*Peter B O'Connor, University of Warwick*



Modulation of precursor and fragment intensities in the fragmentation zone.



ToC-MS plot of Polymyxin B with UVPD.

Poster number: **IM-PA-049**

## **STREAMLINING WORKFLOW FROM CHARACTERIZATION TO QUALITY CONTROL OF THERAPEUTIC OLIGONUCLEOTIDES IMPURITIES ACROSS IPRP-LC-HRAM-MS PLATFORMS**

Abstract ID: **568**

**Presenting author: Catharina Crone, Thermo Fisher Scientific, Bremen, Germany**

### **Introduction**

In order to support fast growing therapeutic oligonucleotides programs, sensitive and robust analytical strategies are desired to efficiently characterize and monitor these novel modalities and their impurities during development, manufacturing and quality control. IPRP-LC-MS/MS is required to provide base-by-base sequence confirmation and localization of modifications for complete characterization, whereas full MS is often chosen for identity and impurity test to be deployed in the QC lab. In this study, HRAM ddMS<sup>2</sup> data were acquired on an Orbitrap hybrid system coupled with IPRP-LC for characterizing synthetic oligonucleotide FLPs and their impurities. The full MS based monitoring assay was subsequently created and transferred to two HRAM full MS systems. Seamless method transfer was demonstrated by obtaining comparable results across 3 systems.

### **Methods**

Synthetic DNA and RNA oligonucleotides with different length and modifications were separated on DNAPac RP column (4 µm, 2.1 × 50 mm) using a Vanquish Horizon UHPLC system. Both full length product and impurities were characterized and quantified using a ddMS<sup>2</sup> method on an Orbitrap Exploris™ 240 mass spectrometer, sequences were identified using Biopharma Finder™ 5.0 software. For the monitoring assay, full MS data were acquired on an Orbitrap Exploris 240 mass spectrometer and two Orbitrap Exploris™ MX mass detectors, and Chromeleon™ CDS software was used for data acquisition, processing and report generation

### **Preliminary data (results)**

Robust and reproducible separation of oligonucleotide FLPs and their impurities were optimized and achieved using a DNAPac™ RP column on the Vanquish™ Horizon UHPLC system. A 9 oligonucleotides mixture ranging from 6mer to 100mer were used as an SST sample to demonstrate system performance. Full scan MS data acquired with a resolution setting of 120,000 at m/z 200, allowing isotopic baseline separation of the oligonucleotides from 6mers to 60mers, leads to accurate mass measurement with sub to low single ppm mass error, whereas a resolution setting of 240,000 at m/z 200 with intact protein mode is required to obtain isotopic baseline separation of the 100mer oligonucleotides with improved S/N. Using comparative analysis feature in the BioPharma Finder oligonucleotide workflow, we can quickly optimize the stepped NCE value or range for different size oligonucleotides. Using the optimal NCE settings, the high-quality ddMS<sup>2</sup> data were obtained on the Orbitrap Exploris 240 mass spectrometer to allow confident identification and relative quantification of FLPs and their low abundance impurities (<0.1%) with complete or nearly complete fragment coverages of oligonucleotides ranging from 6mer to 60mer. FLPs and some of their impurities were then selected for developing the full MS based monitoring assays on the same system which used the enterprise compliance-ready Chromeleon CDS for data acquisition, processing and reporting. Once developed, the monitoring assay was transferred to and performed on two full-MS only Orbitrap Exploris MX mass detectors.

### **Please explain why your abstract is innovative for mass spectrometry?**

Demonstrate total workflow solution from in-depth characterization to robust monitoring of oligonucleotides and their impurities with seamless method transfer

### **Co-authors:**

*Hao Yang, Thermo Fisher Scientific, San Jose, Ca, United States*  
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Poster number: **IM-PA-050**

## **PHOTODISSOCIATION OF ATOMIC CLUSTERS IN A MULTI-REFLECTION TIME-OF-FLIGHT MASS SPECTROMETER**

Abstract ID: **588**

**Presenting author: Paul Fischer, Institute of Physics, University of Greifswald**

### **Introduction**

Multi-reflection time-of-flight mass spectrometry (MR-ToF MS) is known as a powerful tool for precision mass measurements [F. Wienholtz et al., *Nature* 498:346–349 (2013)] and high-resolution isobar separation [R.N. Wolf et al., *Phys. Rev. Lett.* 110:041101 (2013)] in nuclear physics. In atomic and molecular physics, MR-ToF devices are appreciated for their characteristics as electrostatic ion traps [D. Zajfman et al., *Phys. Rev. A* 55:R1577-R1580 (1997)]. At the University of Greifswald, MR-ToF MS is applied for high-resolution investigations of atomic clusters. Specifically, in-trap photodissociation is employed to study fragmentation behaviors.

### **Methods**

An MR-ToF mass spectrometer is used to capture bunches of atomic clusters produced by laser ablation. While the ions revolve between the device's opposing electrostatic mirrors, in-trap deflector electrodes are used to clean precursor species from contaminants with high separation resolving powers. Species of interest are excited by a laser pulse synchronized to their revolution period. Cluster dissociation leads to new (fragment) species lapping between the mirrors with distinct periods, which can be analyzed with high resolution by increasing the post-excitation storage time.

### **Preliminary data (results)**

Cases of interest include the dissociation channels of various cluster species composed of a single element and the changes thereof upon doping with a single atom from a different element. If the elements are close together in mass, high resolving powers are needed to reliably select precursor species and analyze products, which are provided by the MR-ToF mass spectrometer [P. Fischer et al., *Phys. Rev. Research* 1:033050 (2019)]. The MR-ToF principle also allows time-resolved studies due to the periodic nature of the ions' flight path. Fragments that are continuously produced while a bunch of excited precursors is revolving between the mirrors are used to record the dissociation rate as a function of delay time after the excitation [P. Fischer et al., *Phys. Rev. Research* 2:043177 (2020)]. The time-dependent rates are compared to a statistical model, yielding information on the cluster ensemble's temperature or the species' threshold energy

### **Please explain why your abstract is innovative for mass spectrometry?**

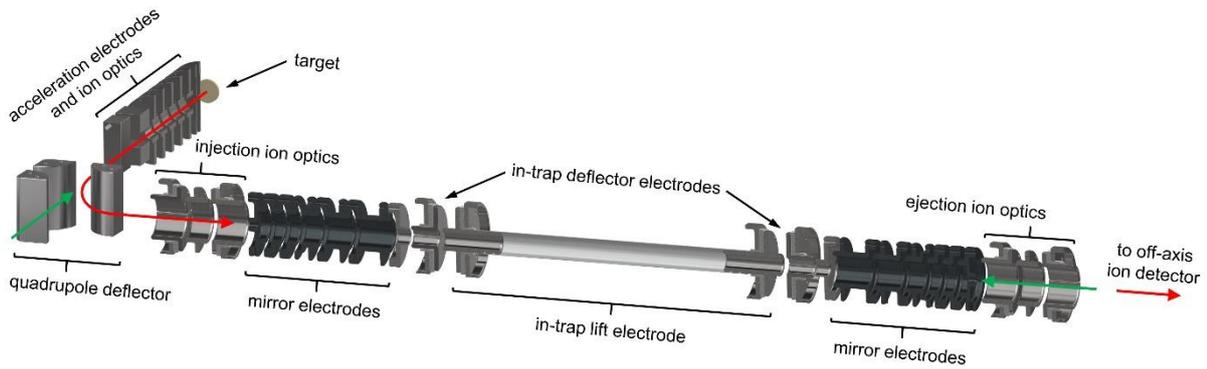
MR-ToF-based photodissociation for the study of cluster fragmentation with high resolving powers for precursor selection and product analysis as well as capabilities for time-resolved investigations.

### **Co-authors:**

*Lutz Schweikhard, Institute of Physics, University of Greifswald*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Experimental setup including multi-reflection time-of-flight mass spectrometer and laser ablation source.

Poster number: **IM-PA-051**

## NITROGEN MUSTARDS-GLUTATHIONE CONJUGATIONS: IN VITRO STUDY

Abstract ID: **616**

**Presenting author: Nurhazlina Hamzah, Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Department of Chemistry, University of Helsinki, P.O. Box 55, FI-00014 Helsinki, Finland.**

### Introduction

Nitrogen mustards (NMs) are classified as toxic chemicals in Schedule 1 in the Chemical Weapons Convention. NMs were defined as blister agents due to their cytotoxic effects. They consist of bis(2-chloroethyl)ethylamine (HN1), bis(2-chloroethyl)methylamine (HN2), and tris(2-chloroethyl)amine (HN3). There are some methods for detecting their hydrolysis products (ethanolamines) as well protein, and DNA adducts in biomedical samples. The methods for analysing protein and DNA adducts of NMs are preferable as they are stable from several weeks to months after exposure. One option to detect the NMs in biomedical samples is to study their conjugation with glutathione (GSH). GSH helps protect cells from potentially toxic electrophiles, and these reactions have been attributed primarily to the detoxication process.

### Methods

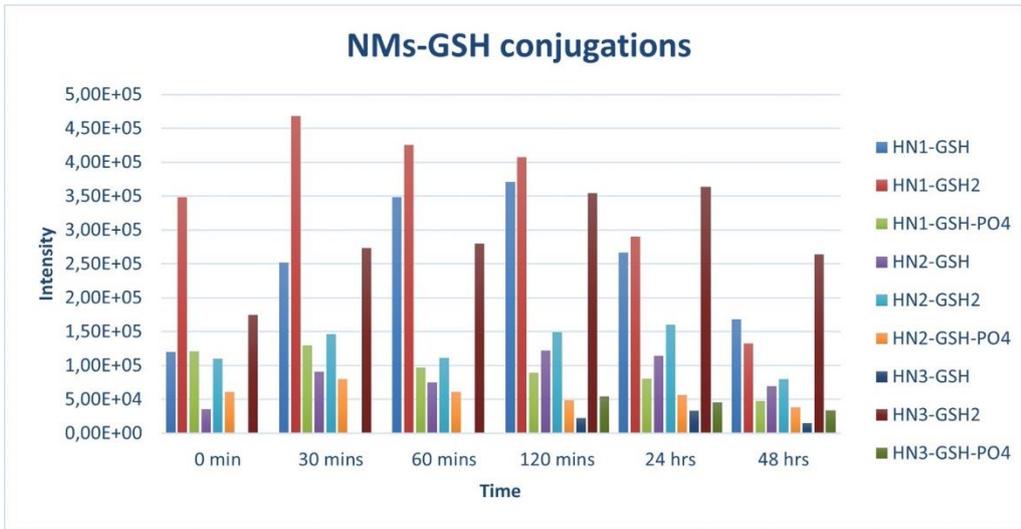
The *in vitro* study was designed to identify new metabolic biomarkers of NMs conjugates with GSH. Individual NMs were spiked into the reaction mixtures consisting of 1 mg/mL human liver S9 fraction containing microsomes, 3 mM of GSH and 0.5 mM of NADPH in 100 mM phosphate buffer (pH 7.4). Six different incubation times ranging from 0 to 48 hours were studied to monitor the formation of the NMs-GSH conjugates in the reaction mixture. The analysis was carried out in full scan mode using the LC-MS/MS. The accurate masses of the conjugates were confirmed using the LC-HRMS/MS.

### Preliminary data (results)

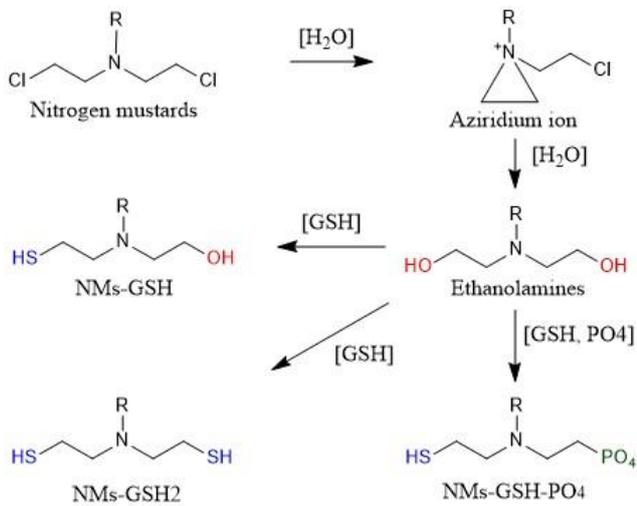
There are three different conjugates found for each NMs which are monogluthionyl, digluthionyl and phosphorylated conjugates. The monogluthionyl conjugates included HN1-GSH, HN2-GSH and HN3-GSH. Other conjugates are the digluthionyl conjugates consisting of HN1-GSH<sub>2</sub>, HN2-GSH<sub>2</sub>, HN3-GSH<sub>2</sub>, and the phosphorylated conjugates, namely HN1-GSH-PO<sub>4</sub>, HN2-GSH-PO<sub>4</sub>, and HN3-GSH-PO<sub>4</sub> for HN1, HN2, and HN3, respectively. Digluthionyl conjugates formed immediately after adding each NMs to the reaction mixtures, followed by monogluthionyl and phosphorylated conjugates. Digluthionyl conjugates showed the highest intensities and are considered primary conjugates in this study. All conjugates are formed primarily in the presence of GSH. The reactivity of the NMs in the reaction mixtures also can be described as follows: HN1>HN2>HN3. Conjugation between NMs and GSH happens when the NMs side chain (leaving group) is replaced by the sulphur atom from the GSH molecule. These reactions occur more quickly than the formation of EAs. When observed for 48 hours, only ethanolamines and NMs-GSH conjugates were produced in this study. The instrument analysis took less than 8 mins per sample. All the measured masses of the conjugates were compared to exact theoretical masses, and errors were calculated. The mass error tolerance by the Organisation for the Prohibition of Chemical Weapons for Biomedical Proficiency Tests states that the mass error should be less than 2.5 ppm for the reported chemicals, showing that these results are acceptable. These GSH metabolites can be used to verify NMs toxification from biological samples like urine.

### Please explain why your abstract is innovative for mass spectrometry?

LC-HRMS/MS was used to confirm the elemental compositions of nine new metabolic biomarkers since no reference chemicals were available.



The proposed NMs-GSH conjugates at different incubation times.



Proposed metabolic route of NMs-GSH conjugations for in vitro study.

Poster number: **IM-PA-052**

## NON-TARGETED ANALYSIS OF CHEMICALS MIGRATING FROM BEER CANS

Abstract ID: **620**

**Presenting author: Ana Kovačič, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia**

### Introduction

Food safety regulations concerning food contact materials (FCM) rely on the screening of known chemicals migrating from packaging materials. To assess the safety of FCM and to reduce human exposure to chemical residues, the identification of potential migrants, including non-intentionally added substances, is required. Accordingly, there is a need for non-targeted analytical investigations, especially improving the most time-consuming step, namely identifying unknown residues.

### Methods

In this study, we performed migration tests from beer cans coated with epoxy resins ( $n = 12$ ) using suitable simulant (20% ethanol), 100% methanol, and beer not yet in contact with packing material. Advanced instrumentation was based on high performance liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry (HPLC-QTOF-MS). The sample preparation, instrumental analysis, and post-acquisition data processing were optimised based on 30 target chemicals to enrich a broad range of compounds from tested matrices.

### Preliminary data (results)

The consistency of data processing with different software tools was confirmed by analysing the same raw data files using MZmine2, ADAP chromatogram builder and MSMS peaklist builder algorithm and an in-house developed algorithm in Python. The results showed high coherence with an overlap of more than 80% of mass features between different processing tools. The number of potentially relevant mass features resulting from all tested workflows was reduced to approximately 370 relevant mass features in negative ionisation mode using OPLS-DA (VIP number  $> 1.5$ ). Initially, the annotation of filtered mass features was based on suspect screening (expanded csv-database of possible leaching chemicals from FCM) performed in R using the modified Shiny app. Next, MS/MS spectra of relevant mass features were submitted to SIRIUS 4 to annotate the potential elemental composition and, where possible, chemical composition to relevant mass feature. The results from SIRIUS were compared with *in-silico* prediction of freely available software (MetFrag and CFM-ID) and MS/MS libraries (MassBank and mzCloud). The annotation was successful for more than 40 mass features identified at confidence level 1 to 4, e.g., bisphenols, BADGE derivatives, 4-[11-(4-Hydroxyphenyl)-1-methylbenzo[b]fluoren-11-yl]phenolate, and derivatives of phthalates. Finally, the presence of annotated mass features will be searched in the extract of beer samples.

### Please explain why your abstract is innovative for mass spectrometry?

The developed non-target screening strategy using advanced mass spectrometry-based methods, including strategies for data reduction and annotation of unknown compounds, will reduce the uncertainty in identifying non-targeted compounds.

### Co-authors:

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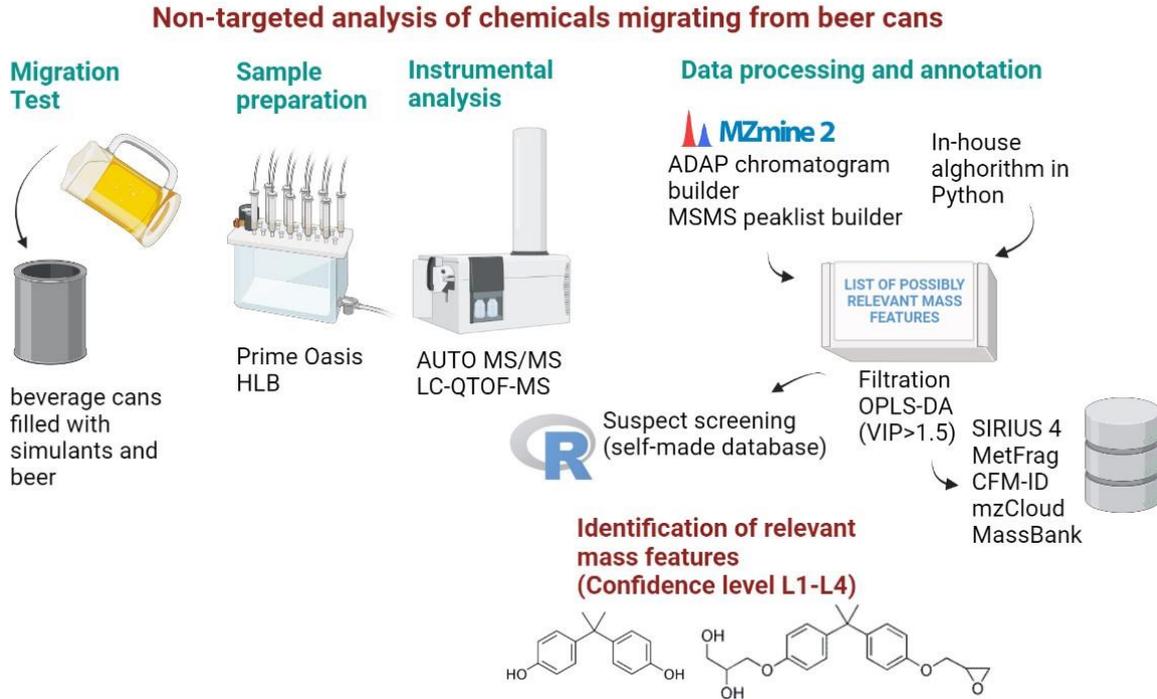
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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Non-target workflow for the analysis of chemicals migrating from FCMs.

Poster number: **IM-PA-053**

## **IMPROVEMENT OF DATA QUALITY IN FT-ICR MS ANALYSIS OF COMPLEX MIXTURES BY INDEPENDENT ACQUISITION AND PROCESSING OF TIME-DOMAIN TRANSIENT DATA**

Abstract ID: **663**

**Presenting author: Alessandro Vetere, Max-Planck-Institut für Kohlenforschung**

### **Introduction**

Mass resolution, mass accuracy, sensitivity and acquisition speed are four vital parameters for the mass spectrometric analysis of crude oil or related samples. Unfortunately, not all of them can be optimized together. While high mass resolution and accuracy can be obtained using Fourier transform mass spectrometers, such as FT-ICR MS, they are typically optimized using stronger fields or longer transient acquisition times – both approaches sacrificing sensitivity, a crucial parameter especially for complex samples.

An alternative approach is the utilization of the more performant absorption mode FT (aFT) instead of the normally applied magnitude mode at the single scan level. More importantly, the aFT mass spectra, being the unreduced data format, open up new avenues for data quality improvement, as presented here.

### **Methods**

Samples from different complex crude oil mixtures were analyzed using a 7 T FT-ICR MS (LTQ FT Ultra, Thermo Scientific, Bremen, Germany), equipped with an external high-performance data acquisition system (FTMS Booster X3, Spectroswiss, Lausanne Switzerland). The independent acquisition of the time-domain transients was performed in parallel with the conventional mass spectra acquisition using various ionization methods, measurement parameters (full scan, spectral stitching, replicates), and sample introduction methods (direct infusion and LC-MS). Data processing (aFT mass spectra generation), analysis and visualization were performed using software packages Peak-By-Peak (Spectroswiss), Composer (Sierra Analytics, Modesto, CA, USA) and Origin (OriginLab, Northampton, MA, USA).

### **Preliminary data (results)**

The utilization of spectral stitching is known to greatly improve the dynamic range and data depth and hence the sensitivity regarding crude oils and related complex samples. Normally, this approach can only be followed by summing up individual spectra. When using an independent device (FTMS Booster X3) for the additional recording of transient information, additional features become available. Apart from the ability to generate aFT mass spectra, individual spectra can now be summed and averaged on the transient level before doing the actual FT. Thus, S/N thresholding is delayed until after obtaining the final mass spectrum, which can lead to an increase in detected signals of around 50%.

Another possibility is the summation of transients across different experiments. This approach appears especially useful, when coupling the mass spectrometer to a chromatographic separation, such as HPLC or to an ion mobility separation. In these cases, the number of scans across a chromatographic peak as well as the acquisition time per scan are limited by the chromatographic time resolution required. A minimum of spectral data depth – i.e. multiple mass spectra – can typically only be obtained by decreasing the acquisition time and therefore also the mass spectrometric resolution. Summing and averaging over multiple replicates of the same measurement eliminates this problem and can lead to a substantial increase in detected signals.

### **Please explain why your abstract is innovative for mass spectrometry?**

Independent and optimized acquisition of time-domain transients from FT-ICR MS leads to a dramatic increase in data quality by enabling the optimization of mass resolution, acquisition time and sensitivity simultaneously.

### **Co-authors:**

*Wolfgang Schrader, Max-Planck-Institut für Kohlenforschung*

Poster number: **IM-PA-054**

## ESTABLISHING A HIGH THROUGHPUT WORKFLOW FOR COMPREHENSIVE YEAST PROTEOMICS

Abstract ID: **700**

**Presenting author: Marian Scherer, U. Distler, O. Vydzhak, R. Danalatos, N. Lohner, B. Luke, P. Baumann, S. Tenzer**

### Introduction

Budding yeast is a well-studied model organism in many areas in biology and many proteomic workflows have been specifically tailored for this organism to improve proteome coverage. Recently a complete draft of the yeast proteome with >5600 proteins has been described, which required extensive prefractionation and is thus not suitable for the analysis of larger sample cohorts. To quantitatively characterize the yeast proteome of a large array of mutants involved in senescence, we optimized lysis and semiautomated sample preparation protocols. Liquid chromatography-mass spectrometry (LC-MS) analyses were performed using an EvoSep One system, ion mobility-enhanced data-independent acquisition (diaPASEF) and data processing with DIA-NN. The optimized LC-MS-based label-free quantitative (LFQ) proteomics platform enables the reproducible high-throughput characterization of yeast proteomes.

### Methods

Total proteins were extracted from yeast cells using alkaline preincubation and boiling in 1% SDS. Extracts were centrifuged at 13.000xg 15 min and supernatants transferred to 96well microtiter plates. Semiautomated sample preparation using a Biomek i7 liquid handling robot (Beckman Coulter) performing an adapted FASP protocol including reduction, alkylation, tryptic digestion and peptide recovery. Peptides were loaded on Evotips and analyzed by LC-MS using an EvoSepOne (60 samples/day method) coupled to a Bruker timsTOF Pro-2 mass spectrometer (diaPASEF, 0.9 s cycle time). Library-free search of rawdata using a yeast UniProt FASTA database and LFQ was performed in DIA-NN.

### Preliminary data (results)

Reproducible protein extraction is a key factor for reproducible quantitative proteomic analyses. Here, we compared several extraction methods, and the combination of alkaline preincubation with boiling in SDS provided a fast, reproducible and high-yield extraction. To ensure reproducibility of sample preparation, we optimized a semiautomated 96-well FASP workflow, which significantly increased throughput and minimized the required manual handling time. The samples in the automated 96-well format show high correlation of precursor quantities with values between 0.93 and 1. For LC-MS analysis, several instrument setups were evaluated. After several optimization steps, the combination of a EvoSep One LC system running with a sample throughput of 60 samples per day (24-minute gradient), and a timsTOF Pro-2 platform in diaPASEF mode provided comprehensive proteome coverage of up to 4000 proteins in a single run with a 1% false discovery rate (FDR). Taking advantage of the high-speed acquisition of the timsTOF Pro-2, we adapted a diaPASEF isolation scheme to the sample type and LC gradient. This resulted in a method with a cycle time of 0.9s which provided an average number of three data points per peak at FWHM (full width at half maximum), resulting in good quantitative precision. On average, more than 40.000 peptide precursors were identified per sample corresponding to over 40.000 stripped peptide sequences and over 4200 protein groups across the whole dataset as reported by DIA-NN. Notably, for more than 3000 protein groups, a data completeness of 100% was achieved.

### Please explain why your abstract is innovative for mass spectrometry?

We have established a reproducible workflow for yeast proteomics that enables the identification and quantification of 4000 protein groups at a throughput of 60 samples per day.

Poster number: **IM-PA-055**

## **DEVELOPMENT AND VALIDATION OF AN ANTIBIOTIC ANALYSIS METHOD IN THE BACTERIAL EXTRACTS BY ORBITRAP LC-HRMS**

Abstract ID: **809**

**Presenting author: Christine Enjalbal, University of Montpellier**

### **Introduction**

Antimicrobials play a central role in modern medicine. However, due to the widespread and untargeted past use of antibiotics, many resistances have developed. The research of new bioactive compounds from the natural samples becomes an essential strategy for new antibiotic discovery.

In order to identify and characterize the unknown complex structures, the advanced mass spectrometer is required with a high accuracy to achieve their generic chemical formula and with MS<sup>n</sup> fragmentation to characterize fragments. The goal of our project, ATB-Discover, is to discover interesting molecules in complex samples so that new antimicrobials can end up in the market.

### **Methods**

Detection, MS<sup>2</sup> as well as MS<sup>3</sup> fragmentations and data treatment were performed on a high-resolution mass spectrometer (LC-HRMS, Orbitrap ID-X, Compound Discoverer, Thermo Scientific) were optimized through a selection of ten standard antibiotic molecules belonging to different molecular families. This "ideal" mix was analyzed in two different matrices (solvent and sample matrix) to be as closed as possible with the real samples. Workflow optimization was carried out to achieve a robust LC-MS<sup>3</sup> method recording reliable results before data treatment. Such LC-MS<sup>3</sup> method was then validated through four main criterions : Specificity, Carryover, Repeatability and Intermediate Precision.

### **Preliminary data (results)**

The results obtained from this validation were acceptable. For the repeatability criterion, the retention time of all molecules interday show a RSD < 2 % in both matrix and solvent sample. The relative intensities of MS<sup>2</sup> fragment ions for all standard molecules intraday have a RSD < 30 %.

### **Please explain why your abstract is innovative for mass spectrometry?**

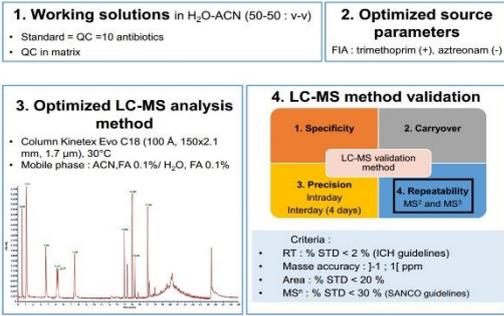
Evaluation of MS<sup>2</sup> and MS<sup>3</sup> repeatability

### **Co-authors:**

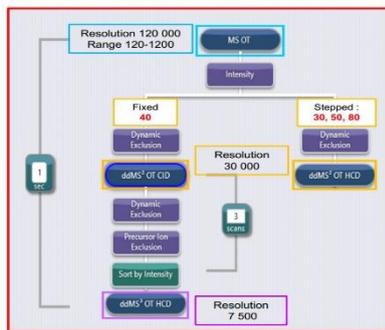
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*Mikail Berdi, DEINOVE*  
*Noémie Fayeulle, DEINOVE*  
*Sarah Ployon, DEINOVE*

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



validation of an antibiotic analysis method



MS3 workflow

Poster number: **IM-PA-056**

## IMPURITY PROFILING OF ORGANIC BY-PRODUCTS OF A NEWLY DEVELOPED GD BASED CONTRAST AGENT

Abstract ID: **821****Presenting author: Sonja Weishaupt, University of Münster, Institute of Inorganic and Analytical Chemistry**

### Introduction

As medicinal products, paramagnetic Gd(III)-based MRI contrast agents (GBCAs) are subject to the European Medicines Agency guidance. Due to the high dose of GBCAs in the range of about 1.2 g Gd per examination, the European guideline requires identification of impurities that exceed a content threshold of 0.05 mass%. Therefore, a thorough analysis of the product for possible by-products is necessary. In case of paramagnetic substances as GBCAs, structure elucidation is difficult, as no structural information can be obtained using  $^1\text{H-NMR}$  spectroscopy. Instead, techniques such as electrospray ionisation high resolution mass spectrometry (ESI-HRMS) and MS/MS experiments are used. For quantitative impurity profiling of Gd-containing by-products, high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) enables significantly better detection limits than HPLC-UV analysis.

### Methods

A reversed phase (RP)-HPLC column with a phenyl phase was used to separate the analytes. For identification of organic by-products, an HPLC-ESI-HRMS system with an Orbitrap mass analyzer was used. An HPLC-UV system and an HPLC-ICP-MS system were used for quantification. The open-source programme MZmine3 was used for the development of a database-supported data analysis.

### Preliminary data (results)

For the quantitative determination of organic Gd-containing by-products, an HPLC-ICP-MS method was developed. With a value of 15 nmol/L, the ICP-MS technique achieves a LOQ that is lower by a factor of 10 than the UV determination. Furthermore, by detecting the  $^{158}\text{Gd}$  signal, the elemental analytical method allows species independent quantification via external calibration with a Gd-containing standard substance. The identification of unknown by-products is performed via HPLC-ESI-HRMS. Gd-containing compounds show isotopic patterns characteristic for the element Gd, which facilitates the finding of signals of possible by-products. The isotopic pattern also allows conclusions to be drawn about the number of complexed Gd atoms. The exact mass detected via HPLC-ESI-HRMS enables the calculation of possible molecular formulae within a mass accuracy range of 5 ppm. Based on this, structural proposals for the compound are developed, which can be supported by additional MS/MS experiments. Due to the large data volumes of HPLC-ESI-HRMS and MS/MS analyses, the use of automated data processing based on a database of already identified by-products offers a helpful tool. The exact mass of the main isotope and the characteristic isotopic pattern of Gd can be used for automated identification of signals of known by-products. For this purpose, an additional data filter function was added to the open-source data analysis programme MZmine3, which allows to check the detected signals for isotope patterns that match the by-products.

### Please explain why your abstract is innovative for mass spectrometry?

For quantification of chemically similar Gd complexes, a new HPLC-ICP-MS method was developed. The identification and development of structural proposals of Gd-containing compounds is done via HPLC-ESI-HRMS or MS/MS.

### Co-authors:

*Wiebke Holkenjans, Bayer AG, Research & Development, Pharmaceuticals*  
*Sandra Balzer, Bayer AG, Research & Development, Pharmaceuticals*  
*Steffen Heuckeroth, University of Münster, Institute of Inorganic and Analytical Chemistry*  
*Uwe Karst, University of Münster, Institute of Inorganic and Analytical Chemistry*

Poster number: **IM-PA-057**

## **MULTIPLE DISSOCIATIONS AND MULTI-STAGE FRAGMENTATIONS CAPABILITIES WITH ACQUIREX WORKFLOW OF ORBITRAP IQ-X TRIBRID MS FOR SMALL MOLECULES STRUCTURE CHARACTERIZATION**

Abstract ID: **834**

**Presenting author: Jingjing Huang, Thermo Fisher Scientific**

### **Introduction**

Impurity profiling and structure characterization by LCMS is an integral part of drug R&D. High resolution mass spectrometer with multiple dissociations and high level MS/MS ( $MS^n$ ) capabilities can provide crucial fragmentation fingerprint information for confident API and drug product impurity profiling and structure characterization.

In this study, Adefovir Dipivoxil impurity profiling was conducted using an LCMS system consisting of a Thermo Scientific Vanquish UHPLC and an Orbitrap IQ-X Tribrid mass spectrometer. HCD and CID dissociation techniques with  $MS^n$  fragmentation were utilized to obtain in-depth structure information. AcquireX, a real-time decision-making data acquisition workflow, was also used to optimize  $MS^n$  quality. The information-rich  $MS^n$  data were processed using "Compound Discoverer" software.

### **Methods**

LCMS analyses were carried out on a Vanquish UHPLC with DAD detector coupled with Orbitrap IQ-X Tribrid MS. Adefovir Dipivoxil (0.25 mg/mL in ACN:H<sub>2</sub>O at 1:3 ratio) was chromatographically separated on a Hypersil GOLD Vanquish column (2.1X150 mm 1.9  $\mu$ m) with gradient elution using mobile phase A: H<sub>2</sub>O/10 mM ammonium formate, and B: ACN:H<sub>2</sub>O (9:1)/10 mM ammonium formate.

The high resolution full scan and HCD, CID of  $MS^n$  data were collected in positive mode using data-dependent acquisition (DDA) with and without the AcquireX background exclusion workflow.

UVPD was conducted for selected compounds structure characterization.

### **Preliminary data (results)**

The preliminary results showed that MS method with HCD, CID  $MS^n$  acquisition provided rich structure information. Major and minor impurities were detected by processing the HRAM and higher level MS/MS ( $MS^n$ ) spectra using Compound Discoverer.

A number of trace impurities were detected with similar  $MS^2$  fragments. In order to discern and determine the structures of these impurities, high quality MS/MS and higher order  $MS^n$  spectra were necessary.

IQ-X MS methods allowed parallel data acquisition using HCD and CID with multiple stage MS/MS ( $MS^n$ ) on a single precursor and its product ions with high mass accuracy, which yielded extensive amounts of structural information and the linkage between them to facilitate confident impurity structure characterization.

In addition, AcquireX automatic background subtraction was used to optimize the  $MS^n$  spectra acquisition. The results showed that the AcquireX workflow improved impurity ID quality by effectively enhancing  $MS^n$  triggering compared with data-dependent acquisition (DDA).

This study showed that the Orbitrap IQ-X tribrid MS enabled data acquisition using multiple dissociation techniques for multiple stage MS/MS (or  $MS^n$ ) fragmentation, which provided comprehensive information for small molecule structure elucidation. In addition, the AcquireX automatic background exclusion workflow further improved  $MS^n$  efficiency and quality.

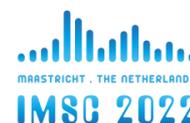
**Please explain why your abstract is innovative for mass spectrometry?**

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours



Multiple dissociations and multi-stage fragmentations capabilities with AcquireX workflow of Orbitrap IQ-X Tribrid MS for confident small molecules structure characterization.

UVPD can provide complementary information for certain compound structural identification.

### **Co-authors:**

*Kate Comstock, Thermo Fisher Scientific*

*Sarah Robinson, Genentech, A Member of the Roche Group*

*Brandon Bills, Thermo Fisher Scientific*

*Vlad Zabrouskov, Thermo Fisher Scientific*

Poster number: **IM-PA-058**

## **DEVELOPMENT OF A CERTIFIED REFERENCE MATERIAL FOR THE DETERMINATION OF ARSENIC, LEAD AND MERCURY IN LIPSTICK**

Abstract ID: **849**

**Presenting author: Deborah Sim, Health Sciences Authority**

### **Introduction**

Toxic elements are banned as intentional ingredients in cosmetic products, as these may be absorbed through skin. Even at low levels, the elements can accumulate in the body and over time cause adverse health effects. The toxic elements of most concern include arsenic, lead and mercury. However, there are very few available cosmetic certified reference materials (CRMs) in the market and none for lipstick. CRMs are important to the testing laboratories as these are used for method validation or as quality control (QC) materials. The objectives of this project were to develop high accuracy methods for the measurement of arsenic, lead and mercury in cosmetic material, and to develop the first lipstick CRM containing all three toxic elements.

### **Methods**

The lipstick material was formulated by a Singapore-based pharmaceutical company using ingredients suitable for cosmetic products. Known amounts of toxic elements were spiked into the lipstick material and homogenised. A stabiliser for mercury was successfully identified and added into the material. This was crucial in the preparation of lipstick CRM containing mercury. Homogeneity, short and long-term stability studies were carried out using inductively-coupled plasma mass spectrometer (ICP-MS). Assignment of certified values and long-term stability study were carried out by isotope dilution mass spectrometry (IDMS) for lead and mercury and standard addition for arsenic using ICP-MS and high resolution ICP-MS, respectively.

### **Preliminary data (results)**

The development of the CRM, including procedures for sample preparation, homogeneity study, stability study, assignment of certified values and uncertainty evaluation, was carried out according to the requirements of ISO/IEC 17025, ISO 17034 and ISO Guide 35.

The toxic element contents in the material were found to be homogenous. They were also stable for a period of 14 days at maximum allowable temperature during transportation at 50 °C and over a period of at least 34 months at storage condition at ambient temperature. Using the validated methods, the lipstick material was certified for the toxic elements arsenic, lead and mercury. The assigned values are traceable to the International System of Units (SI) through the use of standard reference materials from National Institute of Standards and Technology (NIST). Each certified value came with a measurement uncertainty evaluated using internationally-accepted GUM approach .

The lipstick material was used to organise an accuracy-based proficiency testing (PT) programme for Singapore testing laboratories and was also successfully launched as the first lipstick CRM (HRM-2012A). This CRM has been purchased by local and overseas testing laboratories for method validation or as QC materials.

Subsequently, a Supplementary Comparison and parallel Pilot Study on Elements in Lipstick Material (APMP.QM-S17 & -P38) was organised by HSA for the Inorganic Analysis Working Group (IAWG), under the auspice of the Asia Pacific Metrology Programme (APMP). It was a good platform where the accuracy of our developed method can be benchmarked against different measurement procedures used by other metrology institutes worldwide.

### **Please explain why your abstract is innovative for mass spectrometry?**

High accuracy methods were developed for the certification of arsenic, lead and mercury in the first lipstick CRM, using exact-matching IDMS and the standard addition approach.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Certified Reference Material Production

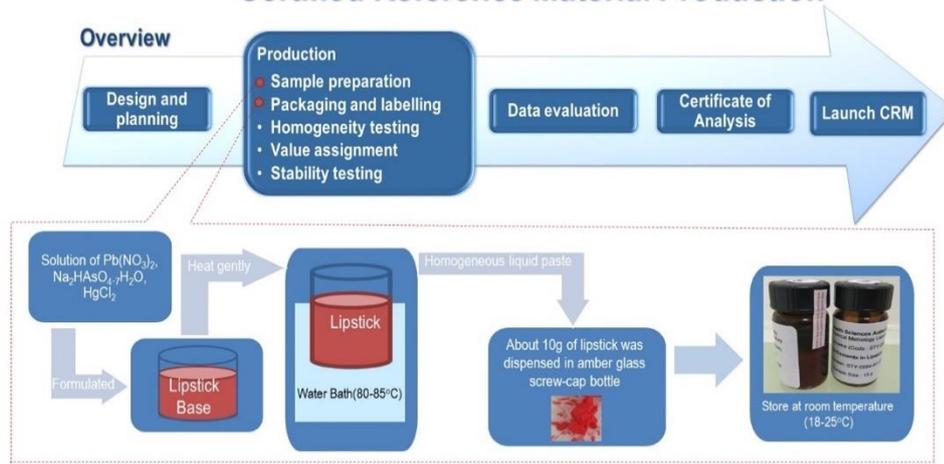


Figure 1: Overview of the production of certified reference material (In accordance with ISO/IEC 17025, ISO 17034 and ISO Guide 35)

Poster number: **IM-PA-059**

## **ENSURING CONFIDENT ANALYSIS OF EXTRACTABLES AND LEACHABLES SCREENING USING HIGH-RESOLUTION QUADRUPOLE TIME OF FLIGHT TECHNOLOGY**

Abstract ID: **878**

**Presenting author: Gordon Kearney, Waters Corporation**

### **Introduction**

Due to concern about the safety of components from plastic, it is crucial to screen for and identify potential extractables and leachables (E&L) in pharmaceutical packaging and medical devices. There are several challenges when undertaking these studies to meet the regulations currently in place. For example, analytical instrumentation needs to be highly sensitive to detect low level components to meet expected screening thresholds. Additionally, the ability to quantify and identify E&L compounds from the screening step on the same analytical platform is also important. Here, we describe an E&L screening experiment using liquid chromatography and a benchtop high-resolution quadrupole time of flight mass spectrometer (LC-QToF HRMS) combined with a screening software solution to address some of these challenges.

### **Methods**

Three commercial nasal sprays were purchased. Neat solution (leachables) was removed prior to extraction of the nasal container closure system with isopropanol for 72 hours at 40 °C (extractables), along with a control blank. The blank, extracted samples, and neat solutions were spiked with an internal standard and injected in triplicate. An E&L system suitability (SST) mix and a calibration series were also run. The instrument was an ACQUITY™ Premier connected to the Xevo™ G3 QToF MS with electrospray ionization. Data was reviewed in the UNIFI™ application out of the waters\_connect™ platform.

### **Preliminary data (results)**

The Xevo G3 QToF MS was used in MS<sup>E</sup> mode which enabled full acquisition of the accurate mass information of both precursor and fragment ions. Mass accuracy for all detected compounds had a mass error of less than 3 ppm which aids library matching and elemental composition calculation to ultimately aid full characterization. The instrument has had updates to the ion optics and detection system to maximize transmission and proved to be highly sensitive and reproducible for the SST mix. An increase in response up to 10 times, compared to previous iterations, was seen for analytes within the SST mix and retention times showed RSDs below 0.1%.

Using the screening software, a step-by-step workflow solution, streamlines data interpretation. Components found in the samples above the analytical threshold were screened against a library to find matches against accurate mass, retention times, and mass fragments. A comparison tool and elucidation toolkit were employed to find and characterize unidentified components unique to or elevated in the sample. For example, a compound found at  $m/z$  368.4253, was putatively assigned as a surfactant using the structural elucidation toolkit. Implementing quantitation into the workflow was also investigated, exploring calibration curves and response factors. Using a calibration curve of the internal standard, the concentration of the internal standard spiked into the samples could be calculated within 8 % of the known value.

### **Please explain why your abstract is innovative for mass spectrometry?**

With the Xevo G3 QToF, confident identification of E&L components in complex matrices is enabled through novel ion optics and detection system which maximize transmission.

### **Co-authors:**

*Rachel Sanig, Waters Corporation*

*Jayne Kirk, Waters Corporation*

*Cristian Cojocariu, Waters Corporation*

*Andrew Feilden, Hall Analytical Laboratories Ltd*

Poster number: **IM-PA-060**

## **SEPARATION OF MONOMERIC FLAVONOIDS BY CYCLIC ION MOBILITY MASS SPECTROMETRY**

Abstract ID: **896**

**Presenting author: Carlo de Bruin, Wageningen University & Research**

### **Introduction**

Analytical techniques such as liquid/gas chromatography coupled to mass spectrometry (LC/GC-MS) are widely used for the characterization of the complex mixture of (isomeric) proteins, carbohydrates, lipids, and phytochemicals in food products. Such products contain isomers that are challenging to separate, while they can possess different reactivity and bioactivity. Phytochemicals such as flavan-3-ols (catechins) possess various stereoisomers that differ in their chemical properties. Even though the flavan-3-ol epimers (e.g. epicatechin (EC) and catechin (C)) can be resolved by LC-MS, the analysis is time-consuming and there is lack of fast and direct ways to individually monitor these epimers during (auto-)oxidation reactions. Recently, cyclic ion mobility mass spectrometry (cIMS-MS) has been introduced as a tool for high resolution separation of analytes.

### **Methods**

Epimer standards were dissolved in 50% MeOH and analysed by direct infusion cIMS-MS with a flow of 10  $\mu\text{L}/\text{min}$ . Typical IMS parameters were: travelling wave (TW) velocity 250 m/s and TW static height 10 V. All samples were spiked with either NaI or LiCl to induce the formation of sodium and or lithium adducts, respectively.

### **Preliminary data (results)**

Our results show that formation of sodium and lithium adducts enhanced IMS separation of catechin epimers, compared to deprotonation and protonation. Sodiated ions of all four catechin epimer pairs from green tea samples were fully resolved by cIMS-MS in less than 300 milliseconds. After achieving separation, the semi-quantification of catechin epimers within cIMS-MS was also studied. Ratio determination of sodiated EC:C was tested in a range of 1:5 and vice versa (within the linear range 50-1200  $\mu\text{g}/\text{mL}$ ). Ionization differences between EC and C were corrected for using an ionization correction factor (ICF). Using this approach, semi-quantification was successful, with an average standard deviation of 4.7%. Afterwards, the method was tested on a real green tea sample. Standard addition was used to correct for matrix effects. The EC:C ratio in green tea was determined to be 1.6:1 (507 and 319  $\mu\text{g}/\text{mL}$  of EC and C, respectively). Semi-quantitative properties make this method valuable to rapidly monitor isomerization reactions and reactivity of individual isomers of flavonoids, for example during (auto-)oxidation of tea. Finally, we demonstrated that this method can also be used to separate sodium adducts of two sets of flavonoid positional isomers (i.e. morin, tricetin, and quercetin; and kaempferol, fisetin, luteolin, and scutellarein). This shows the potential of our cIMS-MS approach to be extended to a wider variety of flavonoid isomers in the future, which will aid in the in-depth characterization of complex flavonoid-rich food samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

Our study shows that cIMS-MS, a novel IMS-MS technique, can be used for fast in-depth characterization of flavonoid isomers in complex food samples.

### **Co-authors:**

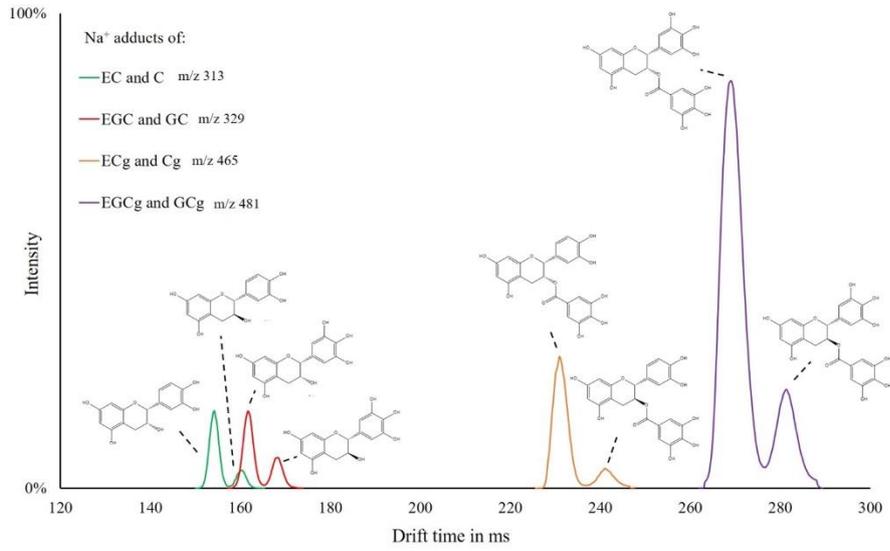
*Maria Hennebelle, Laboratory of Food Chemistry, Department of Agrotechnology and Food Sciences, Wageningen University, Wageningen, The Netherlands*

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*Wouter de Bruijn, Laboratory of Food Chemistry, Department of Agrotechnology and Food Sciences, Wageningen University, Wageningen, The Netherlands*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Separation of four catechin pairs of isomers in green tea

Poster number: **IM-PA-061**

## **SICYLIA-TMT: A MULTI-STEP LABELLING STRATEGY FOR GLOBAL REDOX PROTEOMICS**

Abstract ID: **904**

**Presenting author: Sergio Lilla, CRUK Beatson Institute Glasgow**

### **Introduction**

High levels of reactive oxygen species can cause oxidation of different classes of molecules causing profound consequences on cellular functions. ROS have also been shown to be a signal intermediate which modulates enzyme activity, or triggers signalling cascades. Notably, Cysteines are an important stress sensor that swiftly responds to external stimuli. In 2018 we have developed SICyLIA, a new method that enables accurate measurement of Cysteine oxidation levels in cells and tissue using light or stable-isotope labelled, heavy, iodoacetamide (IAA) as readout for cysteine thiol oxidation(1). Here we further develop the method, taking advantage of TMT technology, to improve the throughput and to include the portion of the redox proteome that we could not measure in the original method.

### **Methods**

To block swiftly free thiols, we add light IAA to lysis buffer. Reversibly-oxidised Cysteines are then reduced, and newly generated thiols blocked with heavy IAA. This creates two labelled populations of free and reversibly-oxidised thiols, further labelled with TMT. Peptides are then fractionated using high-pH chromatography. Each fraction is analysed using a Q-Exactive HF or Lumos. Data are processed with MaxQuant querying SwissProt database. Three-step normalisation of peptide reporter ion intensity is performed with Perseus: by median intensities, by protein levels and to compare heavy-light IAA TMT, by internal reference scaling. Regulated cysteines are determined using student T-test.

### **Preliminary data (results)**

The new SICyLIA-TMT method was benchmarked against the same samples analysed with the original SICyLIA method ("Acute oxidation model"). Results show that the SICyLIA-TMT outperform the old method in respect to the number of peptides robustly quantified (>9000 in the old SICyLIA; >12000 light and >1000 heavy in the SICyLIA-TMT). Additionally, sample preparation and mass spec time were both halved.

The SICyLIA-TMT method was then applied to cancer-associated fibroblast cells, stimulated with non-lethal amount of hydrogen peroxide (HP). The oxidation state can now be determined on the free and reversibly-oxidised thiol fraction simultaneously. Annotation with UniProt "Feature keys" show that many quantified peptides, contain functionally relevant Cysteines, such as active sites and disulphide bonds.

The two thiol fractions can also be analysed together to calculate stoichiometry, under the assumption that, for each Cysteine residue, the summed intensities of free and reversible oxidised thiol fraction represent 100% of the residue. This allows calculating Cysteine oxidation occupancy (% of oxidation). To do that the heavy and light peptides are further normalised using the Internal Reference Scaling method or LIMMA. Clustering analysis of the Cysteine oxidation occupancy identified at least 3 groups with discrete changes in Cysteine oxidation states. Key examples are the active sites of GAPDH and Peroxiredoxin 6, which showed divergent profiles: while the oxidation occupancy of the active site of GAPDH dramatically increased upon HP treatment, those of the active site of PRDX6 decreased, likely to counteract the action of HP.

### **Please explain why your abstract is innovative for mass spectrometry?**

This method enables simultaneous quantification of free and reversibly-oxidised thiol fraction and determines Cysteine oxidation occupancy at global proteome level.

### **Co-authors:**

*Samuel Atkinson, Institute of Medical Sciences, University of Aberdeen, CRUK Beatson Institute Glasgow*  
*Jiska van der Reest, VIB Center for Cancer Biology, Ghent, CRUK Beatson Institute Glasgow*  
*Sara Zanivan, CRUK Beatson Institute Glasgow*

Poster number: **IM-PA-062**

## **FLOW INJECTION ANALYSIS MASS SPECTROMETRY FOR HIGH THROUGHPUT QUANTIFICATION IN COMPLEX MATRICES**

Abstract ID: **910**

**Presenting author: Wouter Coppes, DSM BioData & Translation - Center Analytical Innovation**

### **Introduction**

Recent developments in robotics and automation have cleared the way to prepare biological samples in high-throughput. This allows research and development in multiple fields to have more comprehensive workflows as the number of experiments that can be done in a time frame is greatly expanded. A challenge for analytical teams is to be able to measure large numbers of samples, with fast and robust methods that still have acceptable accuracy and reproducibility. With the main advantage of Flow Injection Analysis Mass Spectrometry (FIA-MS) being speed, other method parameters were evaluated to see if the increased throughput can be achieved without compromising too much.

### **Methods**

FIA-MS was evaluated as a fast quantification tool for multiple biological matrices, using liquid extracts or supernatants that were directly injected on a SCIEX TripleTOF 6600 system coupled to a Shimadzu UHPLC system. FIA-MS was also compared to a, ultra-fast isocratic method using a short SEC guard column applied directly on the spray needle. Target compounds varied from amino acids to lipids and the results were compared to other analytical methods for evaluation of the accuracy, sensitivity and reproducibility. Analysis times were as low as 45 seconds sample-to-sample, with the limitation coming from the autosampler.

### **Preliminary data (results)**

Using <sup>13</sup>C-labelled internal standards

To evaluate the performance of FIA-MS for polar analytes in aqueous extracts, amino acids were analyzed in yeast extracts after dilution and addition of <sup>13</sup>C- labelled internal standards to correct for ionization effects. In Figure 1, a comparison was made with the standardized AccQ-tag method for amino acids compared to non-derivatized amino acids analyzed by FIA-MS. Due to the high abundance of amino acids, high dilution is possible and with labelled standards this leads to good accuracy for many amino acids, as illustrated in the figure.

Organic liquid-extracts

Compounds that are more non-polar in an aqueous matrix rich of salts can be extracted using organic solvents and is a solution to prevent suppression by salts. To evaluate FIA-MS performance in extracts without availability of isotope labelled standards, oleic acid was quantified using FIA-MS after liquid-extraction into organic solvent. A good correlation was found of oleic acid quantified by NMR and by FIA-MS of multiple fermentation broth samples showing the accuracy of the FIA-MS is comparable to NMR.

SEC guard column

Next to non-polar compounds or compounds for which labelled internal standards are available, there is also interest in many other less abundant metabolites in aqueous solutions in complex matrices. A proposed workflow is to use a SEC guard column directly connected to the MS spray needle, for maintaining throughput while allowing minor separation of the compounds of interest and salts and avoid matrix effect for quantification. Examples of this approach will be presented on our poster.

### **Please explain why your abstract is innovative for mass spectrometry?**

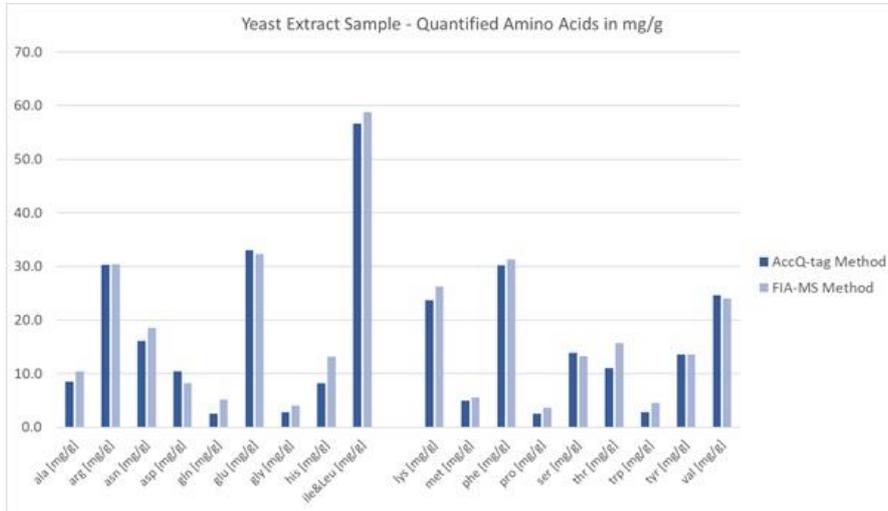
FIA-MS allows ultra-fast (relative) quantification of many compound classes with methods as low as 45 seconds per sample.

**Co-authors:**

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Emilie Usureau, DSM BioData & Translation - Center Analytical Innovation*  
*Leon Coulier, DSM BioData & Translation - Center Analytical Innovation*  
*Erwin Kaal, DSM BioData & Translation - Center Analytical Innovation*



Quantification of free amino acids in yeast extracts (FIA-MS&LC-MS)

Poster number: **IM-PA-063**

## TARGETED FORENSIC SCREENING AND SEMI-QUANTITATION OF DRUGS IN URINE USING A NOVEL HIGH-RESOLUTION ACCURATE-MASS MASS SPECTROMETER

Abstract ID: **928**

**Presenting author: Claudio De Nardi, Thermo Fisher Scientific**

### Introduction

Toxicology laboratories face many challenges, including extremely high volumes of samples in complex matrices and the proliferation of designer drugs. Laboratories must screen and quantify quickly and at low cost. While these challenges can be addressed individually, it is far more difficult to address all of them in a single analytical approach. Here, we present a novel workflow that combines liquid chromatography and high-resolution accurate-mass (HRAM) mass spectrometry to screen and quantify large panels in a single run while retaining the ability to retrospectively interrogate the analytical data for novel or unexpected compounds. Further, we demonstrate that the method, developed on one model of mass spectrometer, can be run successfully on a newer instrument model.

### Methods

Over 1500 standards were analyzed on an HPLC/quadrupole-Orbitrap system. Retention times and HRAM MS/MS spectra were used to generate spectral library and compound database. 101 compounds covering a wide range of compound classes, hydrophobicities, and polarities were selected for proof-of-concept. Mixes of the selected compounds, with 8 internal standards, were spiked into urine in a range of 0.1–2000 ng/mL and diluted 20x with water. Separation occurred using a 15.5-minute HPLC gradient and MS data was collected by full-scan data-dependent MS2 with polarity switching at 60,000 (MS) and 15,000 (MS/MS) FWHM resolution.

### Preliminary data (results)

Data was processed using software optimized for trace compound analysis to determine limits of detection (LOD), quantification (LOQ), and identification (LOI). Limit of detection was defined as the lowest concentration at which a peak, at the correct retention time, could be extracted from full-scan data with 5 ppm mass accuracy. Calibration curves were generated for each compound using the internal standard with the closest retention time. All curves were weighted 1/x and were either linear or quadratic, depending on data fit. Limit of quantification was defined as the lowest concentration for which the back-calculated concentration fell within 30% of nominal. Finally, limit of identification was defined as the lowest concentration at which the peak had both a passing isotopic pattern score and positive library match in the spectral library.

All 101 diverse compounds were detected, quantified, and identified in a single run. Limits of detection ranged from 0.1 to 50 ng/mL. Limits of quantification ranged from 0.1 to 100 ng/mL. Limits of identification ranged from 0.1 to 1000 ng/mL.

For Research Use Only – Not For Diagnostic Procedures

### Please explain why your abstract is innovative for mass spectrometry?

An established HPLC-HRAM MS screening method for drugs of abuse was implemented on a next generation Orbitrap instrument.

### Co-authors:

*Kristine Van Natta, Thermo Fisher Scientific*

*Jingshu Guo, Thermo Fisher Scientific*

*Bradley J. Hart, Thermo Fisher Scientific*

*Sarvesh Iyer, Thermo Fisher Scientific*

*Tanis Correa, Thermo Fisher Scientific*

Poster number: **IM-PA-064**

## DEVELOPMENT OF A LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR THE FREE FATTY ACID PROFILING OF GREEK YOGURT

Abstract ID: **952**

**Presenting author: Christiana Mantzourani, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771, Center of Excellence for Drug Design and Discovery, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771**

### Introduction

Yogurt is an increasingly popular fermented dairy product with high nutritional value, traditionally produced by milk fermentation. Among dairy products, yogurt has received a particular interest due to numerous studies that associate yogurt intake with improved metabolic health and a reduced risk of T2D.<sup>1</sup> Free fatty acids (FFAs) in yogurt are formed during the fermentation process and their sensory characteristics contribute to the organoleptic properties of yogurt. The most common approach to quantify FFAs is the use of gas chromatography combined with either flame ionization detection (GC-FID), or mass spectrometry detection (GC-MS) involving the conversion of fatty acids into the corresponding methyl esters (FAMES).

### Methods

In this work, we present a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the determination of a large set of FFAs in yogurt, avoiding a tedious sample preparation procedure and derivatization. LC-MS/MS measurements were performed with an ABSciex Triple TOF 4600 combined with a micro-LC Eksigent and an autosampler. Electrospray ionization in negative mode was used for the MS experiments. Halo C18 2.7  $\mu\text{m}$ , 90 Å, 0.5 $\times$ 50 mm<sup>2</sup> (Eksigent) was used as a column and the mobile phase consisted of a gradient (A: acetonitrile/0.01% formic acid/isopropanol 80/20 v/v; B: H<sub>2</sub>O/0.01% formic acid).

### Preliminary data (results)

The present LC-HRMS method allows the simultaneous determination of a large set of common and uncommon FFAs in yogurt samples, in a 10-min single run, avoiding any derivatization step. 25 common saturated and unsaturated FFAs, together with 21 saturated hydroxy fatty acids (SHFAs) and 17 oxo fatty acids (SOFAs) were analyzed in 26 cow and 7 sheep Greek yogurt samples. For the first time, a detailed analysis of bioactive SHFAs<sup>2</sup> and SOFAs in yogurt samples was carried out. 10HSA, 7HSA and 16HPA were found at concentrations higher than 50 ng/g. 9OPA, 5OPA, 10OSA, 9OSA and 8OSA were the most abundant SOFAs, at concentrations lower than the corresponding SHFAs. Finally, comparing the concentrations of 63 FFAs in cow and yogurt samples, we observed differences at the concentrations of six common FFAs and five minor oxidized FFAs. Based on these FFAs, principal component analysis (PCA) allowed the discrimination of cow from sheep yogurt samples.

**Acknowledgements:** The research presented was carried out within the framework of a Stavros Niarchos Foundation grant to the National and Kapodistrian University of Athens.

1. Gijsbers, L., Ding, E. L., et al. Consumption of dairy foods and diabetes incidence: A dose-response meta-analysis of observational studies. *Am J Clin Nutr*, **2016**, 103(4), 1111–1124.

2. Kokotou, M. G., Kokotos, A. C., et al. Saturated Hydroxy Fatty Acids Exhibit a Cell Growth Inhibitory Activity and Suppress the Cytokine-Induced  $\beta$ -Cell Apoptosis. *J Med Chem*, **2020**, 63(21), 12666–12681.

### Please explain why your abstract is innovative for mass spectrometry?

The accuracy and the sensitivity provided by LC-HRMS permitted the analysis of the minor ingredients SHFAs and SOFAs in yogurt, allowing the discrimination of cow and sheep yogurt samples

**Co-authors:**

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Charikleia S. Batsika, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771, Center of Excellence for Drug Design and Discovery, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771*

*Maroula G. Kokotou, Laboratory of Chemistry, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 11855*

*George Kokotos, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771, Center of Excellence for Drug Design and Discovery, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771*

Poster number: **IM-PA-065**

## **IMPLEMENTATION OF CCS-M/Z TRENDLINES IN THE IDENTIFICATION PROCESS OF QUATERNARY AMMONIUM COMPOUNDS (QACS) IN INDOOR DUST SAMPLES THROUGH DRIFT TUBE ION-MOBILITY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY**

Abstract ID: **972**

**Presenting author: Lidia Belova, University of Antwerp**

### **Introduction**

Quaternary ammonium compounds (QACs), used as disinfectants and surfactants, are considered contaminants of emerging concern (CECs). Significantly higher QAC levels have been reported in dust and human blood samples collected during the COVID-19 pandemic compared to pre-pandemic time points. However, information on the occurrence and identity of many QACs in environmental matrices is lacking. Ion-mobility mass spectrometry derived collision cross section (CCS) values can serve as a valuable additional identification parameter within suspect screening studies of CECs. For a limited number of QACs, CCS values and CCS- $m/z$  trendlines have been reported in the past. This study aims at expanding this dataset by describing CCS values and CCS- $m/z$  trendlines of additional QAC classes and use this data for compound identification in indoor dust.

### **Methods**

All measurements were conducted on an Agilent 6560 drift tube IM quadrupole time-of-flight mass spectrometer using data-independent acquisition. Reference CCS values of >20 QACs are reported as average values of triplicate measurements. Reference CCS values were implemented in the suspect screening analysis of indoor dust samples collected in Belgium during the winter 2022 alongside a suspect list containing > 500 known and predicted QACs. Matches of experimental with reference CCS values (deviation < 2%) and the alignment of annotated QAC features within the trendlines obtained from reference standards, were added to the identification criteria to increase identification confidence.

### **Preliminary data (results)**

From the reference CCS values of >20 QACs, CCS- $m/z$  trendlines could be calculated for the three QAC classes (benzalkyl dimethylammonium (BAC), alkyltrimethylammonium (ATMAC) and dialkyldimethylammonium (DDAC) compounds). All trendlines were well fitted with linear models resulting in correlation coefficients of  $R^2 > 0.98$ . Additionally, based on the observed trendlines, BACs could be distinguished from the other classes containing only alkyl side chains.

In the investigated dust samples, >10 QACs not included in the target database were identified. First, features were annotated based on a match of the experimental exact masses ( $m/z$  tolerance < 7 ppm) with the formulae included in the suspect list. Consequently, high-energy frames obtained in DIA mode were examined in order to identify characteristic fragments previously defined within the database compilation. The characteristic fragments were linked to the corresponding parent compounds as both shared similar drift times in the IM dimension. Lastly, compound annotations were confirmed by investigating the deviations of the experimental CCS values from the previously obtained CCS- $m/z$  trendlines. This additional identification confidence allowed to decrease the number of false-positive assignments. The high number of identified QACs indicates their increasing occurrence in the indoor environment pointing out the urge for further (quantitative) studies on environmental and biological samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

Investigation of the alignment of experimental CCS values with previously characterized CCS- $m/z$  trendlines as an additional identification criterion.

### **Co-authors:**

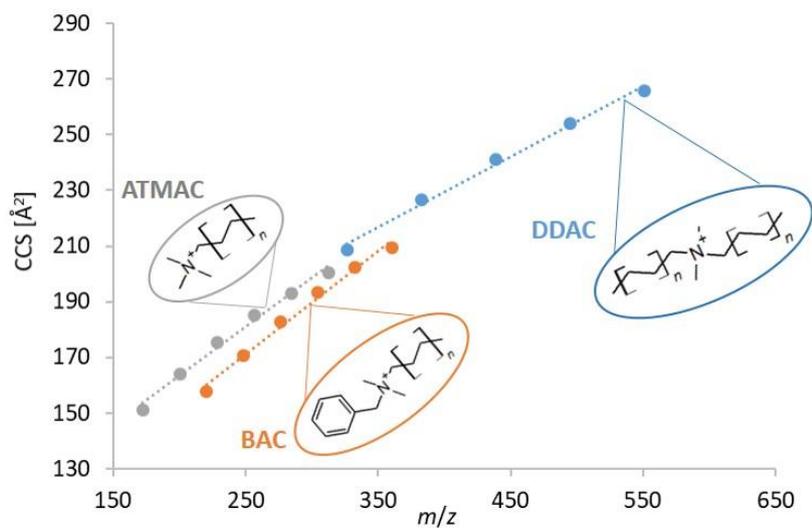
*Giulia Poma, University of Antwerp*

*Alexander L. N. van Nuijs, University of Antwerp*

*Adrian Covaci, University of Antwerp*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



CCS-*m/z* trendlines of three classes of quaternary ammonium compounds.

Poster number: **IM-PA-066**

## DEVELOPMENT OF A LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR THE LIPIDOMICS ANALYSIS OF FREE FATTY ACIDS IN HUMAN PLASMA OF HEALTHY AND DIABETIC SUBJECTS

Abstract ID: **980**

**Presenting author: George Kokotos, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771, Center of Excellence for Drug Design and Discovery, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771**

### Introduction

The presence of free fatty acids (FFAs) in human plasma has been well documented and the alterations in their levels have been associated with insulin resistance and type 2 diabetes mellitus (T2D).<sup>1</sup> 2- and 3-Hydroxy FAs have been reported as minor components of human plasma, however nothing is known about the existence of other saturated hydroxy fatty acids (SHFAs). We have shown that a variety of SHFAs, present in milk and other dairy products, exhibit growth inhibitory activities against human cancer cell lines and suppress  $\beta$ -cell apoptosis.<sup>2</sup> The aim of our work was to explore the presence of SHFAs in plasma and to study their levels, together with those of other oxidized and common FFAs, in plasma of diabetic and healthy subjects.

### Methods

Herein, a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the rapid determination of a large set of FFAs in human plasma is presented, avoiding a tedious sample preparation procedure and pre-treatment. LC-MS/MS measurements were performed with an ABSciex Triple TOF 4600 combined with a micro-LC Eksigent and an autosampler. Electrospray ionization in negative mode was used for the MS experiments. Halo C18 2.7  $\mu\text{m}$ , 90  $\text{\AA}$ , 0.5 $\times$ 50 mm<sup>2</sup> (Eksigent) was used as a column and the mobile phase consisted of a gradient (A: acetonitrile/0.01% formic acid/isopropanol 80/20 v/v; B: H<sub>2</sub>O/0.01% formic acid).

### Preliminary data (results)

In an initial experiment, a "suspect" LC-HRMS approach with reversed-phase chromatography and high mass accuracy MS operating in full scan negative mode was utilized in human plasma samples. Subsequent fragmentation studies pointed to the existence of previously undocumented SHFAs. To address this further, we developed an LC-HRMS method for the simultaneous determination of a large set of common and oxidized FFAs in human plasma samples, in a 10-min single run, avoiding any derivatization step. A total of 35 common saturated and unsaturated FFAs, as well as 39 saturated hydroxy and oxo FFAs, were determined in plasma samples from 29 subjects with type 2 diabetes mellitus (T2D), 14 with type 1 diabetes mellitus (T1D), and 28 healthy subjects. Various regio-isomers of hydroxystearic acid (OSA) and hydroxypalmitic acid (OPA), namely 7HSA, 8HSA, 11HPA, 16HPA, were quantified in human plasma, in addition to 2- and 3-HSA. Alterations in the levels of medium-chain FFAs (C6:0 to C10:0) were observed between the control group and T2D and T1D patients.

**Acknowledgements:** The research presented was carried out within the framework of a Stavros Niarchos Foundation grant to the National and Kapodistrian University of Athens.

1. Sobczak, A.I.S.; Blindauer, C.A.; Stewart, A.J. Changes in plasma free fatty acids associated with type-2 diabetes. *Nutrients* **2019**, *11*, 2022.

2. Kokotou, M. G., Kokotos, A. C., et al. Saturated Hydroxy Fatty Acids Exhibit a Cell Growth Inhibitory Activity and Suppress the Cytokine-Induced  $\beta$ -Cell Apoptosis. *J Med Chem*, **2020**, *63*(21), 12666–12681.

### Please explain why your abstract is innovative for mass spectrometry?

LC-HRMS allowed the fast determination of a large set of FFAs in plasma, indicating the existence of various SHFAs and alterations of medium-chain FAs between healthy and diabetic subjects.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Co-authors:

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Poster number: **IM-PA-067**

## QUANTIFICATION OF ENDOCANNABINOIDS USING A SOLID PHASE EXTRACTION IN COMBINATION WITH MICRO FLUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (SPE- $\mu$ L-MS/MS)

Abstract ID: **992**Presenting author: **Alena Sens, Goethe Universität (Universitätsklinikum)**

### Introduction

The endocannabinoid system is an endogenous physiological regulatory system that occurs in the brain and nervous system of all mammals. The ECS is an extensive biological system and has been studied in the context of various disorders, including Alzheimer's and Parkinson's disease. Endocannabinoids (eCBs) and eCB like substances are low concentrated but highly active lipid mediators and can be quantified in blood-based matrices like plasma or serum as well as tissue and CSF samples. However, the determination of eCBs and eCB-like substances is a challenging task requiring a highly selective and sensitive analytical method. In this study, a solid phase extraction (SPE) protocol in combination with a micro-flow liquid chromatography-tandem mass spectrometry ( $\mu$ LC-MS/MS) method was developed for the analysis of eCBs and eCB analogues.

### Methods

A high-end triple quadrupole mass spectrometer QTRAP 7500 with a Turbo Ion Spray source was used in combination with a M5  $\mu$ LC-system (all Sciex). Measurements were performed in positive ESI mode. Chromatographic separation was achieved using a phenomenex C18 XB column (2.6  $\mu$ m, 2.1  $\times$  50 mm) at a flow-rate of 4  $\mu$ L/min and 10  $\mu$ L injection. For gradient elution water plus 10 mM ammonium formate and 0.1% formic acid (FA) (solvent A) and acetonitrile (solvent B) were used. As  $\mu$ LC is prone to blockage of the column, SPE was chosen for generating clean sample extracts.

### Preliminary data (results)

Due to (pre-)analytical challenges a highly selective, sensitive and standardized sample processing protocol in combination with a sophisticated LC-MS is necessary when analyzing eCBs and eCB analogues. Therefore, a SPE protocol in combination with  $\mu$ LC and high-end MS/MS was used. The SPE protocol was optimized based on the recommendations of the manufacturer (Biotage) resulting in the following final procedure:

1. Conditioning (acetonitrile, 2 mL)
2. Equilibration (1% formic acid solution, 1 mL)
3. Pretreatment (1% formic acid solution, 0.6 mL + 0.2 mL K3EDTA plasma)
4. Sample Load (0.8 mL)
5. Washing (40% methanol, 1 mL)
6. Elution (acetonitrile, 1 mL)

The application of  $\mu$ LC using gradient elution (12 min) provided good analyte separation including baseline separation of 2-AG/1-AG as shown in the chromatogram in *Fig. 1*. Signal areas for AEA ( $1.9 \pm 1.1 \cdot 10^6$ ), 2-AG ( $2.6 \pm 0.7 \cdot 10^6$ ), DHEA ( $2.3 \pm 0.6 \cdot 10^6$ ) and LEA ( $6.0 \pm 2.0 \cdot 10^6$ ) were evaluated for the  $\mu$ LC combination with the QTRAP7500 and compared with data obtained by analyzing comparable samples using a QTRAP6500+ instrument (Sciex) equipped with an Agilent 1260 Infinity II UHPLC and a Waters Acquity UHPLC BEH C18 column (1.7  $\mu$ m, 2.1  $\times$  100 mm). The data indicate a considerably higher sensitivity using the  $\mu$ LC-QTRAP7500 instrument. Signal areas were approximately factor 10 higher than with a UHPLC-QTRAP6500+ instrument (*Fig. 2*). Similar results were achieved for other eCB analogues 1-AG, PEA, OEA, SEA, aLEA, and EPEA (data not shown) promising a better reliable quantification in samples from clinical studies.

## POSTER SESSION A

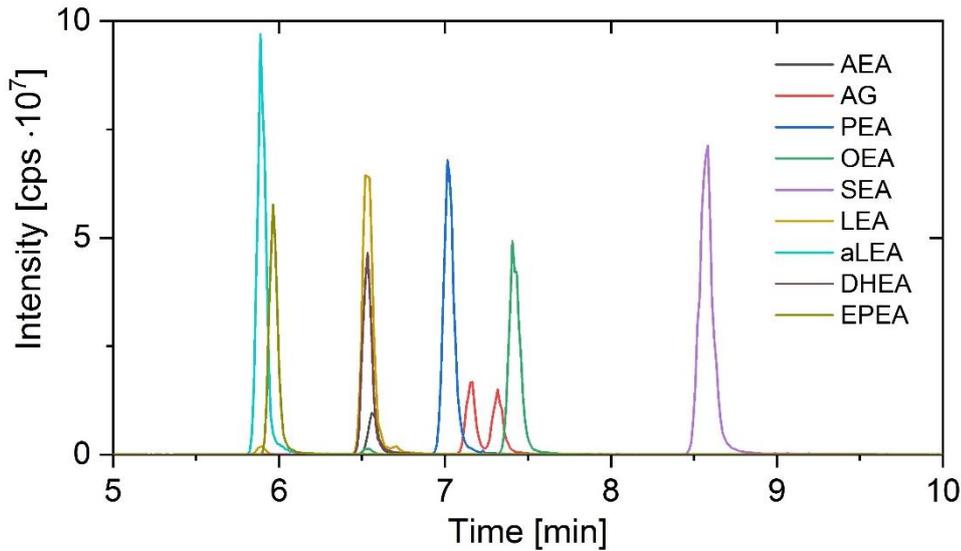
Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Please explain why your abstract is innovative for mass spectrometry?

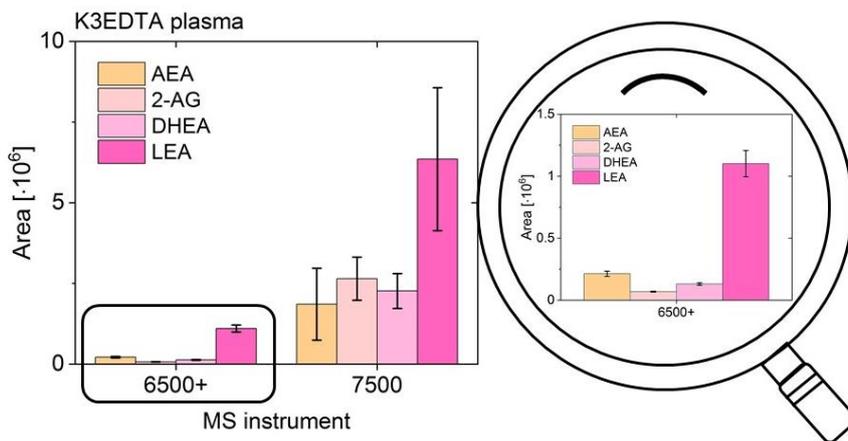
The developed and optimized SPE- $\mu$ LC-MS/MS makes the sensitive and selective quantification of low concentrated eCBs and eCB analogues possible.

### Co-authors:

*Dominique Thomas, Fraunhofer ITMP / Goethe Universität (Universitätsklinikum)*  
*Gerd Geißlinger, Fraunhofer ITMP / Goethe Universität (Universitätsklinikum)*  
*Robert Gurke, Fraunhofer ITMP / Goethe Universität (Universitätsklinikum)*



Chromatogram of eCBs and eCB analogues in standard solutions ( $\mu$ LC-QTRAP7500)



Comparison of signal areas of AEA, 2-AG, DHEA, LEA

Poster number: **IM-PA-068**

## **A NOVEL STABLE ISOTOPE LABELING DATA PROCESSING WORKFLOW FOR LC-MS AND LC-TIMS-MS DATA**

Abstract ID: **1006**

**Presenting author: Sven Meyer, Bruker Daltonics GmbH & Co. KG**

### **Introduction**

LC-MS based labeling experiments offer the ability to detect and quantify the incorporation of isotopically labeled atoms into an organism's metabolites. Ion mobility provides a further dimension for both the separation and annotation of isomeric and co-eluting metabolites in LC-MS analyses of both labeled and unlabeled samples. The additional separation dimension requires streamlined data processing workflows. Here we present a completely integrated approach for the analysis of LC-TIMS-MS and LC-MS measurements to assess differences in incorporation of  $^{13}\text{C}$  or  $^{15}\text{N}$  labels .

### **Methods**

Basis for the described workflow is the creation of target compound lists from e.g. metabolic pathways or established LC-MS methods. The automated creation of extracted ion chromatograms or mobilograms to obtain quantitative information for all possible isotopologue traces of the target molecules is the initial data processing step. The correction for natural occurring isotopes can be optionally performed and the integrated computation of fractional enrichments allows intuitive data interpretation. Batch processing simplifies the elucidation of large-scale tracer experiments. In addition, in-depth manual review options and the possibility to correct chromatographic and mobility peak boundaries can generate high confidence in results.

### **Preliminary data (results)**

Labeling experiment data were acquired on a timsTOF Pro (Bruker) with (timsON) and without (timsOFF) ion mobility separation. Datasets were processed and analyzed using the novel workflow integrated in the TASQ software solution. CCS values, retention times and mass-to-charge values of the target molecules were determined and utilized to extract ion mobility resolved isotopologue chromatogram traces. The extracted ion chromatograms were manually inspected and can provide improved data for labeling experiment analysis.

### **Please explain why your abstract is innovative for mass spectrometry?**

A novel, integrated and streamlined workflow for high confidence analysis of stable isotope labeling data obtained by LC-MS and LC-TIMS-MS

### **Co-authors:**

*Heiko Neuweger, Bruker Daltonics GmbH & Co. KG*

*Ilmari Krebs, Bruker Daltonics GmbH & Co. KG*

*Patrick Groos, Bruker Daltonics GmbH & Co. KG*

*Surendar Tadi, Bruker Scientific LLC*

*Erica Forsberg, Bruker Scientific LLC*

Poster number: **IM-PA-069**

## **POLYMERS OF MICRO(NANO)PLASTIC IN HOUSEHOLD TAP WATER OF THE BARCELONA CITY (SPAIN)**

Abstract ID: **1018**

**Presenting author: Marinella Farre, IDAEA-CSIC**

### **Introduction**

Microplastics (MPLs) are emerging persistent pollutants affecting drinking water systems, and different studies have reported their presence in tap water. However, most of the work has focused on particles in the 100–5 µm range. Here, is a workflow to assess the presence of polymers of micro and nanoplastics (MNPLs), with sizes from 0.7 to 20 µm in tap water samples of the Barcelona Metropolitan Area (BMA) distributed in the 42 postal codes and collected from August to October 2020, is presented.

### **Methods**

The method consisted of water fractionated filtration followed by toluene ultrasonic-assisted extraction and size-exclusion chromatography coupled to high-resolution mass spectrometry with atmospheric pressure photoionization source with negative and positive ionization conditions HPLC(APC)-APPI(±)-HRMS) and normal phase chromatography HILIC LUNA® column and electrospray ionisation source in positive and negative mode HPLC(HILIC)-ESI(±)-HRMS). The acquisition was performed in full scan mode, and the subsequent tentative identification of MNPLs polymers has been based on increasing the confirmation level, including the characterisation of monomers by using Kendrick Mass Defect (KMD) analysis, and confirmation and quantification using standards.

### **Preliminary data (results)**

Polyethylene (PE), polypropylene (PP), polyisoprene (PI), polybutadiene (PBD), polystyrene (PS), polyamide (PA), and polydimethylsiloxanes (PDMS) were identified. PE, PP, and PA were the most highly detected polymers, and PI and PBD were found at the highest concentrations (9,143 and 1,897 ng/L, respectively). A principal component analysis (PCA) was conducted to assess differences in MNPLs occurrence in drinking water, that was provided by the two drinking water treatment plants (DWTPs) suppliers. Results showed that no significant differences (at a 95% confidence level) were established between the drinking water supplies in the different areas of the BMA.

### **Please explain why your abstract is innovative for mass spectrometry?**

The use of high-resolution mass spectrometry and the KMD to confirm the identity of polymers in the samples.

### **Co-authors:**

*Albert Vega-Herrera, IDAEA-CSIC*

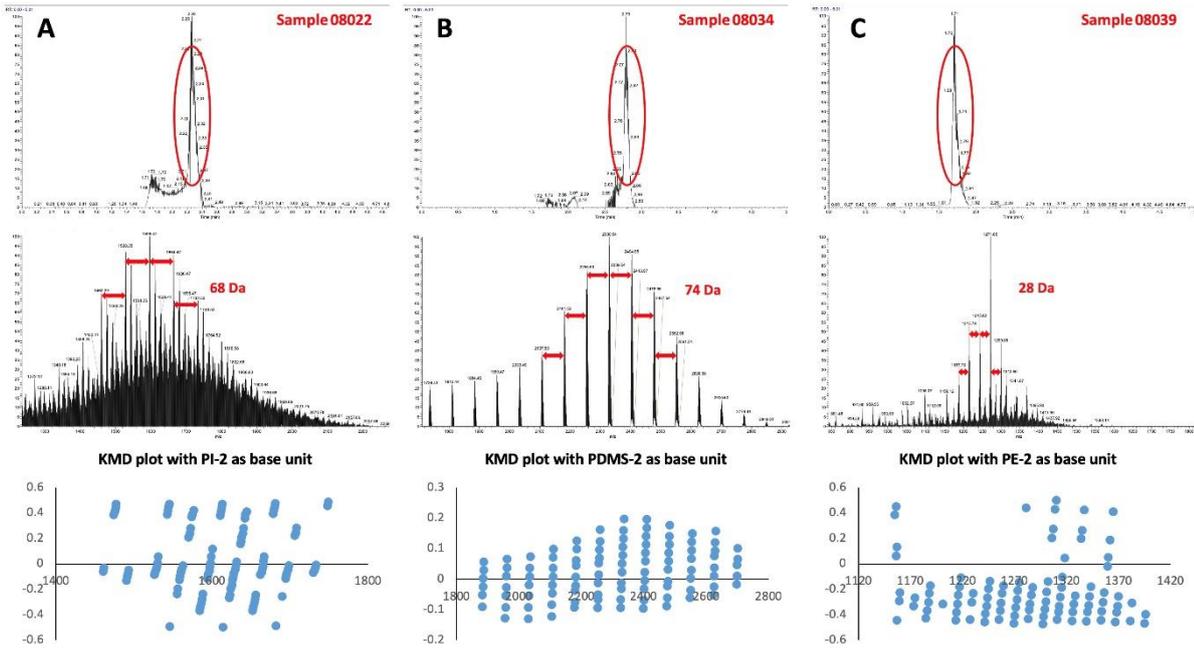
*Marta Llorca, IDAEA-CSIC*

*Cristina M Villanueva, ISGlobal*

*Esteban Abad, IDAEA-CSIC*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
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Examples of identifications by Kendrick Mass Defect analysis.

Poster number: **IM-PA-070**

## LEVERAGING A HIGHER DUTY CYCLE DIA ACQUISITION FOR ENHANCED PROTEOMICS ANALYSIS ON A ZENOTOF 7600 FOR ENHANCED PROTEOMICS ANALYSIS

Abstract ID: **1019**Presenting author: **Yves Le Blanc, SCIEX**

### Introduction

The ability to identify and quantify large number of proteins and peptides is of great importance in translational medicine and life science research. Data independent acquisition (DIA) approaches have shown to surpass data dependent acquisition (DDA) methodologies in terms of protein identifications in complex matrices especially at shorter acquisition speeds. Our new QTOF system equipped with a novel Zeno trap is able to deliver sensitivity gains in variable window SWATH acquisition. The built-in Zeno trap increases duty cycle at MS/MS level to over 90%, allowing for unprecedented gains in sensitivity (5-20x) at MS/MS, resulting in more identifications using Zeno SWATH DIA. We evaluated increases in protein and peptide identifications using Zeno SWATH DIA vs. SWATH DIA at various sample throughputs and protein loads.

### Methods

A ZenoTOF 7600 system using a microflow probe on an OptiFlow ESI source was coupled to an EvoSep One EV-1000 (EvoSep) LC. EvoSep EV1112 column was used for 30 SPD (samples per day) throughput, EV1109 column was used for 60 SPD and 100 SPD, whereas EV1107 column was used for 200 SPD throughput. SWATH DIA and Zeno SWATH DIA methods covered precursor mass range of 400-750 or 400-900 m/z. HeLa (Thermo Fisher Scientific) peptide loads equivalent to 25ng, 50ng and 200ng were injected in triplicate. Data was processed in DIA-NN using an in-house spectral library at 1% FDR.

### Preliminary data (results)

EvoSep was operated at throughputs of 200 SPD (samples per day, 5 min gradient), 100 SPD (11 min gradient), 60 SPD (21 min gradient) and 30 SPD (45 min gradient). When analyzing with Zeno SWATH DIA rather than SWATH DIA, we obtain 39-51% increase in protein group identifications at low (50 ng) peptide loads and 67-88% increase in proteins with quantified with CV under 20% at all SPD throughputs. At higher peptide load (200 ng), with Zeno SWATH DIA we see an increase of 11-25% and 34-59% in total number of protein groups detected and quantified under 20% CV, respectively.

For a 25 ng load, with a 60 SPD method we can detect over 4400 protein groups with 25000 precursors and with a 30 SPD workflow, there are over 5300 protein groups with more than 29000 precursors. At 200 ng HeLa peptide load with a 60 SPD and a 30 SPD method, we are able to identify over 6800 and 7600 protein groups respectively, with nearly 90% of identifications having a CV <20%. There are over 49000 and 63000 precursors for a 200 ng load at 60 SPD and 30 SPD, respectively. A 200 SPD method detects over 4000 protein groups with 20000 precursors for a 200 ng load. Library-free searches with a FASTA database perform well and eliminate the necessity of generating spectral libraries. Largest gains in identifications with Zeno SWATH acquisition are observed at low level loads.

### Please explain why your abstract is innovative for mass spectrometry?

High duty cycle in Zeno SWATH DIA significantly increases the number of identifications at low peptide loads relative to SWATH DIA.

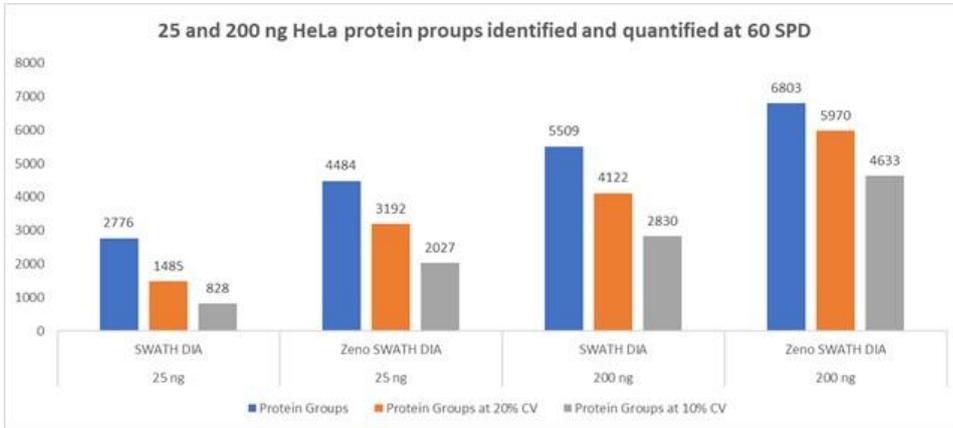
### Co-authors:

*Ihor Batruch, SCIEX*  
*Jason Causon, SCIEX*  
*Naomi Diaz, SCIEX*  
*Tatjana Talamantes, SCIEX*  
*Anjali Chelur, SCIEX*  
*Nic Bloomfield, SCIEX*

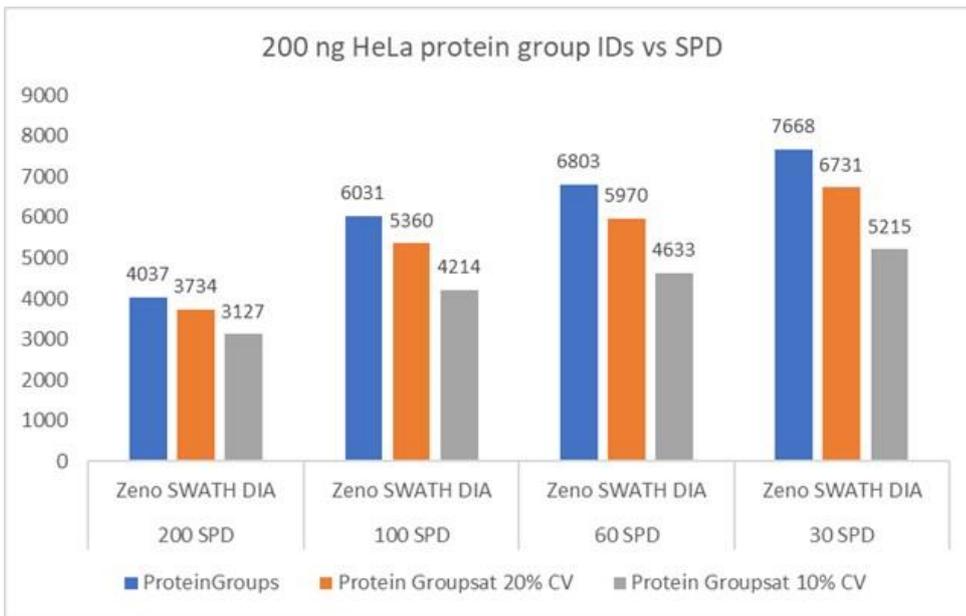
**POSTER SESSION A**

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 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours

Stephen Tate, SCIEX  
 Pavel Ryumin, SCIEX  
 Jose Castro-Perez, SCIEX



Protein group identifications with SWATH and Zeno SWATH DIA



Protein group identifications with Zeno SWATH DIA vs SPD

Poster number: **IM-PA-071**

## **A COMPLETE AND AUTOMATED SAMPLE PREPARATION STRATEGY FOR HIGH-THROUGHPUT AND STANDARDIZED PROTEOMICS APPLIED TO A CLINICAL COHORT OF PATIENT PLASMA SAMPLES**

Abstract ID: **1021**

**Presenting author: Ole B. Hoerding, Evosep**

### **Introduction**

Mass spectrometry-based clinical proteomics is becoming increasingly popular and provides a powerful set of technologies, with the potential to revolutionize health care and enable precision medicine. We specifically designed and developed the Evosep One for high throughput applications, with a focus on clinical analysis of large sample cohorts.

For a widespread adaptation of proteomics in the clinic, the entire workflow from sample preparation through LC-MS and data analysis needs to be fast and robust to enable the required throughput. To meet these requirements, we introduce an automated end-to-end proteomics sample preparation workflow starting from protein lysate to tryptic peptides loaded on Evotips ready for injection on the Evosep One applied to a clinical cohort of patient plasma samples.

### **Methods**

HeLa cells were harvested in boiling 5% SDS buffer. Plasma samples were collected from 48 patients, diagnosed with systemic sclerosis and 48 healthy control individuals. A complete and automated sample preparation workflow was integrated on an Opentrons OT-2 robot utilizing protein aggregation capture (PAC), followed by on-bead trypsin digestion, and automatic loading of the resulting peptides onto Evotips using a specially designed pneumatic 8-channel module from Evosep (prototype). The device has a footprint of a 96 well microtiter plate, fits one position in the robot and uses positive air pressure to move liquid through Evotips.

### **Preliminary data (results)**

Here we describe an automated end-to-end workflow, where all manual interventions poses a risk of introducing variations and sample handling errors. Using HeLa protein lysate, we optimized the PAC protocol for high-throughput automation on the Opentrons-2 resulting in a complete workflow for 96 samples starting from protein to digested peptides, loaded on Evotips in less than 2 hours and applied this to a clinical cohort of 96 plasma samples. The entire protocol was designed for a 'single touch' fully automated approach, carefully considering consumption of pipette tips, buffer trays and digestion conditions to fit the Opentrons framework and finally with integrated loading of peptides on Evotips, which serve as storage until analysis. To minimize sample preparation time, we utilized a short 1 h digestion at room temperature with optimized digestion buffers providing the same reproducible digestion efficiency as protocols with overnight digestion at 37°C.

To demonstrate clinical applicability, we tested a sample cohort of 96 plasma samples collected from 48 patients diagnosed with the incurable autoimmune disease, systemic sclerosis, where diagnosis in an early phase is still difficult due to lack of symptoms, and compared these to plasma samples from 48 healthy control individuals. We digested 1 ul of plasma with our automated workflow and analyzed the samples with the 60 samples per day method on the Evosep One connected to an Orbitrap Exploris 480 mass spectrometer in DIA mode. We consistently quantified 340 proteins with high reproducibility covering 5 orders of magnitude in dynamic range.

### **Please explain why your abstract is innovative for mass spectrometry?**

A fully automated end-to-end workflow is fundamental to facilitate large-scale standardized proteomics. This workflow is specifically developed for integration with the Evosep One to enable analysis of large sample cohorts.

### **Co-authors:**

*Dorte B. Bekker-Jensen, Evosep*

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*Ole Østergaard, University of Copenhagen*

*Lasse Falkenby, Evosep*

*Line Vinderslev Iversen, Statens Serum Institut, Bispebjerg University Hospital, Odense University Hospital*

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*Nicolai Bache, Evosep*

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Poster number: **IM-PA-072**

## IMPLEMENTATION OF PASEF AND IMPROVED IONIZATION CHARACTERISTICS IN IMMUNOPEPTIDOMICS

Abstract ID: **1027**

**Presenting author: Daniel Flender, University of Antwerp, Centre for Proteomics, Vito, Unit Health**

### Introduction

Immunopeptidomics is a powerful tool to identify MHC-bound peptides presented on the cell surface directly. In particular, identifying neoantigens and tumour-specific antigens is crucial for developing and improving immunotherapies. Despite advances in technical sensitivity, identifying immunogenic peptides remains an intricate approach. Contrary to tryptic peptides, the MHC binding motif can account for poor ionization characteristics and in addition to the partially low abundance of immunopeptides. Furthermore, the constrained length of 8-12 amino acids for immunopeptides yields often singly charged precursor ions, hindering the distinction between fragmentation spectra and background signal. Therefore, improvement in sensitivity for immunopeptides and an increase in the number of obtained fragmentation spectra and their quality are decisive for in-depth coverage and *de novo* sequencing of the immunopeptidome

### Methods

This project aims to increase the number of identification by using two approaches; 1) the use of trapped ion mobility mass spectrometry and 2) implementing labelling techniques to increase the charge state of immunopeptides. In this study, we use immunoprecipitation to isolate MHC complexes from Jurkat cells and elute MHC-I bound peptides for a comparative analysis between the timsTOF Pro and the Q-Exactive. PEAKS was used for the data analysis, and the UniProt human reference proteome was used as the target database. Before LC-MS/MS, isobaric labelling was applied to the immunopeptides with a tandem mass tag.

### Preliminary data (results)

The timsTOF Pro has the edge over conventional mass spectrometers in the field of immunopeptidomics since it employs trapped ion mobility spectrometry to separate and capture immunopeptides. The tims accumulate all precursor ions and release them sequentially according to their mobility in an electrical field. This allows to separate singly and multiply charged ions from each other and background signal. In immunopeptidomics, this results in less background and higher sensitivity for low abundant peptides. Our data show that the PASEF approach improves the quality of fragmentation spectra, which results in a higher certainty of *de novo* sequences.

Comparing the timsTOF Pro to the Q-Exactive reveals an advantage for the timsTOF Pro, as it yields a significantly higher number of identifications of unique peptides and less co-eluting peptides resulting in less ambiguous fragmentation spectra. However, the measurement on the Q-Exactive results in several unique identifications which are not observed in the timsTOF Pro measurement, indicating a complementarity between both instruments.

Additionally, the isobaric labelling of immunopeptides was found to promote the formation of multiply charged precursor ions and therefore enhance the number of unique identifications. Overall the combination of trapped ion mobility and isobaric labelling indicates a 2-3 fold increase in the number of identifications compared to previous studies using the same amount of sample input

### Please explain why your abstract is innovative for mass spectrometry?

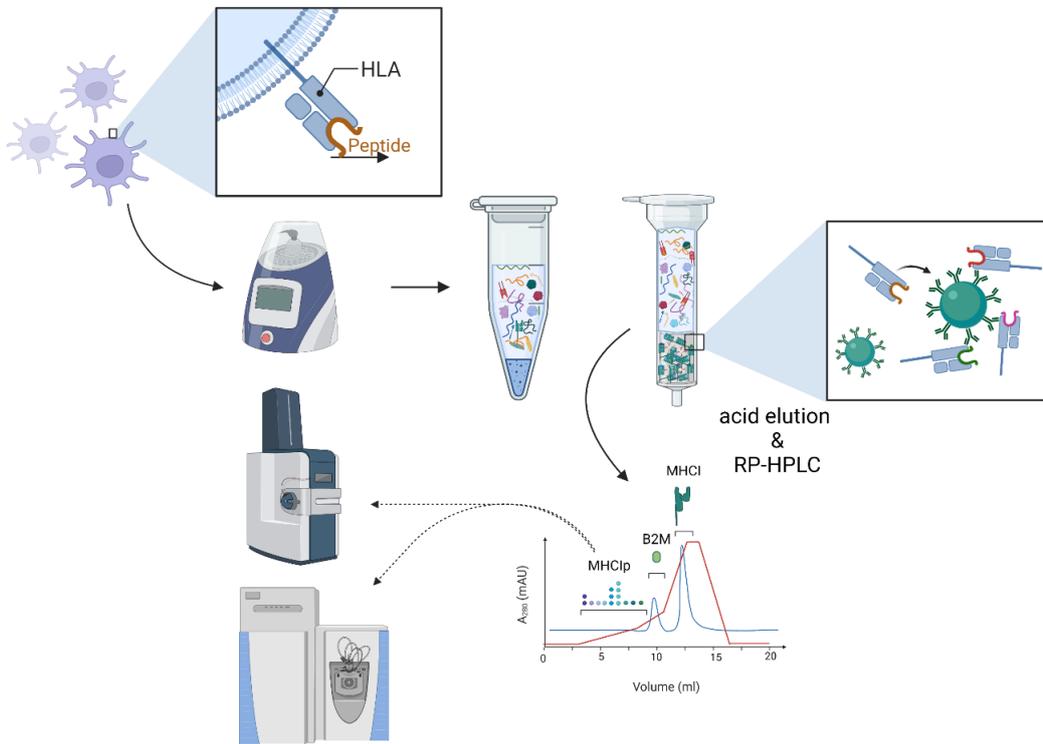
The current set-up using the timsTOF Pro and isobaric labelling indicates the preeminent approach for the most complete immunopeptidome coverage

### Co-authors:

*Elise Pepermans, University of Antwerp, Centre for Proteomics, Vito, Unit Health*  
*Kurt Boonen, University of Antwerp, Centre for Proteomics, Vito, Unit Health*  
*Geert Baggerman, University of Antwerp, Centre for Proteomics, Vito, Unit Health*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
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Immunopeptidomics workflow for the Identification of MHC-I bound peptides

## Session: Instrumentation and methods

Poster number: **IM-PA-073**

### ENHANCED LAAPPI-MS FOR IMAGING APPLICATIONS

Abstract ID: **79**

Presenting author: **Juha-Pekka Hieta, University of Helsinki**

#### Introduction

Infrared (IR) laser ablation atmospheric pressure photoionization (LAAPPI) is an ambient MS method that allows analysis and imaging of untreated and matrix-free biological samples. IR-LAAPPI can sample molecules from deeper parts of the sample than UV-MALDI, which allows its use for depth profiling and is a major advantage in the imaging of samples such as plant leaves, which cannot be sectioned or otherwise prepared without significant metabolic changes. Recent work on decreasing the sampling spot size of OPO IR lasers has significantly improved the achievable lateral resolution of LAAPPI for MS imaging purposes.

#### Methods

LAAPPI is based on IR laser ablation (IRLA), which can perform accurate, matrix-free spot-to-spot sampling for MS analysis with good repeatability as the IR laser energy is absorbed by the sample water. IRLA sampling results in efficient ablation of sample molecules to the gas phase, after which they are subsequently ionized with an ionization method of choice, such as atmospheric pressure photoionization (APPI). APPI provides good ionization efficiency for nonpolar neutral compounds that may be poorly ionized by electrospray ionization (ESI).

#### Preliminary data (results)

Recent results of LAAPPI-MS imaging of animal and plant tissues show that the method can now acquire spatially more accurate distribution images of small molecules than previously demonstrated. The acquired MS images match well with the histological features of rodent brain and *Arabidopsis thaliana* leaves, even at the single cell level. The analysis of brain tissue shows that LAAPPI can acquire complementary biological information to ESI-based methods, such as LAESI, as it can more efficiently ionize nonpolar compounds. Furthermore, the analysis of complex, multi-layered structure of leaves demonstrated the feasibility of LAAPPI-MS to depth profiling.

#### Please explain why your abstract is innovative for mass spectrometry?

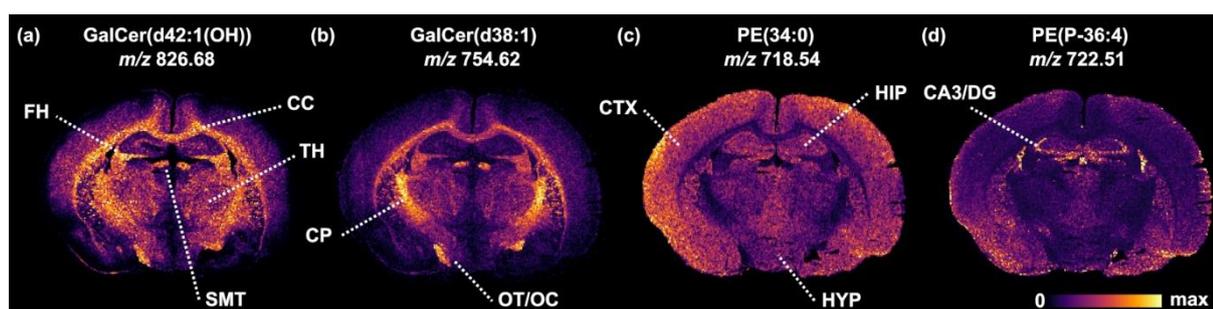
The enhanced LAAPPI-MS now allows imaging of polar and nonpolar 50–1000 Da molecules from untreated biological samples with a good lateral resolution.

#### Co-authors:

*Nina Sipari, University of Helsinki*

*Heikki Rääkkönen, University of Helsinki*

*Risto Kostainen, University of Helsinki*



**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

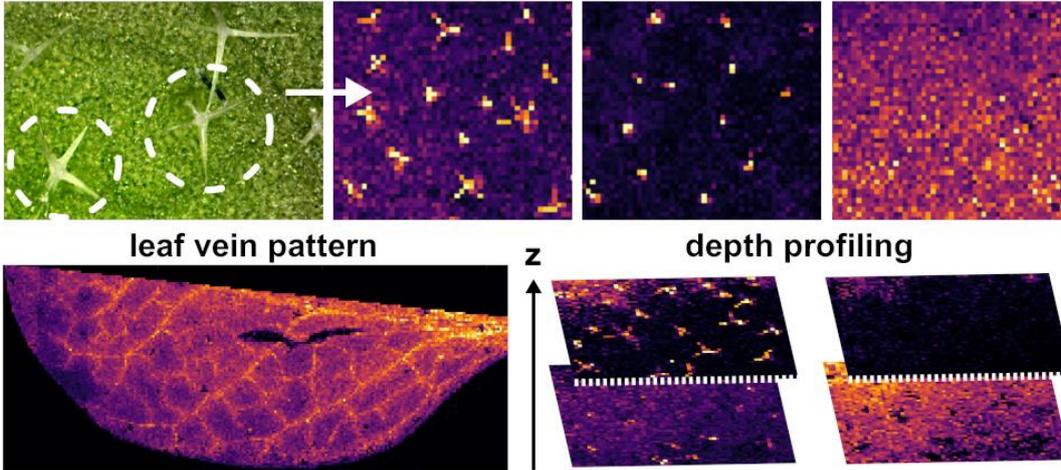
LAAPPI-MS images of 20 µm thick rat brain tissue sections.

## LAAPPI-MS imaging of *A. thaliana* leaves

single-cell imaging of trichomes

flavonoids

cuticular wax



LAAPPI-MS images of *Arabidopsis thaliana* leaves.

Poster number: **IM-PA-074**

## **MS SPIDOC: MASS SPECTROMETRY MEETS SINGLE PARTICLE IMAGING**

Abstract ID: **104**

**Presenting author: Thomas Kierspel, Deutsches Elektron-Synchrotron DESY, Centre for Structural Systems Biology CSSB, European XFEL GmbH, Leibniz Institute for Experimental Virology (HPI)**

### **Introduction**

Native mass spectrometry (MS) enables the ionization and transfer of structurally intact non-covalent protein complexes into the gas-phase. As such, it is a perfect tool to study proteins and their assembly intermediates in a mass and conformation specific manner. However, structure follows function, and the structural information that can be gained with techniques like top-down MS or ion mobility is limited. Accordingly, other experimental approaches such as X-ray diffractive imaging are necessary to get a full understanding of the proteins and their assemblies.

### **Methods**

MS SPIDOC (Mass Spectrometry for Single-Particle Imaging of Dipole Oriented Protein Complexes) is a Horizon 2020 funded research and innovation program [1] aiming at the combination of both experimental techniques. In particular, well established methods from MS like  $m/z$  selection, ion trapping or ion mobility are adapted as part of the sample delivery system for X-ray diffraction.

### **Preliminary data (results)**

In contrast to conventional diffractive imaging of crystallized proteins, the proteins here are delivered as single particle without the need for crystallization. This increases naturally the requirement to the X-ray source. Thus, single-particle X-ray diffractive imaging (SPI) is only conducted at X-ray free electron lasers [2], the worlds brightest X-ray sources in the world.

This contribution will highlight the ongoing efforts of the MS SPIDOC consortium to develop this sample delivery system for the use at beamlines of the European XFEL [3]. The current state of the designing and manufacturing of the instrument prototype will be presented as well as the results of the first testing of individual component modules. Furthermore, simulations as well as experimental device design for particle dipole orientation in strong electric fields will be previewed.

[1] <https://www.ms-spidoc.eu>

[2] Seibert et al., *Nature* **470**, 78–81 (2011)

[3] Kadek et al., *Drug Discov. Today Technol.* 08 (2021)

### **Please explain why your abstract is innovative for mass spectrometry?**

What makes MS SPIDOC unique is that we use several techniques developed for native MS and extend its potentials to alternative observation methods such as X-ray diffraction.

### **Co-authors:**

*Alan Kadek, BIOCEV - Institute of Microbiology CAS, Leibniz Institute for Experimental Virology (HPI), European XFEL GmbH*

*Kristina Lorenzen, European XFEL GmbH*

*Charlotte Uetrecht, School of Life Sciences, University of Siegen, Leibniz Institute for Experimental Virology (HPI), Deutsches Elektron-Synchrotron DESY, Centre for Structural Systems Biology CSSB, European XFEL GmbH*

Poster number: **IM-PA-075**

## **DESI AND/OR LA-REIMS? ADJACENT AUTOMATED AMBIENT TECHNIQUES FOR THE PRECISE IDENTIFICATION OF CANCER TISSUE**

Abstract ID: 112

**Presenting author: Gabriel Stefan Horkovics-Kovats, Waters Research Center Kft., Eötvös Loránd University**

### **Introduction**

Chemical imaging using mass spectrometry is an emerging technology that can be used for applications in various scientific fields. One example is the precise identification of cancer tissue from sectioned samples. Recently developed laser-assisted rapid evaporative ionization mass spectrometry (LA-REIMS) and automated desorption electrospray ionization (AutoDESI) are ambient imaging techniques that require no specific sample preparation and are capable of each metabolic profiling of the target tissue. Since these are two different methods, the advantages and disadvantages of each technique must be defined to discover optimized application areas and combination possibilities. To compare the two techniques, a set of veterinary and human samples including fresh and formalin-fixed sections were measured on both imaging setups. The aim is to compare spectra quality and content regarding image resolution.

### **Methods**

The LA-REIMS imaging setup consists of an optical parametric oscillator and a self-made optical path focusing on the sample surface and a commercially available motorized stage.

The AutoDESI imaging setup consists of a DESI-XS sprayer, heated transfer line and automation enabling the automatic measurement of 80 slides (Figure 1).

The generated aerosol (LA-REIMS) and the extracted solvent (AutoDESI) are introduced into a Xevo™ G2-XS ToF MS (Waters Corporation) for analysis. Multivariate statistics including Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were used for point-by-point pixel classification and model generation.

Xevo is a trademark of Waters Technologies Corporation

### **Preliminary data (results)**

With both setups, tumor tissue could be successfully classified from healthy tissue and visualized in high resolution (Figure 2). The power density of the LA-REIMS can be adapted to specific sample requirements by optical components, like lenses and neutral density filters, and software control. For example, core biopsy samples can be measured with robust settings, which is difficult with AutoDESI due to very precise and focused sprayer settings. A usable signal was achieved with LA-REIMS with power densities between 34.08-106.04 W/cm<sup>2</sup>, which define the achieved spot sizes and thus the overall image resolution. The nondestructive AutoDESI method allows not only re-scanning of the same sample, but also oversampling by spraying the sample, thus enabling even higher resolutions. However, this spray-motion prevents accurate measurement of uneven samples. On the other hand, AutoDESI can obtain signals from smeared samples, while a sample thickness of at least 7 microns is recommended for LA-REIMS. When measuring fresh samples, LA-REIMS achieved a signal sensitivity in the 10<sup>\*e</sup>4 region, while it was one magnitude higher (10<sup>\*e</sup>5 region) with AutoDESI. In the case of FFPE samples, LA-REIMS achieved a tissue specific signal sensitivity in the 10<sup>\*e</sup>3 region, compared to almost no signal with AutoDESI. The overall results were put into comparison to showcase LA-REIMS spectra featuring more abundant PEs with ammonia loss (e.g. 699.5 m/z) and AutoDESI spectra featuring more abundant PIs (e.g. 885.5 m/z). Both techniques enable a robust, routine, unattended operation allowing efficient measurement of large cohort samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

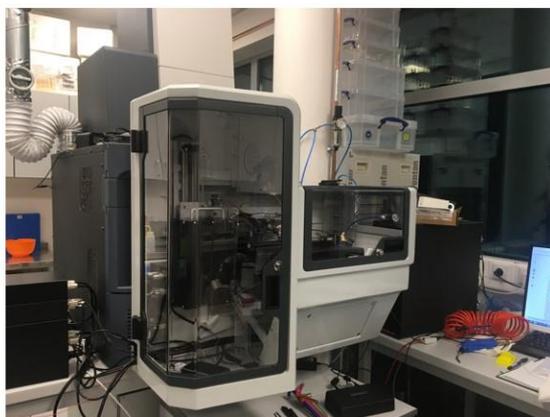
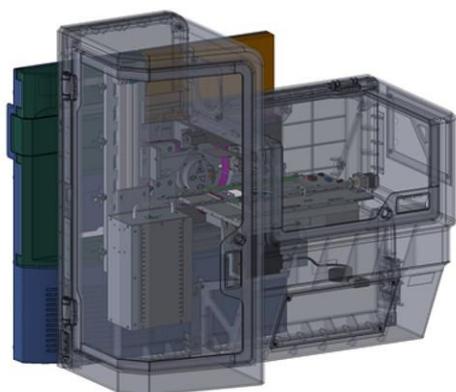
Fully automated high, throughput sample section imaging from microscope slides with LA-REIMS and AutoDESI imaging setups for pathological application areas.

### **Co-authors:**

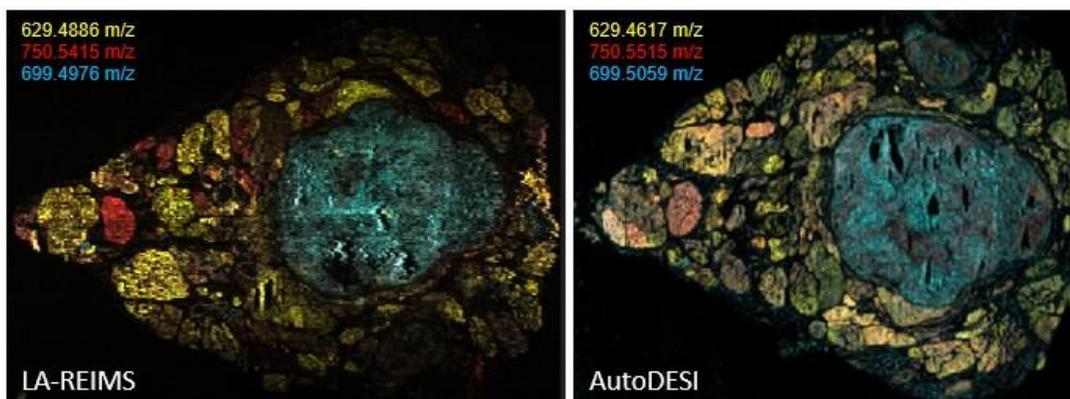
**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Richard Schäffer, Waters Research Center Kft.*  
*Csaba Hajdu, Waters Research Center Kft.*  
*Gitta Schlosser, Eötvös Loránd University*  
*Julia Balog, Waters Research Center Kft.*



AutoDESI model (left) and application (right)



Tubular carcinoma of dog sample: LA-REIMS (left), AutoDESI (right)

Poster number: **IM-PA-076**

## **MS IMAGING OF INTACT 100 KDA MEMBRANE AND SOLUBLE PROTEIN ASSEMBLIES**

Abstract ID: **140**

**Presenting author: Oliver J Hale, University of Birmingham**

### **Introduction**

Nanospray-desorption electrospray ionization (Nano-DESI) has been demonstrated for spatially resolved analysis of intact protein assemblies directly from tissue under native-like conditions. In the present work, protein assemblies of approx. 100 kDa were analysed by nano-DESI analysis of eye lens tissue. We demonstrate mass spectrometry imaging (MSI) and spatially resolved, top-down analysis of an integral membrane protein assembly, Aquaporin-0 (Aqp0), and soluble tetramers of the beta-crystallin protein family, allowing extensive characterisation.

### **Methods**

Fresh frozen sheep eye lenses were cryosectioned and thaw mounted onto glass microscope slides. Sections were analysed without further sample preparation. Nano-DESI MS and MSI was performed with a non-denaturing solvent comprised of 200 mM aqueous ammonium acetate and the detergent C8E4 at 2x critical micelle concentration.

Nano-DESI was performed on an Orbitrap Eclipse with flow rate =2  $\mu$ L/min and spray voltage approx 1.2 kV. Aqp0 contained within detergent micelles was released by application of collision voltage in the source region of the mass spectrometer. Top-down analysis was performed using collisional dissociation (HCD) and proton transfer charge reduction (PTCR).

### **Preliminary data (results)**

Aqp0 is a homotetrameric integral membrane protein assembly (assembly molecular weight: 113 kDa), featuring 6 transmembrane helices and 2 intramembrane helices per subunit. The assembly was solubilized and ionized by directly sampling the tissue with a non-denaturing solvent system and mass spectrometry compatible detergent, allowing extensive characterization in a spatially resolved top-down approach. The intact mass of the Aqp0 homotetramer was measured by deconvolution of full scan MS mass spectra and PTCR MS<sup>2</sup> mass spectra. Signals for phosphorylated Aqp0 incorporated in the assembly were also detected. The mass of Aqp0 subunits was measured by isolation and HCD of tetrameric Aqp0 and deconvolution of subunit ion signals and enabled confirmation of the tetrameric stoichiometry of the intact assembly. Sequence information for Aqp0 was obtained by pseudo-MS<sup>3</sup>; removal of the micelle and dissociation of the tetrameric assembly in the mass spectrometer source region by elevated collision voltage, followed by isolation and HCD of subunit ions. Sequence coverage was approx. 11%. Fragment ions were detected from specific regions of the subunit, including the C-termini of both Asp-residues in the Aqp0 sequence. Soluble hetero and homo beta-crystallin tetramers (approx. 94 kDa) were imaged and identified in a similar manner. Ion images showed the different distributions of protein assemblies throughout the lens; for example, a beta-crystallin homotetramer was located within the lens nucleus, while Aqp0 was most abundant within the lens cortex.

### **Please explain why your abstract is innovative for mass spectrometry?**

Mass spectrometry imaging and top-down analysis of intact membrane and soluble protein assemblies of approx. 100 kDa molecular weight directly from tissue.

### **Co-authors:**

*Helen J Cooper, University of Birmingham*

Poster number: **IM-PA-077**

## **MASS SPECTROMETRY IMAGING PROVIDES INSIGHTS INTO THE FATE OF CARNITINE AND ACYLCARNITINES IN ISCHEMIC MOUSE BRAIN**

Abstract ID: **178**

**Presenting author: Leonidas Mavroudakos, Department of Chemistry - BMC, Uppsala University, Sweden**

### **Introduction**

Brain is occupying about 2 % of the total body's mass but it requires almost 20 % of the body's energy. Oxidation of glucose is the primary source of energy for the brain in comparison with muscle, where  $\beta$ -oxidation of fatty acids prevails. Nonetheless, the enzymes that are necessary for energy production from the utilization of fatty acids can be found in neurons of the brain. It has also been shown that energy production can occur from the oxidation of fatty acids in the brain. L-Carnitine can be found in cells and tissue as free carnitine as well as acylcarnitines and assists in transportation of long-chain fatty acids in the mitochondria for subsequent  $\beta$ -oxidation. Therefore, carnitine and acylcarnitines are important molecules involved in the energy metabolism.

### **Methods**

Here, we have used an ambient mass spectrometry imaging (MSI) technique for studying the distribution of carnitine and acylcarnitines in ischemic mouse brain. Specifically, we employed nanospray desorption electrospray ionization (nano-DESI) MSI with appropriate internal standards doped in the solvent for quantitative analysis. Further, we used suitable correction factors to account for the differential ionization efficiency of the various acylcarnitines and obtained unbiased quantitative results. Thin tissue sections from an ischemic mouse model (middle cerebral artery occlusion, MCAO) were analyzed.

### **Preliminary data (results)**

Our preliminary data demonstrate that acylcarnitine was depleted and that medium and long-chain acylcarnitines were accumulated in the ischemic area. This could be attributed to the supporting role of acylcarnitine under starvation conditions by assisting with reacylation of membranes. Further, we speculate that the accumulation is a result of prohibited utilization of fatty acids under ischemic conditions due to higher oxygen demand for  $\beta$ -oxidation compared to glucose oxidation, which in combination with increased generation of superoxide enhances oxidative stress in the neurons. Collectively, we show that nano-DESI MSI of thin brain tissue sections from ischemic model provides unique insights into the energy metabolism of brain during ischemia that promotes further understanding on the biochemical processes of stroke.

### **Please explain why your abstract is innovative for mass spectrometry?**

Use of ambient mass spectrometry imaging with no sample preparation for gaining insights into brain energy metabolism.

### **Co-authors:**

*Ingela Lanekoff, Department of Chemistry - BMC, Uppsala University, Sweden*

Poster number: IM-PA-078

## TOWARDS CELLULAR RESOLUTION OF TRYPTIC PEPTIDES IN MASS SPECTROMETRY IMAGING: A FOCUS ON ENZYME APPLICATION AND REPRODUCIBILITY

Abstract ID: 211

**Presenting author: Bastian Jahreis, Bastian Jahreis, Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Germany, Andreas Römpf, Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Germany**

### Introduction

MALDI-mass spectrometry imaging (MALDI-MSI) is a unique analytical technique which has a variety of applications in bioanalytical sciences including the investigation of proteins under *in situ* conditions. Besides direct ionization and detection of small proteins using high spatial resolution MSI, imaging of bigger proteins and subsequent identification remains difficult. Here, tryptic digestion of proteins on tissue and MS imaging of tryptic peptides is the method of choice when protein identification is necessary. However, a complex sample preparation including chemical treatment of tissue sections and enzyme application results in limitations in achievable lateral resolution and sensitivity. This work is focused on the optimization of a dedicated MS imaging workflow to achieve high resolution MALDI-MS imaging of tryptic peptides.

### Methods

After storage at  $-80^{\circ}\text{C}$ , mouse brain sections ( $12\mu\text{m}$  thickness) were dried in a desiccator. Lipids and salts were removed from tissue by washing with a series of ethanol and water. Digestion was performed by spraying trypsin sequentially on tissue using a semi-automatic pneumatic sprayer. Between each spray, tissue sections were placed inside a digestion chamber at  $37^{\circ}\text{C}$ . Sections were incubated for 2h after the last spray. 2,5-DHB matrix was applied using a pneumatic spraying device. MS imaging was performed using an AP-SMALDI5-AF (TransMIT GmbH, Giessen, Germany) ion source attached to a Q-Exactive-HF (Thermo Scientific, Bremen, Germany).

### Preliminary data (results)

Trypsin spray application was investigated and optimized to achieve high spatial resolution MS imaging data of tryptic peptides. A modified semi-automatic pneumatic spraying device was developed. Several parameters were investigated including enzyme flow rate and volume, gas pressure and needle geometry used for nebulization of the trypsin solution. Here, an optimized sample preparation workflow is presented, allowing high spatial resolution MS imaging of tryptic peptides. In addition, high mass resolution and accuracy allows identification of tryptic peptides based on accurate mass. Our workflow allows reproducible imaging of tryptic peptides in mouse brain sections at  $20\mu\text{m}$  and  $10\mu\text{m}$  pixel size with high mass resolution ( $R > 100000$  FWHM) and mass accuracy ( $\text{RMSE} < 3$  ppm). MALDI-MS imaging at  $10\mu\text{m}$  pixel size showed that the spatial distribution of tryptic peptides is highly influenced by the trypsin application method and that even small changes can lead to peptide delocalization. In addition, peptide ion detection at these high lateral resolutions is also affected. Therefore, a second trypsin application method is presented resulting in a higher number of protein identifications and possible MS imaging down to a pixel size of  $20\mu\text{m}$ .

### Please explain why your abstract is innovative for mass spectrometry?

We developed a high-resolution bottom-up MS imaging workflow to visualize tryptic peptides at  $10\mu\text{m}$  pixel size in mouse brain tissue.

Poster number: **IM-PA-079**

## A FAST METHOD FOR LIPIDS SCREENING USING TLC AND MASS SPECTROMETRY IMAGING

Abstract ID: 276

Presenting author: **Sophie Rappe, Mass Spectrometry Laboratory-ULiège**

### Introduction

Lipids share diverse biological functions and locations in the body while being structurally vast class of compounds. Disturbances in lipid biosynthesis are involved in numerous human diseases such as cancer and neurodegenerative and cardiovascular diseases. Nowadays MALDI mass spectrometry imaging (MSI) has gained enough robustness to efficiently map the spatial distribution of lipids in histological thin sections. In the case of liquid extracts, (U)HPLC-MS is used for quantification. Alternatively, direct infusion allows rapid qualitative lipids pattern evaluation. In this work, we developed a new method for non-targeted and semi-quantitative lipids profiling using radial separation on thin layer chromatography plate (TLC) coupled with MALDI MSI detection.

### Methods

Here we propose a method of direct transfer of the lipids from the sample to the TLC. A fast pre-separation of lipid families according to the hydrophobicity of their polar head on the normal phase MALDI-TLC support should limit the MALDI ion suppression effect while adding an additional identification parameter: the retention factor. Lipid family separations are tested with different thicknesses of the silica phase, both in linear and radial modes. The MALDI images of the plates were recorded using either a TOF and FT-ICR mass analyzer. Kendrick Mass Defects filtering was used for fast and non-targeted data analysis.

### Preliminary data (results)

Fast lipid screening would ideally avoid pretreatment of biological samples such as liquid-liquid or solid-liquid extraction. The lipids were directly transferred by sample-support contact. The nature of the eluent mixture for lipid family separation on TLC was optimized and several separation steps can be performed sequentially. The choice of the mode of separation, linear or radial, was guided by practical considerations allowing improved sample handling and constrained duration MSI detection. Additionally, the radial mode allows a larger number of samples to be run on the same TLC plate with minimal sample handling. Rapid image acquisition of few lines of pixels passing through the center of each spots allowed the automated acquisition and provided twice the separation image on both sides of the diameter. Other separation directions are still available for replicates or further deeper analysis, including ion mobility measurements. During sample preparation, two major factors were identified to affect the MS images: matrix application and silica thickness. The matrix application is a critical step as the matrix must reach the analytes that can be embedded inside the TLC silica pores. The optimal amount of sprayed matrix depends on the silica thickness and the co-crystallization efficiency was different according to the deposition method of the matrix solution. Fluctuations in topology and local accumulation of matrix had to be avoided to retain the optimal lateral resolution, mass resolving power, and mass accuracy.

### Please explain why your abstract is innovative for mass spectrometry?

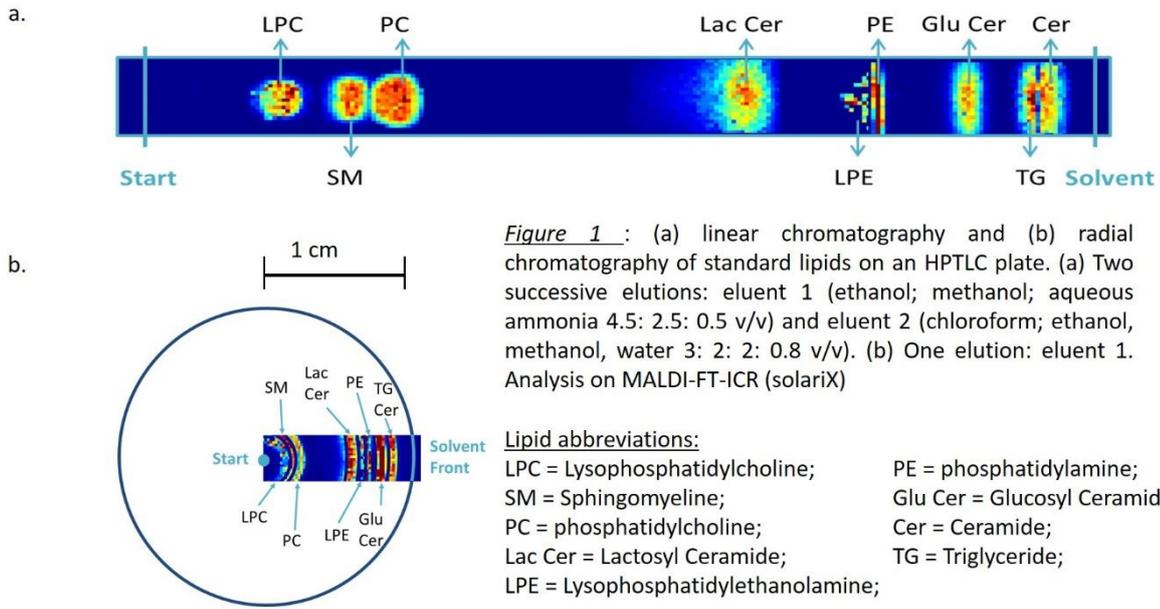
Non-targeted and semi-quantitative method lipids profiling on radial thin layer chromatography coupled with MALDI imaging and fast data analysis.

### Co-authors:

*Johann Far, Mass Spectrometry Laboratory-ULiège*  
*Gauthier Eppe, Mass Spectrometry Laboratory-ULiège*  
*Edwin De Pauw, Mass Spectrometry Laboratory-ULiège*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Poster number: **IM-PA-080**

## **REVEALING ESOGASTRIC CANCER SPECIFICITY BY MALDI-MS IMAGING AND SPIDERMASS**

Abstract ID: **306**

**Presenting author: Léa Ledoux, Univ. Lille, Inserm, CHU Lille, U1192 - Protéomique Réponse Inflammatoire Spectrométrie de Masse - PRISM, F-59000 Lille, France.**

### **Introduction**

With about 951,000 cases each year, esogastric cancer (EC) is the fifth most often-diagnosed cancer worldwide. EC encompasses a variety of different adenocarcinoma types and subtypes such as the tubular, the papillary, the mucinous and the PCC types the latest including SRC and NOS subtypes. PCC is a very aggressive type, occurring in young patients, and extremely difficult to diagnosed because of its diffuse character. In particular, it is difficult to distinguish PCC intraoperatively and this is at the origin of the relapse of a half of patients. The objective is, thus, to use MALDI-MSI to reveal specific lipid profiles of ECs and confirm them using the SpiderMass to setup a more specific intraoperative diagnostic to assist surgeons in the removal of the entire tumor.

### **Methods**

A cohort of EC excised tissues, obtained from the FREGAT clinico-biological database, were sectioned on a cryostat (Leica CM 1510S). Three sections were collected, one (5µm) for the H&E staining, the second (20µm) for SpiderMass and the last one for (12µm) the MALDI-MSI. The MALDI-MSI analysis was performed in both polarities on a MALDI-TOF (Rapiflex, Bruker) at 50µm spatial resolution using norharmane as matrix (7mg/mL) deposited with an HTX Imaging TM-Sprayer. The SpiderMass analysis was performed on a Q-TOF instrument (Xevo, Waters) in both polarities. The data were processed on SCILS (MALDI-MSI and SpiderMass) and AMX (SpiderMass).

### **Preliminary data (results)**

The 60 (30 healthy and 30 cancers) EC sections that were analyzed by MALDI-MSI in both polarities were submitted to multivariate analysis. Each EC type and subtype were showing a different and specific molecular profile enabling a clear discrimination between them. The markers discriminating the different types and subtypes were identified by MS<sup>2</sup> and manually annotated. Very interestingly, MALDI-MSI clearly revealed tissue heterogeneity also in healthy tissues, enabling to differentiate tissue specific and cancer specific features. Because of strong similarity between MALDI and WALDI MS spectra (93%), the same cohort was analyzed with the SpiderMass. SpiderMass images were very like the MALDI ones leading to the identification of the same discriminative markers. The SpiderMass data were then used to build classification models for typing and subtyping the EC using machine-learning and deep-learning. Due to the important heterogeneity of the healthy tissue, deep learning clearly gave better results. The models were further validated in blind. Finally, the SpiderMass was evaluated in the pathology lab and compared to the gold standard histology process to assess its specificity and sensitivity.

### **Please explain why your abstract is innovative for mass spectrometry?**

Developing a novel and fast intraoperative diagnostic based on MS for esogastric cancer with better performances than the gold standard.

### **Co-authors:**

*Nina Ogrinc, Univ. Lille, Inserm, CHU Lille, U1192 - Protéomique Réponse Inflammatoire Spectrométrie de Masse - PRISM, F-59000 Lille, France.*

*Yanis Zirem, Univ. Lille, Inserm, CHU Lille, U1192 - Protéomique Réponse Inflammatoire Spectrométrie de Masse - PRISM, F-59000 Lille, France.*

*Florence Renaud, Mucines, Différenciation et cancérogénèse épithéliale, UMR-S 1172, University of Lille.*

*Guillaume Piessen, Mucines, Différenciation et cancérogénèse épithéliale, UMR-S 1172, University of Lille.*

*Michel Salzet, Univ. Lille, Inserm, CHU Lille, U1192 - Protéomique Réponse Inflammatoire Spectrométrie de Masse - PRISM, F-59000 Lille, France.*

*Isabelle Fournier, Univ. Lille, Inserm, CHU Lille, U1192 - Protéomique Réponse Inflammatoire Spectrométrie de Masse - PRISM, F-59000 Lille, France.*

Poster number: **IM-PA-081**

## **FAST MOLECULAR PROFILING VIA TARGETED SINGLE-CELL MALDI MSI**

Abstract ID: **332**

**Presenting author: Christian Croissant, CeMOS / Mannheim University of Applied Sciences**

### **Introduction**

In recent years, the characterization of tissue on the single-cell level has become an important method for studying biological processes with high spatial resolution. In addition, the investigation of isolated samples is currently exploited to perform e.g. high-throughput MSI for the identification of the lipid and peptide composition of organelles. Here, we present a platform for single-cell MSI to target lipidomic and metabolic profiles of individual and multiplexed sample types. This allows the investigation of e.g. metabolic changes in a variety of fundamental biological applications. In addition, by use of that platform various combinations of drugs and substrates can be assayed at once with a single-cell representing the reaction vessel.

### **Methods**

Cells were seeded on DMA (droplet micro array) slides and subsequently imaged optically. Cell positions were identified and transferred to a mass spectrometer for single-cell MSI. Molecular data was collected with highest mass accuracy, and profiles of the individual cells were extracted. The molecular composition of the individual cell types was identified by multivariate statistics or ML (machine learning) and linked to parameters of the specific cell assay.

### **Preliminary data (results)**

Optical images were acquired and used for targeted MALDI of selected sample positions. Data was analyzed, and general lipidomic and metabolic profiles were identified. Differences in samples were taken into account for further in-depth look and linked to sample type. Replicates of single cells allow for a cell type specific generalization and distinction between cells of different types via their molecular profile. Specific markers for molecular classification of cell types were identified and confirmed in a supervised approach.

### **Please explain why your abstract is innovative for mass spectrometry?**

Single-cell molecular profiling of individual and multiplexed samples for cell fingerprinting and high-throughput drug screenings.

### **Co-authors:**

*Stefan Schmidt, CeMOS / Mannheim University of Applied Sciences*

*Johanna Huber, CeMOS / Mannheim University of Applied Sciences*

*Carsten Hopf, CeMOS / Mannheim University of Applied Sciences, Medical Faculty / Heidelberg University*

Poster number: **IM-PA-082**

## SOFT-MATERIAL SURFACE SAMPLING WITH ULTRASONIC METHOD FOR MASS SPECTROMETRY

Abstract ID: **333**

**Presenting author: Ari Salmi, Electronics Research Laboratory, Department of Physics, University of Helsinki, FI-00014, Finland**

### Introduction

Biological samples can benefit from liquid immersion by preventing, e.g., drying. High-intensity ultrasound has been employed for sample preparation, e.g. in biology, by utilizing an ultrasonic homogenizer to extract ingredients from plant tissue, but the method does not allow localized sampling of the biomaterial. We have developed an ultrasound technique that aims to resolve this issue. We employ focused ultrasound in three ways: a) to desorb material from the sample surface by localized cavitation erosion, b) to align the ultrasound transducer and to monitor the desorption process via an acoustic feedback loop, and c) to induce an acoustic streaming field that aids in the liquid sampling process. This proposed technique permits analysis of samples with non-flat sample topography in a liquid immersion.

### Methods

We used a custom-made high-intensity focused ultrasound (HIFU) setup to perform surface sampling of plant leaves, having distinct height profiles, submerged in water. Liquid sampling was performed during the desorption process using a liquid handling system developed for our purpose. The collected liquid samples were transferred for subsequent chemical analysis, performed by electrospray ionization mass spectrometry (ESI-MS). Mass spectra of controls and desorption samples were used to identify mass peaks corresponding to ions of specific biomolecules.

### Preliminary data (results)

Four test desorptions were performed on different desorption sites of a basil leaf, Figs. 1&2. The desorption sites showed material removal from the leaf with a spatial resolution of 500  $\mu\text{m}$ .

### Please explain why your abstract is innovative for mass spectrometry?

The focused-ultrasound-based surface sampling method permits studying non-flat samples in liquid immersion from both perspectives: mechanical and chemical contrast.

### Co-authors:

*Tom Sillanpää, Drug Research Program and Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014, Finland*

*Jere Hyvönen, Electronics Research Laboratory, Department of Physics, University of Helsinki, FI-00014, Finland*

*Petri Lassila, Electronics Research Laboratory, Department of Physics, University of Helsinki, FI-00014, Finland*

*Joni Mäkinen, Electronics Research Laboratory, Department of Physics, University of Helsinki, FI-00014, Finland*

*Axi Holmström, Electronics Research Laboratory, Department of Physics, University of Helsinki, FI-00014, Finland*

*Topi Pudas, Electronics Research Laboratory, Department of Physics, University of Helsinki, FI-00014, Finland*

*Riikka Lepistö, Drug Research Program and Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014, Finland*

*Clare Strachan, Drug Research Program and Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014, Finland*

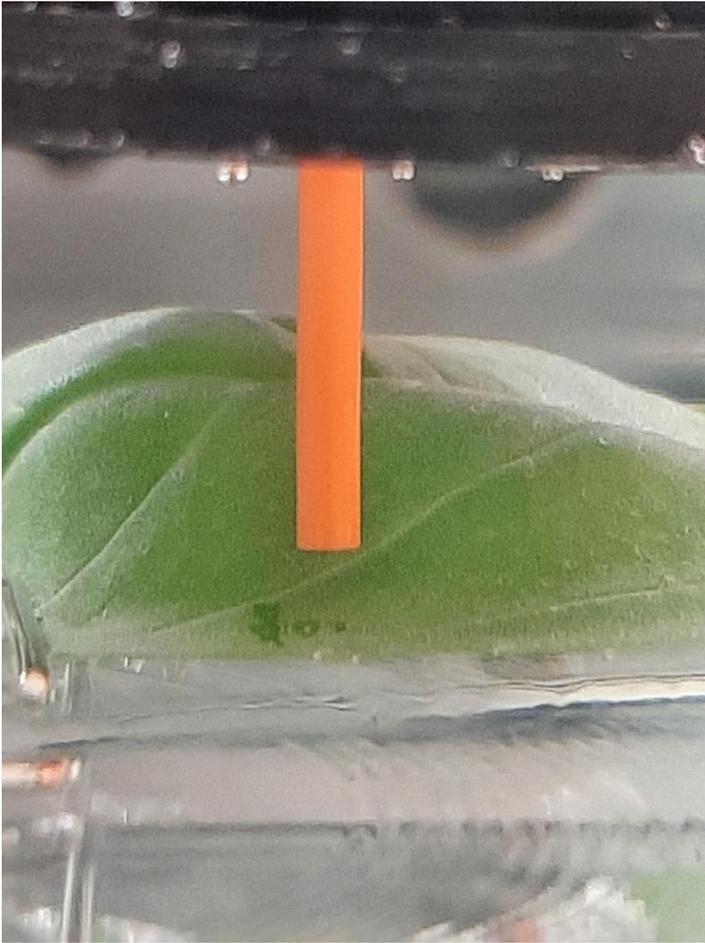
*Antti Kuronen, Department of Physics, University of Helsinki, FI-00014, Finland*

*Tapio Kotiaho, Drug Research Program and Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014, Finland*

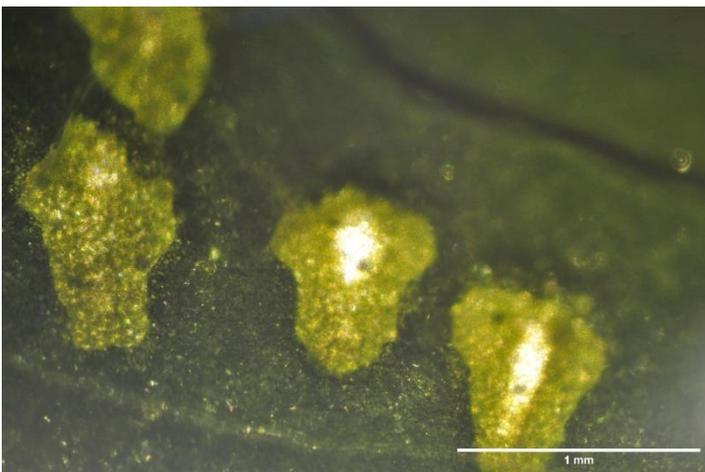
*Edward Hægström, Electronics Research Laboratory, Department of Physics, University of Helsinki, FI-00014, Finland*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



*Basil leaf attached to a glass cover slide in water.*



*Optical microscope image of the leaf surface.*

Poster number: **IM-PA-083**

## **REVISITING THIN LAYER CHROMATOGRAPHY BY MASS SPECTROMETRY IMAGING AND IN SOURCE DECAY (ISD)**

Abstract ID: **351**

**Presenting author: Edwin De Pauw, Mass Spectrometry Laboratory, Uliege**

### **Introduction**

Rapid screening methods of the molecular composition of complex samples are growing as first analytical line. For non-volatile compounds, most of the applications are based on infusion methods using ESI. On the other side, thin layer chromatography is a well-known separation method with analytical applications in many fields of molecular sciences. Mass Spectrometry is a method of choice for TLC plates analysis as it brings molecular information, particularly useful for the identification of unknowns. MALDI imaging is a promising candidate for the TLC plates analysis including biopolymers sequencing thanks to in source decay.

### **Methods**

Critical parameters have been tested to improve the TLC MALDI coupling. The performances of TLC have been largely improved using thinner supports (10 to be tested, 100, 200 microns). Several matrices and matrix deposition methods have been used (9AA, DHB, CHCA, ...) A method has been developed to avoid the mass shift, keeping high resolution and mass accuracy across the whole image to facilitate the identification of unknowns. "In Source Decay". has been transposed to TLC. Limits of detection have been explored for biopolymers (proteins, oligonucleotides).

### **Preliminary data (results)**

Among ionization methods, MALDI is particularly interesting when coupled with TLC as it can be used without extraction of the analytes, keeping a memory of the sample for further analysis. Thanks to the progress in Imaging Mass Spectrometry (IMS) instrumentation, it is now possible to use MALDI IMS to visualize the several whole elution profiles (up to 6 per plate) in a workable time. During MALDI ionization, ions receive an amount of internal energy that can lead to fragmentation. The relative "hardness" of the matrix can be modulated and therefore the fragmentation extent. In source decay fragmentation can be chemically assisted by the MALDI matrix. ISD has been transposed to TLC. Reactive matrices induce in source fragmentation that can be used for fast sequencing long tags of biopolymers, a growing application for biopharmaceutical quality control. The addition of ion mobility in the experimental workflow is a clear prospect for future developments as emerging from preliminary data. The recent developments in TLC MALDI will be presented together with specific applications in the biomolecules field with a specific focus to MALDI imaging as powerful detector.

### **Please explain why your abstract is innovative for mass spectrometry?**

This work shows the first application of MALDI imaging including in source decay for the analysis of TLC plates

### **Co-authors:**

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Poster number: **IM-PA-084**

## **INNOVATIVE MASS SPECTROMETRY APPROACH BASED ON MSI-GUIDED TARGETED METABOLOMIC TO IDENTIFY AND CHARACTERIZE NEW NATURAL PRODUCTS SPECIFIC TO BACTERIAL CO-CULTURE.**

Abstract ID: 352

**Presenting author: Pierre Burguet, University of Liege**

### **Introduction**

Co-culture is an innovative approach extensively used to stimulate and enhance the diversity of metabolites, produced by various microorganisms. The analysis and the discovery of new secondary metabolites from bacterial co-culture can be performed using many analytical methods. Within this context, mass spectrometry and its related techniques, have been recognized as the techniques of choice for their huge potential in the discovery and elucidation of compounds of interest. However, the conventional approach relies mainly on a non-targeted metabolomic LC-MS of the extracts and can be difficult to find interesting compounds within the very complex matrix. Therefore, we decided to develop a new approach based on mass spectrometry imaging data to detect and identify new metabolites with a targeted metabolomics analysis.

### **Methods**

High resolution mass spectrometry imaging (MSI) is used to explore the spatial distribution of molecules of mono and co-culture of bacteria in gel-based growing media. These data have been processed with our In-House Python-based software to specifically extract the signal exclusively produced by the interaction compared to the control condition. The metabolome is then extracted from the interaction region using various organic solvents by solid-liquid extraction. The latter are then analyzed by MALDI-FT-ICR to identify which condition of solvent has extracted the compounds of interest. The latter is selected for being analyzed by targeted metabolomics (LC-MSn) to identify the metabolites.

### **Preliminary data (results)**

We challenged our innovative mass spectrometry approach on two randomly selected bacteria species, *Escherichia coli* and *Streptomyces coelicolor*, grown on ISP2 agar medium. As explained before, this bacterial interaction aims to stimulate the expression of different ions specific to the co-culture.

High resolution MSI of mono and co-culture of *S.coelicolor* and *E.coli* were acquired in an untargeted manners to observe the production and distribution of metabolites. We then compared the image of the interaction between *S. coelicolor* and *E. coli* with the images of the control monocultures. Thus, out of the several thousand peaks present in the raw mean spectrum (> 2000), only ten are retained after comparison. Of the peaks retained, more than half correspond to metabolites expressed by *E. coli* only upon interaction with *S. coelicolor*.

These ions identified by IMS can then be extracted and analyzed by MS/MS in order to structurally elucidate these ions. In order to do so, the metabolome will then be extracted from the interaction region using various organic solvents by solid-liquid extraction, with the aim of extracting most of the microbial metabolites. The latter will be analyzed by MALDI-FT-ICR to identify which condition of solvent has extracted the compounds of interest. The latter will be selected for being analyzed by targeted metabolomics (LC-MSn) to identify the metabolites, for example thanks to the comparison of MS2 spectra with different databases containing standard metabolite spectra such as METLIN and MassBank.

### **Please explain why your abstract is innovative for mass spectrometry?**

The whole approach corresponds to a complete and fast analysis method being very useful for the detection and identification of new metabolites and can easily be used routinely.

Poster number: **IM-PA-085**

## DOPANT-ASSISTED MALDI-2-MSI: A DOUBLE BOOSTER FOR HIGHLY RESOLVED IMAGING OF LIPIDS AND METABOLITES

Abstract ID: **363****Presenting author: Christoph Bookmeyer, Institute of Hygiene, University of Münster, University Rovira i Virgili, Tarragona**

### Introduction

In MALDI-MS imaging, the range of analyte classes that are amenable to the analysis is often limited by low ionization yields and ion suppression effects. Postionization techniques can dramatically increase the sensitivity. Our recently introduced MALDI-2 technique utilizes a second pulsed UV laser that intersects the expanding MALDI plume. In elevated pressure conditions, ample additional matrix ions are produced triggering complex gas-phase reactions. This together results in strongly increased ion yields for a wide range of analyte classes. Here we show the addition of ionization-mediating dopants such as acetone to the ion source for dopant-assisted MALDI-(DA)-2-MSI to further enhance secondary ionization processes, and how this leads to an additional boost for highly resolved imaging of lipids and further metabolites.

### Methods

16  $\mu\text{m}$ -thick tissue sections of porcine brain homogenate and mouse cerebellum and spleen were coated with 2,5-dihydroxyacetophenone (DHAP) matrix using a standard sublimation protocol. MALDI-2-MS(I) was performed using a modified dual ion-funnel MALDI/ESI-Injector (Spectrograph, operated at  $\sim 8$  mbar, Figure 1) coupled to a Q Exactive Plus Orbitrap mass analyzer. A Nd:YAG laser with 266 nm wavelength was used to initiate postionization. Acetone, toluene, anisole, chlorobenzene, and 2-propanol were tested as dopants and were passively introduced into the ion source via capillaries and valves.

### Preliminary data (results)

Experiments targeting the optimization of key experimental parameters were conducted with sections of porcine brain homogenate. We investigated the effects of partial dopant vapor pressures of the different dopants and several further relevant parameters, such as inter-laser pulse delay and total in-source pressure. Using the optimized settings, the analytical abilities of MALDI-DA-2-MS(I) were investigated with mouse tissue sections. Exemplary mass spectra and MSI comparing both measurement modes are plotted in Figure 2.  $[\text{M}+\text{H}]^+$  resp.  $[\text{M}-\text{H}]^-$  ion species of glycerophospholipid, sphingolipids, and many further lipid and metabolite classes were registered with signal intensities that exceeded the ones observed with MALDI-2 by up to another order of magnitude. Compared to standard MALDI-MSI, the overall ion boost was thus as high as 3-4 orders of magnitude for numerous compounds (e.g., cholesterol, hexosylceramides). Due to the broadly enhanced signal intensities also of low-abundant analyte species, the number of tentatively annotated analytes (based on accurate mass) could be increased by 70% compared to regular MALDI-2 and by a factor of 8 for standard MALDI, respectively.

Using dopants other than acetone yielded comparable pattern of (de-)protonated ion species, albeit with lower analyte and higher background signal intensities. Dopants like toluene and anisole are known for undergoing electron-transfer reactions forming molecular ions. The absence of these radical species in the obtained mass spectra thus indicates a high level of chemical interaction of the excited compounds in the dense MALDI plume, which results in predominant proton-transfer reaction products.

### Please explain why your abstract is innovative for mass spectrometry?

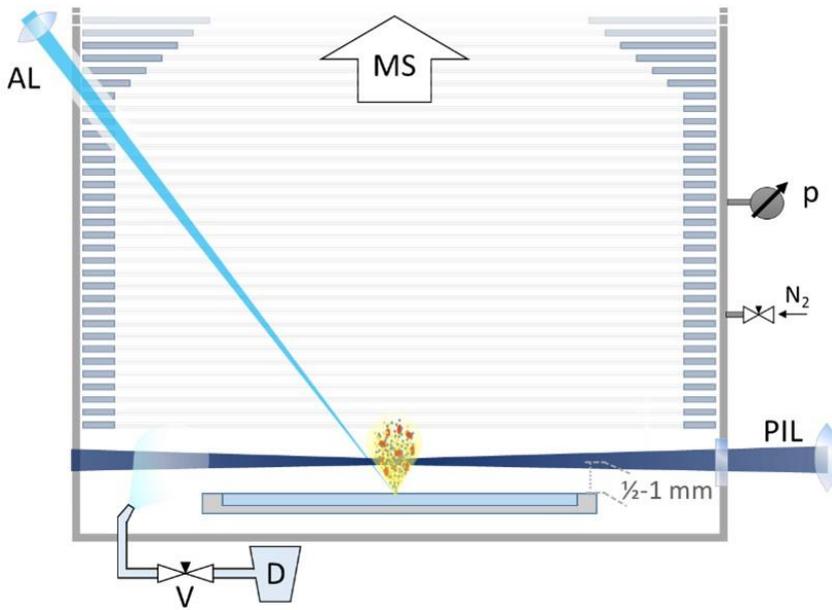
First demonstration of dopant-assisted MALDI-2-MSI to increase the analytical depth of a MALDI imaging experiment even beyond the MALDI-2 boost.

### Co-authors:

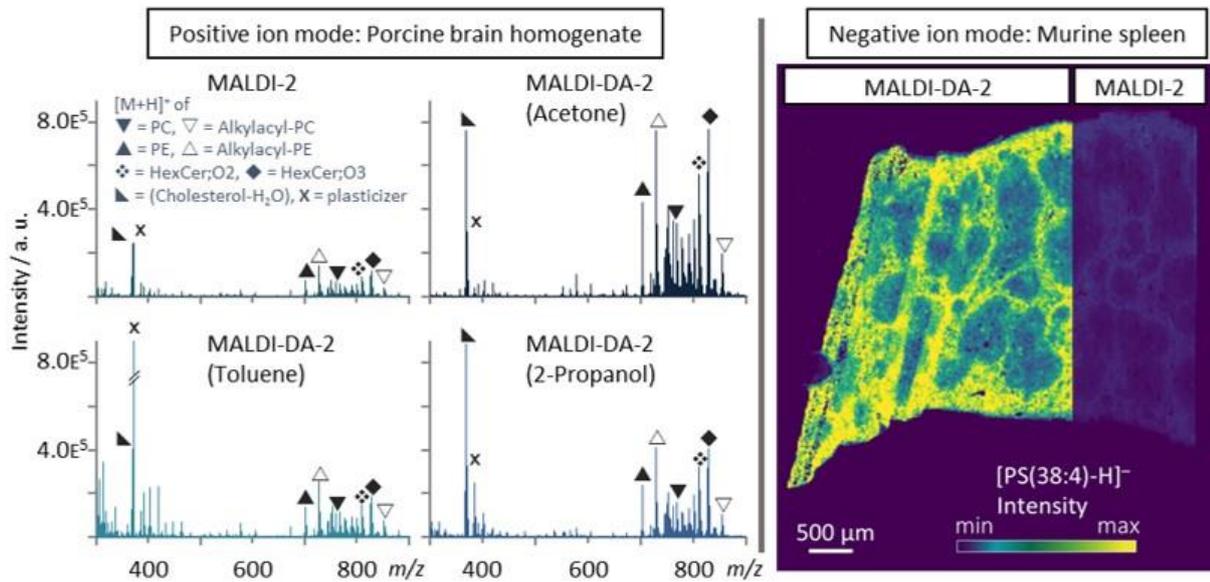
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POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Schematic of dopant-assisted MALDI-(DA)-2 in a Spectrograph dual-ion-funnel.



Comparing MALDI-2-MS(I) with different dopants.

Poster number: **IM-PA-086**

## **CORRELATIVE CHEMICAL IMAGING TO UNDERSTAND NEURITIC PLAQUE PATHOLOGY IN ALZHEIMERS DISEASE**

Abstract ID: **382**

**Presenting author: Srinivas Koutarapu, University of Gothenburg**

### **Introduction**

Alzheimer's disease (AD) is the most common form of dementia, with the majority cases belonging to sporadic variant (sAD). Despite over 100 years of research, the pathological mechanisms underlying AD are still unclear. Accumulation and aggregation of amyloid- $\beta$  ( $A\beta$ ) peptides into extracellular plaques is the trademark of AD pathology. Amyloid plaque deposition presents itself with a very heterogenous phenotype involving structurally different plaque types. Specifically, neuritic plaques have been identified to be associated with both neuronal cell death and Tau pathology, the other major hallmark of AD. However, the factors that promote formation of neuritic plaques remain elusive.

### **Methods**

For this we have been using imaging mass spectrometry (IMS) and Immunohistochemistry (IHC) together with hyperspectral microscopy for correlative chemical imaging of polymorphic plaque pathology in human post mortem brain tissue of sAD patients (n=5). MALDI IMS and IHC performed on the consecutive, mirror faced section to annotate and correlate peptide signatures with immunohistochemical profiles of neuritic plaques and cored plaques. Hyperspectral microscopy of IHC and fluorescent amyloid staining based luminiscent conjugated oligothiophene (LCO) was used for in situ characterization of amyloid fibrillisation and classification of structurally distinct plaque morphotypes.

### **Preliminary data (results)**

IMS elucidates the complex spatial distribution patterns of peptide expression on one brain section while IHC (RTN3-Tau)/LCO-Co staining on the consecutive section equips with the hyperspectral information to delineate the tau morphological signatures of neuritic plaque. MALDI analysis of  $A\beta$  peptides showed a significant difference of  $A\beta_{1-40}/A\beta_{1-42}$  and  $A\beta_{3Pe-42}/A\beta_{1-42}$  between cored and neuritic plaques. Specifically,  $A\beta_{1-40}/A\beta_{1-42}$  was increased in cored plaques while  $A\beta_{3Pe-42}/A\beta_{1-42}$  was higher in neuritic plaques. Here A clear correlation pattern was found between IMS and LCO emission data.

### **Please explain why your abstract is innovative for mass spectrometry?**

Multimodality imaging including Imaging Mass Spectrometry and Immunohistochemistry allows to visualise age-related pathological processes and chemical complexity in amyloid  $\beta$  morphologies like neuritic plaques and cored plaques.

Poster number: **IM-PA-087**

## MASS SPECTROMETRY OF CHIRAL MIXTURES BY MICRO-GC CHIRAL-MS

Abstract ID: **442**

**Presenting author: Maurice Janssen, MassSpecpecD BV**

### Introduction

Mass spectrometry is chirally blind, it cannot directly distinguish the two enantiomers of chiral molecules. Enantiomer-specific identification of chiral molecules in multi-component mixtures is extremely challenging. Many established techniques for single-component analysis fail to provide selectivity in multi-component mixtures and lack sensitivity for dilute samples. Here we show how enantiomers of chiral mixtures can be differentiated by a micro Gas Chromatograph coupled to a Supersonic Molecular Beam Mass Spectrometer using laser-based PhotoElectron Circular Dichroism (PECD), microGC-chiralMS. Laser-based PECD has been shown to be an extremely sensitive chiroptical method for the direct measurement of enantiomeric excess in chiral molecules [1-4]. The chiral asymmetry of PECD is typically 1-10%, two to three orders of magnitude better than the chiral asymmetry obtained in conventional (absorption) (V)CD/ROA.

### Methods

Following the separation of a mixture of (chiral) molecules injected in a microGC, the output flow of a normal GC column is expanded in vacuum via a micro-fabricated de Laval nozzle and crossed by an ionizing circular-polarized ultrafast laser pulse. Ions and electrons are detected sequentially on a time- and position sensitive detector. The Time-of-Flight of the ions provides the mass of the molecules as they elute from the column. The forward-backward PECD asymmetry in the angular distribution of the photoelectrons is detected by Velocity Map Imaging and is directly proportional to the enantiomeric excess of the eluting chiral molecule.

### Preliminary data (results)

Here we report on the design, construction and progress evaluation of a newly developed (prototype) chiral Mass Spectrometer that integrates a microGC with a cold supersonic molecular beam Time-of-Flight Velocity Map Imaging spectrometer [5]. The microGC-chiralMS spectrometer was developed *in silico* using state-of-the-art computational techniques including MOLFLOW+, OpenFOAM-DSMC and SIMION. The novel prototype chiral MS apparatus is very compact and utilizes a specially micro-fabricated de Laval nozzle that provides optimal coupling of the microGC output flow in a supersonic molecular beam to the ultrafast laser photoionization geometry. The laser ionization operates at a repetition rate of 150 kHz and provides a typical MS TOF-window of about 7 microsec. In this presentation we will report the mass resolution and typical mass spectra observed by photoionization of benchmark chiral molecules like fenchone, limonene and others. Furthermore, we will present GC-MS spectra obtained after injection of mixtures of (volatile) chiral molecules typically used in flavor and fragrances. The PECD asymmetry is analyzed to give the (R/S)-enantiomeric excess of the various compounds that elute from the microGC. We will report the latest status of the new chiral Mass Spectrometer.

### References

- [1] C.S. Lehmann *et al.*, *J.Chem.Phys.* **139**, 234307 (2013)
- [2] M.M. Rafiee Fanood *et al.*, *Nature Communications* **6**, 7511 (2015)
- [3] M.H.M. Janssen, *The Analytical Scientist*, **May**, 44 (2016)
- [4] M.H.M. Janssen and I. Powis, *Current Trends in Mass Spectrometry*, **May**, 16 (2017)
- [5] Project microGC-chiralMS, financially supported by the European Fund for Regional Development and OPOost (EFRO-00949, 2020-2023).

**Please explain why your abstract is innovative for mass spectrometry?**

The novel microGC-chiralMS spectrometer combines various innovations related to microGC hyphenation via micro-fabricated de Laval nozzles, high-rep rate ultrafast laser photoionization MS and chiral VMI photoelectron spectroscopy.

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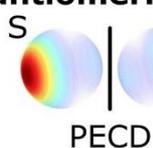
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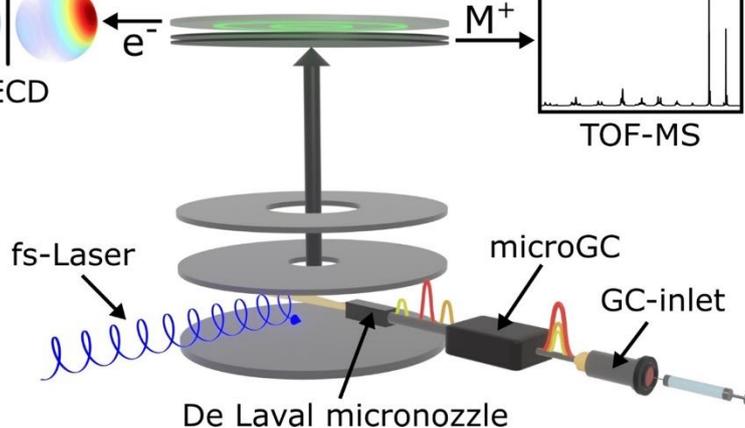
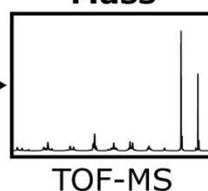
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*Cas Damen, Saxion University of Applied Sciences*

**Enantiomeric excess**



**Mass**



The novel microGC-chiralMS spectrometer for mixtures of chiral molecules.

Poster number: **IM-PA-088**

## LASER DESORPTION – RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY, A NOVEL METHOD FOR SAMPLE PREP-FREE TISSUE IMAGING AND CANCER SURGERY

Abstract ID: **597**Presenting author: **Daniel Simon, Imperial College London, Rosalind Franklin Institute**

### Introduction

Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is a novel technique that allows the rapid analysis of different biological samples (food, microbes, and human/animal tissues) without the need for sample preparation. The technique was successfully utilised *in vivo* human surgeries where the technique is capable of classifying the histological status of cancerous and healthy tissues. The accurate tissue diagnosis is highly dependent on the accuracy of the reference tissue models. An infrared laser-based mass spectral imaging platform using nanosecond and prototype picosecond lasers was developed with the aim to generate REIMS data from single cells, which is capable provide molecular histopathology data for chemically guided surgical interventions. To show the capacity of the system a multi-omics LD-REIMS study was designed using FFPE colorectal cancer TMAs.

### Methods

The instrument consists of a REIMS source installed on a Xevo G2-S QToF (Waters) mass spectrometer. Motorized 2D XY stage coupled with laser optics was used as a sampling platform for all imaging experiments. iKnife experiments were performed using a prototype handpiece coupled with Covidien ForceTriad diathermy generator. Intelliguide CO<sub>2</sub> laser (OmniGuide) at 10600nm, Opolette HE2731 OPO laser (Opotek), 2700–3100nm and a prototype picosecond infrared laser were used to map the laser parameters. 12µm fresh frozen pork liver, rat, mouse brain and formalin-fixed paraffin-embedded (FFPE) pork liver and human colorectal cancer tissue microarrays (TMA) were used as samples.

### Preliminary data (results)

The spot size achieved with our setup after optimisation was 70µm with the CO<sub>2</sub> laser, around 50µm with the OPO laser and 10µm with the picosecond laser, fluence at ablation point was found to be around 50J/cm<sup>2</sup> with the commercial lasers and 3e<sup>-2</sup>J/cm<sup>2</sup> with the picosecond setup. Different raster size images were acquired, and at 20µm raster sizes, tissue structures were observable on the mouse brain hippocampus regions. The superior beam quality of the picosecond laser allows <10µm raster imaging, proving the capacity to do single-cell resolution imaging. Optimal wavelength was found to be 2900–2950nm which correlates with the absorption maximum of water content of tissues. Pulse widths between msec-psec range were tested, finding the picosecond pulse structure to be better for ablation due to the thermal load being less on organic molecules due to shorter pulse widths. The molecular coverage of the Laser Desorption – REIMS is comparable to the iKnife data and other imaging techniques, numerous metabolites and lipids were observed during experiments. Human breast tumour, rat brain and pork liver molecular images were successfully acquired. The molecular changes observed in the data show good correlation with the histopathological status of tissues. A multi-omics LD-REIMS imaging study was performed on TMA slides, the setup was compared with and without xylene treatment samples and no significant differences were found between the two, proving the method to be a sample-preparation free method for analysing FFPE samples; larger cohorts of samples are under analysis for the large-scale study.

### Please explain why your abstract is innovative for mass spectrometry?

Sample preparation free, high-resolution LD-REIMS imaging for single-cell molecular histopathology with the capability to guide *in vivo* surgical applications.

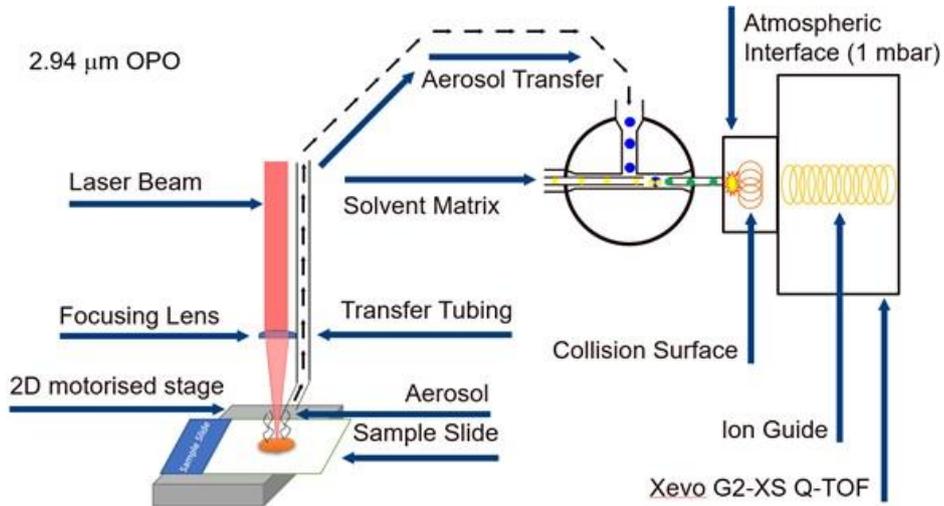
### Co-authors:

Vincen Wu, Imperial College London  
Yuchen Xiang, Imperial College London  
Ronan Battle, Imperial College London  
Helen Huang, Imperial College London  
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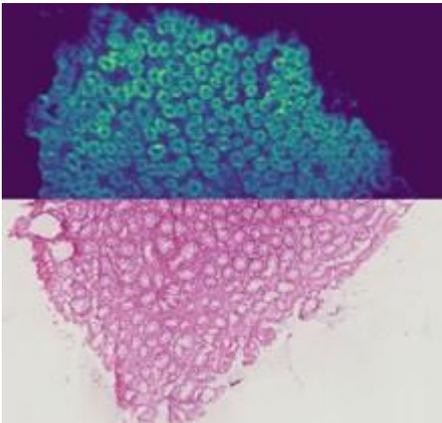
**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Shahd Abuhelal, Imperial College London*  
*Lauren Ford, Imperial College London*  
*Kenneth Robinson, Rosalind Franklin Institute*  
*Julia Abda, Imperial College London*  
*Robert Murray, Imperial College London*  
*Josephine Bunch, National Physical Laboratory, Rosalind Franklin Institute*  
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Schematics of the LD-REIMS setup.



Haematoxylin-Eosin stained image combined single-ion heatmap of PE(36:2)

Poster number: **IM-PA-089**

## **THE CUMULATIVE INFLUENCE OF SALT ADDITIVES COUPLED WITH MATRIX SUBLIMATION/RECRYSTALLIZATION ON MALDI LIPIDS IMAGING**

Abstract ID: **600**

**Presenting author: Mariya Shamraeva, The Moscow Institute of Physics and Technology**

### **Introduction**

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MSI) is a potent way to visualize a wide variety of biomolecules. The sample preparation steps, including the method of MALDI matrix application, influence significantly on a bunch of analytical characteristics. Moreover, mass spectrometry profiling of tissue lipids could be supposedly distorted due to the lipid ionization in the form of protonated, sodium and potassium adducts. The salt doping could provide advantages for the investigation of the contribution of different lipid forms such as CEs (cholesterol esters), DAGs/TAGs (di-/triacylglycerol), PCs (phosphatidylcholine), and SMs (sphingomyelin). Herein, the method of salt brain tissue doping coupled with matrix sublimation and further recrystallization step, facilitating detectability, greater reproducibility, higher sensitivity and better spatial resolution compared with conventional methods, is presented.

### **Methods**

For MALDI-MSI, the rat brain sections were drip-washed by fixed volume of potassium acetate, ammonium acetate, sodium acetate, lithium trifluoroacetate solutions with different ionic strength. Then, the matrix was sublimated with a subsequent recrystallization step, which was used to better co-crystallization of the DHB (2,5-dihydroxybenzoic acid) MALDI matrix with analytes over the sample surface. Profiling and MSI of the tissues sections was performed on the Bruker MALDI-TOF ultrafleXtreme/rapifleX mass spectrometer in positive mode and the signals between  $m/z$  400-1000 were collected.

### **Preliminary data (results)**

The optimization of both salt doping and matrix application was performed. At the first stage, it was made with regard to the investigation of suitable ionic strength of salt solutions for salt doping. At the second stage, it was made with reference to selection of appropriate recrystallization conditions, including solvent composition, temperature and time, required for better lipid visualization. For methods, including salt doping with sublimation and recrystallization, the larger numbers of peaks with higher signal intensities and signal-to-noise ratios were found. In order to compare different techniques, MSI was carried out under the same conditions. The evaluation of the imaging analytical reproducibility was estimated for both methods without recrystallization step and then with it, demonstrating better reproducibility for the latter method. Moreover, without the recrystallisation step, high salt concentrations cause the rapid and poor crystallization of the MALDI matrix leading to the formation of large crystals, especially in the white matter of the brain. The obtained results suggest that salt doping in combination with sublimation and recrystallization steps might provide mass spectrometry imaging of lipids with good reproducibility and high sensitivity.

### **Please explain why your abstract is innovative for mass spectrometry?**

The combination of three steps: salt doping, matrix sublimation, recrystallization under optimized conditions provides a homogeneous distribution of small size crystals and increases the ionization efficiency of different lipids categories.

### **Co-authors:**

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*Sergey Silkin, The Moscow Institute of Physics and Technology*

*Maria Derkach, The Moscow Institute of Physics and Technology*

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Poster number: **IM-PA-090**

## TAKING A CLOSER LOOK AT SAMPLE PREPARATION FOR LIPID DETECTION IN MALDI MSI USING DIFFERENT SPRAYING DEVICES

Abstract ID: **628**Presenting author: **Peter Sandbichler, Technical University of Vienna**

### Introduction

To improve the efficiency of MALDI-MSI experiments and ensure their reproducibility, meticulous sample preparation is essential. Matrix application is a key factor in this process. Properties such as the size of the matrix crystals and the homogeneity of the layer influence the signal intensity and thus the achievable lateral resolution. Furthermore, a uniform size distribution of the matrix crystals across the entire sample must be achieved in order to be able to make a meaningful statement about the distribution of the analytes. We present a comparative study between two sprayers (TM/HTX, iMLayer AERO/Shimadzu) in terms of analyte detection, layer quality and reproducibility of experiments.

### Methods

Snap frozen kidney (mouse) tissue (provided by L. Kenner and C. Vranka, Medical University of Vienna) was cut (10  $\mu\text{m}$  sections), thaw-mounted on microscope slides and vacuum dried before matrix application using different spraying devices. To allow a comparison the matrix was applied to two consecutive tissue sections, using either of the sprayers. Experiments were performed in triplicates to avoid statistical fluctuations. MALDI MSI measurements were either performed on a MALDI-8030 or a MALDI-7090 (both Shimadzu). Data analysis was performed using IMAGEREVEAL MS, OriginPro and Perseus. Deposited matrix layers were characterized by a VHX-5000 (Keyence) digital microscope.

### Preliminary data (results)

In a matrix spray process, changing parameters like temperature, solvent flow, pressure, nozzle distance or stage velocity influences layer thickness, crystal shape, layer homogeneity, drying processes between layer application and in the end matrix layer quality including incorporation of analytes in the matrix crystals. Additionally, the circumstances of matrix application must not be neglected. Increasing the time used for sample preparation has high potential for variations in the measurement results due to the instability of analytes (lipids, metabolites, etc.). In this study we present the comparison of the TM HTX sprayer with an instrument newly introduced on the market, the iMLayer AERO. The newly developed sprayer from Shimadzu provides an inert nitrogen atmosphere to control humidity during spraying and allows the sample to be presented to the spraying nozzle in a vertical position. Our study puts special emphasis on the comparison between vertical and horizontal positioning in terms of analyte localization, by studying analyte localization in consecutive sections either mounted horizontally as in the HTX sprayer or vertically as in the iMLayer AERO. For all experiments, the spraying method was pre-evaluated using a digital microscope. Promising methods were tested on tissue in low-resolution imaging runs on a Shimadzu MALDI-8030 to accelerate method development. If quality criteria were met, consecutive cuts were measured on a Shimadzu MALDI 7090 at a spatial resolution of 20  $\mu\text{m}$  and the performance of the two sprayers was compared in terms of analyte delocalization, ion signal, number of analytes, reproducibility and time.

### Please explain why your abstract is innovative for mass spectrometry?

The influence of vertical positioning in a spraying device as well as the presence of a nitrogen atmosphere during spraying is evaluated in comparison to horizontal positioning under atmospheric conditions.

### Co-authors:

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*Martin Handelshausner, Technical University of Vienna*

*Kenta Terashima, Research and Development Department, Analytical & Measuring Instruments Division, Shimadzu Corporation*

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Poster number: **IM-PA-091**

## **THE OPTIMAL PARAMETERS OF FLOW FOCUSING MECHANISM FOR MASS SPECTROMETRY IMAGING OF CLINICAL SAMPLES**

Abstract ID: **632**

**Presenting author: Vincen Wu, Imperial College London**

### **Introduction**

In contrast to the solvent droplets produced by a mechanism known as electrospray, flow focusing is a common technique applied in microfluidics system to control and manipulate the formation of droplets. This flow focusing mechanism was further developed by Gañan-Calvo and Forbes to be compatible with mass spectrometry for surface analysis and imaging of explosion, narcotics, and artificial fingerprints, termed as desorption electro-flow focusing ionization (DEFFI). To determine the optimal parameters for the imaging of clinical samples, we examined different parameters and have found that the shapes of the distal tip of emitters, gas pressure, solvent flow rate, distance between emitter and orifice, distance between sprayer and surface, and voltage, to be essential for improving its spatial resolution and sensitivity.

### **Methods**

The parameters are described as followed: (1) emitter tip shape; (2) inner diameter of emitter; (3) solvents; (4) gas pressures; (5) distance between emitter and orifice; (6) orifice diameter; (7) distance between the sprayer and the surface. 5 lines of 5 mm were acquired on pork liver section with 1 scan/sec at an acquisition speed of 100  $\mu\text{m}/\text{ms}$ . Afterwards, rhodamine slide were used for determining the diameter of impact surface area.

### **Preliminary data (results)**

Based on the results, the distance between the orifice and emitter, solvent flow rate, gas pressure, voltage, and the shape of the distal tip of the emitter were important for the desorption and ionization of ions, and the focusing of primary droplets. High voltage is particularly effective at lower gas pressure to focus primary droplets that would have otherwise been stuck inside the sprayer. Higher voltages decreases the impact surface area, while maintaining similar ion intensities. Higher gas pressure increases ion intensity, while decreasing the impact surface area, which could be due to higher velocity impact of droplets against the surface. The higher the gas pressure, the higher the intensity, so this parameter is limited by the sprayer. A short distance between the orifice and emitter was found to be optimal, as it greatly affected the impact surface area. The larger the distance, the more difficult it is to focus the emitted jet stream, thereby increasing the impact surface area, as well as yielding lower ion intensity. Increase in solvent flow rate also increases the overall signal intensity, which was to be expected, as more ions can be solubilized and desorbed. However, the increase of solvent flow rate also increases the impact surface area. The tapered tip of the emitter seems to produce smaller and more symmetrical and spherical impact surface area when compared with a bluntend emitter. For the comparison of different solvents, methanol was the better choice in terms of higher ion intensity and smaller impact surface area.

### **Please explain why your abstract is innovative for mass spectrometry?**

A wide range of parameters have been examined for DEFFI to increase sensitivity and spatial resolution for imaging of clinical tissues

### **Co-authors:**

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Poster number: **IM-PA-092**

## **THE SEPARATION OF HOMOGENEOUS REGIONS OF INTEREST IN MASS SPECTROMETRY IMAGING DATA WITHOUT DIMENSION REDUCTION**

Abstract ID: **661**

**Presenting author: Stanislav Pekov, Skolkovo Institute of Science and Technology, Russia, Moscow Institute of Physics and Technology, Russia**

### **Introduction**

Common methods for clustering pixels in mass spectrometry images are based on dimension reduction algorithms. During such procedures, each pixel is assigned to one of the clusters; however, some borderline pixels do not represent the molecular composition of any single tissue type but the superposition of cells from different tissue types. This effect is especially notable for the samples of the boundaries of highly malignant brain tumors where non-malignant and cancer cells are thoroughly mixed and have intricate geometry due to the specific form of the neurons and glial cells. Thus, it became complicated to determine whether the spectra represent the cells' lipid content of one tissue type or not. A method for region detection concerning the nature of mass spectrometric data is urgently needed.

### **Methods**

Mass spectra of mouse brain tissues and human brain tumor tissues were obtained with MALDI-TOF and MALDI-Orbitrap instruments. The spectra were transformed into multidimensional vectors, and the homogeneous regions were separated by the density of vectors in the space of the original dimension with the help of a cosine similarity metric.

### **Preliminary data (results)**

The high spatial resolution achieved by modern mass spectrometry imaging instruments allows investigating lipid alterations through heterogeneous tissue samples, which is especially interesting for the carcinogenesis investigation. However, the borderline pixels attributing to one of the regions may corrupt the determined lipid profiles of the tissues, so the regions with homogeneous mass spectra should be determined. The accurate separation of the regions with cells of only one tissue type is possible only with the implementation of the method based on the physical nature of the data. The spectrum to vector transformation is mathematically correct and retains the physical meaning of the data: each dimension corresponds to ions with a specific  $m/z$  value. Further determination of vector density allows to filter the borderline pixels and find homogeneous regions. Implementing the cosine similarity metric simplifies the calculations as no specific normalization or dimension reduction is required. Additionally, the spectra similarity evaluation helps assess whether each of the detected clusters should be considered a significant one or only as of the transition zone between major regions.

### **Please explain why your abstract is innovative for mass spectrometry?**

The novel approach for the homogeneous regions of interest detection with regard to the mass spectrometric nature of data is proposed.

### **Co-authors:**

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Poster number: **IM-PA-093**

## MULTIDRUG QUANTIFICATION BY MALDI-MSI

Abstract ID: **708**

**Presenting author: Michael Tuck, Institut de Chimie & Biologie des Membranes & des Nano-objets, CNRS, Université de Bordeaux**

### Introduction

Tuberculosis is the second most lethal disease caused by a single infectious agent, behind SARS-CoV-2, with 1.5 million dead in 2020 and 10 million sickened worldwide. Yet, unlike Covid-19, *Mycobacterium tuberculosis* (Mtb) has affected the earliest hominids, making it the deadliest microbe of all time. Tuberculosis is treated with a panel of multiple drugs due to its persistent nature with second/third line regimens often employed. The rate of multidrug resistance is up 10% and has become an increasing health crisis. Whether these drugs are localizing to the necrotic granulomas, where Mtb resides, and at what concentrations remains unclear. For the simultaneous analysis of multiple drugs, a quantitative matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) strategy is developed for the first time.

### Methods

Mtb-infected mice are treated with a common regimen of anti-bacterial drugs. For quantitative MALDI-MSI, a mimetic tissue is prepared by homogenizing rat liver and spiking aliquots with drug standards. Homogenates are serially frozen to form a mimetic tissue with seven calibration points for each drug quantified. A linear regression on each drug is performed to correlate MALDI-MSI signal intensity with the known drug concentrations. Tissues are cryosectioned at 12  $\mu\text{m}$  and sublimated with 1, 5-diaminonaphthalene. A rehydration step is performed for analyte extraction. After, high mass resolving power MALDI-MSI is conducted with an AP-SMALDI110 (TransMIT) and QExactive Orbitrap (Thermo).

### Preliminary data (results)

Mouse samples contain up to five anti-tuberculosis drugs: rifampicin, isoniazid, pyrazinamide, ethambutol and clofazimine. Mimetic models were generated with a therapeutically-relevant concentration range for each drug. MSI sensitivity for many drugs was high, even at 5  $\mu\text{m}$  spatial resolution. Analytes in these samples have been previously quantified with liquid chromatography mass spectrometry. Concurrent quantification of multiple drugs with MALDI-MSI is challenging because of differing extraction conditions, limits of detection/quantification and analyte affinities for protonation/deprotonation. Considering this, a mimetic tissue was successfully fabricated with seven points of calibration ranging from 575 to 464,000 ng/g for clofazimine, and 6,000 to 37,000 ng/g for ethambutol for the quantification of dosed tissue. Initial quantitative MSI acquisitions were conducted at 30  $\mu\text{m}$  spatial resolution. MSI datasets of dosed mouse lungs show a linear correlation between the MS signal intensity and that of the mimetic tissue, with an  $r^2$  value of 0.99, facilitating their comparison. By annotating a histological staining after MSI analysis in Qupath (Queen's University Belfast), concentration data can be extracted from each pixel. With this, we show clofazimine for example, localizing to the vascularized foamy macrophages in excess of 200,000 ng/g, the cellular rim at 100,000 ng/g, the outer caseum at less than 50,000 ng/g and the necrotic caseum, which contains most of clofazimine's target site, at less than 25,000 ng/g. Due to drug recalcitrance, and the cocktail of medication required for the successful treatment of tuberculosis, it is important for our biological collaborators to localize and quantify multiple drugs in one analysis.

### Please explain why your abstract is innovative for mass spectrometry?

For the first time to our knowledge, the simultaneous localization and quantification of multiple drugs, necessary in a Tuberculosis model, has been achieved using a quantitative MALDI-MSI workflow.

### Co-authors:

*Florent Grélard, Institut de Chimie & Biologie des Membranes & des Nano-objets, CNRS, Université de Bordeaux*

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Poster number: IM-PA-094

## COMPARISON OF MAPPING STRATEGIES IN LA-ICPMS: SINGLE PULSE, MULTIPLE DOSAGE, AND 2D OVERSAMPLING / DECONVOLUTION

Abstract ID: 720

Presenting author: Martin Šála, National institute of chemistry

### Introduction

In recent decade the Laser ablation- Inductively Coupled Plasma Mass Spectrometry mapping has seen a big advancement in speed, connected to the design of novel fast aerosol delivery systems and better image quality, benefiting from systematic studies of fundamentals of synchronisation of laser sampling, aerosol delivery and acquisition in MS.

### Methods

The imaging quality of several 0D, 1D, and 2D LA-ICPMS strategies were compared for mapping of (ultra)low-concentration samples, via computational modeling and confirmed by experimental work. Different beam sizes, dosages and oversamplings have been taken in account.

### Preliminary data (results)

Acquisition of counts in LA-ICPMS mapping can be performed for each single laser pulse separately or by summing the counts of multiple laser pulses. Conventionally, pixels in an LA-ICPMS map are associated with spot-resolved single laser pulses (zero-dimensional, 0D), but also sub-pixel convolution strategies are in use, associated with one-dimensional (1D) or two-dimensional (2D) overlapping laser shots, and where possible followed by deblurring. Several combination of the parameters yielding described imaging strategies were modeled *in silico* and assessed by structural similarity (SSIM) index to identify the highest image quality between the tested parameter sets. Similar experiments were then executed in practice to confirm the modeling results.

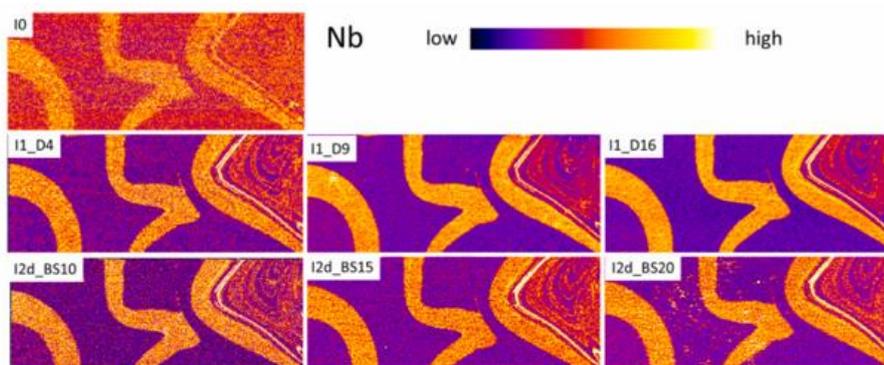
### Please explain why your abstract is innovative for mass spectrometry?

The data presented will be helpful to make the right decision about the best possible LA-ICPMS mapping strategy (and similar techniques regarding sampling; e.g. MALDI) for the highest image quality.

### Co-authors:

Johannes T. van Elteren, National institute of chemistry

Dino Metarapi, National institute of chemistry



Niobium images depicting different mapping strategies of murrina decorative glass.

Poster number: **IM-PA-095**

## **ACCURATE PRE-PROCESSING OF MASS SPECTROMETRY IMAGING DATA WITH HIGH SPECTRAL RESOLUTION**

Abstract ID: 727

**Presenting author: Peter Horvatovich, University of Groningen**

### **Introduction**

Mass spectrometry imaging (MSI) is a technique that promises comprehensive molecular information with high spatial resolution from tissue. Label-free imaging with high-resolution mass spectrometry (MSI) provides large datasets, which contains the spatial distribution of all compounds that can be sampled from tissue section into the mass spectrometer.

One of the most important tasks of MSI data pre-processing is accurate extraction of ion images, with minimized mixing of signals from different compounds. Shifts in the mass-to-charge ratio ( $m/z$ ) of molecular peaks present a major obstacle that can make it impossible to distinguish one compound from another and perform ion image extraction accurately. Accurate, fast and interactive assessment of large MSI data volumes without loss of information require optimized data structure and ion image extraction approach.

### **Methods**

The software written in c++ is open source and python bindings allows efficient integration with other popular MSI package such as Cardinal and MSIQuant. MSIWarp is available as open source package at GitHub:  
<https://github.com/horvatovichlab/MSIWarp>

### **Preliminary data (results)**

Here, we present a label-free  $m/z$  alignment and ion image extraction approach that is compatible with multiple instrument types and makes no assumptions on the sample's molecular composition. We present the data structure and fast information dependent extraction of several thousands of ion images from MSI data obtained with high resolution mass spectrometer (Orbitrap). Our spectral alignment approach, MSIWarp, finds an  $m/z$  recalibration function by maximizing a similarity score that considers both the intensity and  $m/z$  position of peaks matched between two spectra. To deal with particularly misaligned or peak-sparse spectra, we detect and exclude spurious peak matches with a tailored random sample consensus (RANSAC) procedure. MSIWarp is applicable to data sets from both time-of-flight (TOF) and FT instruments, and, crucially, performs alignment using centroided spectra. We present the complete workflow and show the performance on anticancer drugs spiked-in tissue sections as well extraction of ion images of lipids in mouse bladder tissue section.

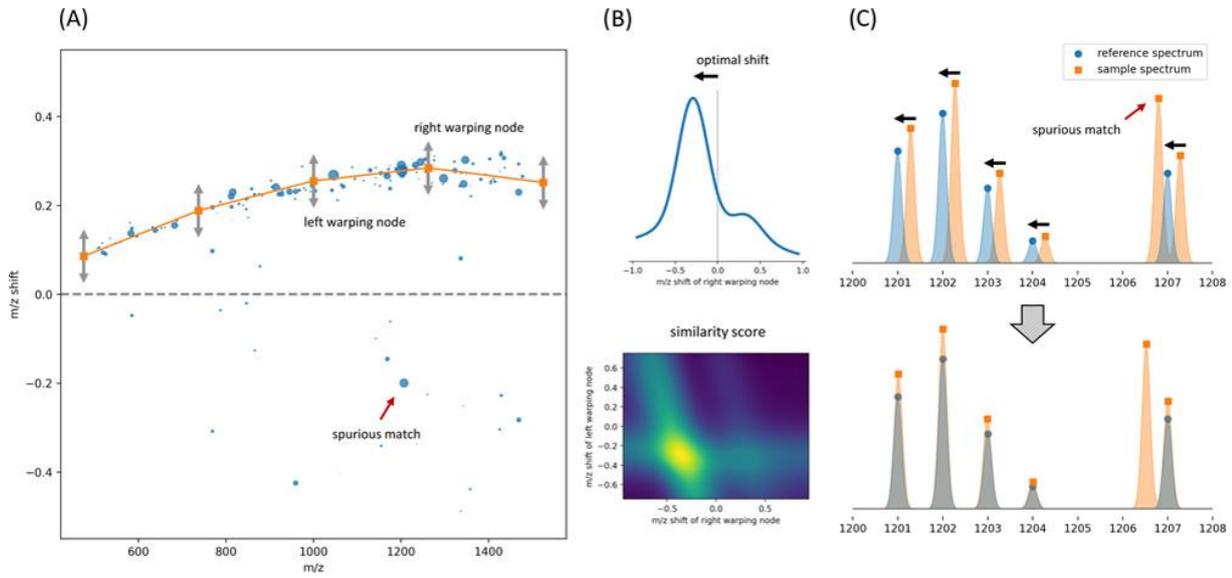
$m/z$  alignment provide considerable improvement for ion image extraction of compounds in datasets with large shifts and allows extraction of cleaner ion image for isotopes that are very close in  $m/z$ . Our results show, that shifts in  $m/z$  are influenced by local ion intensity and this space charge effects in Orbitrap instrument, while shifts in TOF instrument are independent from ion intensity and most related to tissue topology.

### **Please explain why your abstract is innovative for mass spectrometry?**

- accurate automatic alignment of mass spectra in MSI dataset
- accurate extraction of ion images from MSI data obtained with high resolution mass analysers
- comparison with other packages (MSIQuant, Cardinal)

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Scheme of the MSIWarp mass spectra automatic alignment algorithm.

Poster number: **IM-PA-096**

## **AP-SMALDI ORBITRAP IMAGING OF SMALL AND COMPLEX BIOLOGICAL OBJECTS**

Abstract ID: 734

**Presenting author: Bernhard Spengler, Justus Liebig University**

### **Introduction**

Atmospheric-pressure scanning microprobe MALDI mass spectrometry imaging (AP-SMALDI MSI) on orbital trapping mass spectrometers allows to disclose distinct morphologic distributions of lipids, peptides, drugs and metabolites in complex biological samples with highest sensitivity under ambient conditions. [1] High resolution and high accuracy of orbital trapping instruments with SRIG or ion funnel inlet allow to derive precise biological information from tissues, cells and sub-cellular structures.

### **Methods**

We developed various AP-SMALDI Orbitrap systems for high-performance imaging of planar and non-planar (three-dimensional) surfaces. Coaxial imaging normal to the sample surface was employed for highest lateral resolution on Orbitrap Exploris 120, 240 and 480. The imaging systems provide autofocusing operation, high-speed analysis and surface-topography imaging by three-dimensional scanning. Laser spot size and thus achievable non-oversampling lateral resolution goes down to 1.4  $\mu\text{m}$  in diameter [2, 3].

### **Preliminary data (results)**

As a challenging field of application, we studied unicellular and multicellular parasites, regarding both, their anatomical molecular structure and their chemical interactions with the environment and with their hosts. In these experiments, the instruments were operated in various modes, especially to cope with non-flat or non-perfect tissue sections at high lateral resolution. The optimal lateral resolution of 5  $\mu\text{m}$  pixel size in a 5- $\mu\text{m}$ -spot-size instrument was achieved even from non-flat surfaces such as intact bisexual *Schistosoma mansoni* worm couples by using the instrument's pixelwise autofocusing capability.

Three-dimensional scanning of surfaces is enabled by the unique geometry of our setup, including a sample irradiation normal to the sample surface. The orthogonal setup provides a topography-independent positioning in x- and y-direction, a prerequisite for fast and precise focusing and three-dimensional surface imaging.

Regarding the Orbitrap mass spectrometer, ion transmission, inlet system, and ion trapping were optimized for the different Orbitrap Exploris instruments, Exploris 120 MS and Exploris 240 MS, using SRIG, and Exploris 480 MS, using an ion funnel inlet system. Synchronization between imaging system and Orbitrap was set up to achieve high acquisition speed of, for example, 20 pixels per second on an Orbitrap Exploris 240 instrument.

[1] B. Spengler B, *Anal Chem* 2015, 87, 64–82. [2] M. Kompauer et al., *Nature Methods* 2017, 14, 90-96. [3] M. Kompauer et al., *Nature Methods* 2017, 14, 1156-1158.

### **Please explain why your abstract is innovative for mass spectrometry?**

Most recent hybrid Orbitrap MS instrumentation has been applied to high-resolution MS imaging of very small or three-dimensional biological objects.

### **Co-authors:**

*Karl-Christian Schaefer, TransMIT GmbH*

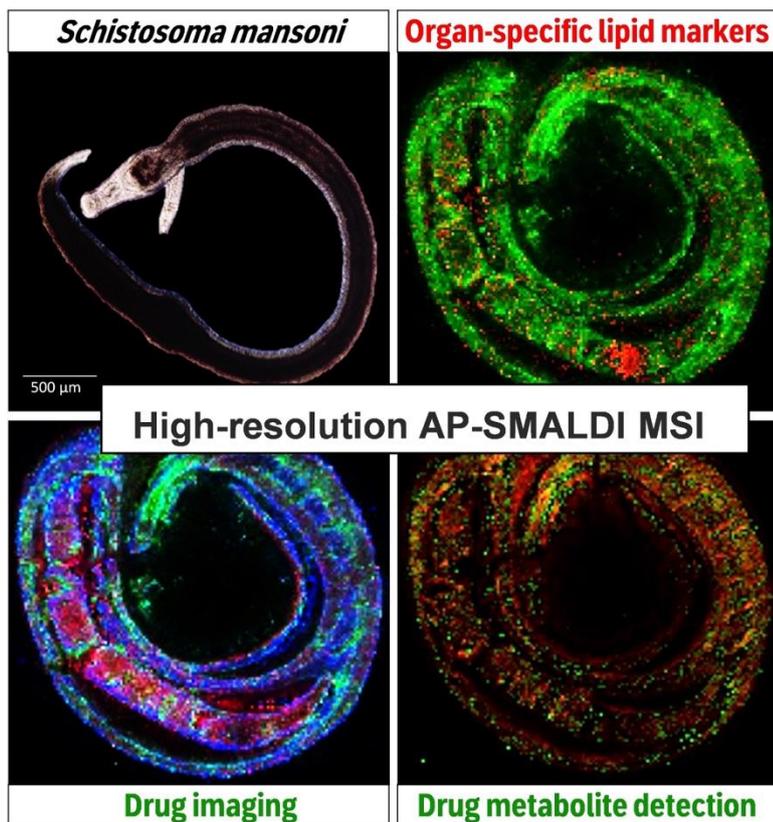
*Max A. Mueller, TransMIT GmbH*

*Julian Schneemann, Justus Liebig University*

*Kerstin Strupat, Thermo Fisher Scientific (Bremen) GmbH*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



MS imaging of Schistosoma mansoni worm couples at high resolution



AP-SMALDI5 AF (TransMIT GmbH) coupled to Orbitrap Exploris 240 (Thermo)

Poster number: **IM-PA-097**

## HIGH RESOLUTION IMAGING PLATFORM INCORPORATING MALDI AND DESI

Abstract ID: **803**

**Presenting author: Mark Towers, Waters Corporation**

### Introduction

One key issue in imaging mass spectrometry (MSI) is analyte identification, particularly with regards to small molecules (metabolites/lipids). This is due to the abundance of molecular species with similar masses within a small mass range and no chromatographic separation. One way to improve compound identification confidence is through increased mass resolution and mass accuracy. To achieve this with a trapping style instrument requires a long scan duration. Here we demonstrate a multi reflecting-Time-of-Flight(ToF) instrument providing a mass resolution of >200,000 FWHM and a mass accuracy <500ppb with a scan speed of up to 10 Hz. The instrument supports both matrix assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI) sources, specifically designed for imaging applications.

### Methods

For instrument assessment by MALDI and DESI several tissue types were analyzed. Frozen tissues were sectioned, at 12µm for MALDI and 18µm for DESI. MALDI sections were sprayed with alpha-Cyano-4-hydroxycinnamic acid (CHCA) or 2, 5-dihydroxybenzoic acid (DHB) using a TM-Sprayer (HTX). DESI sections required no additional sample preparation. MALDI images were acquired with a range of pixel sizes (<15-200µm), laser focusing and attenuation were adjusted for maximum sensitivity. For DESI, 2µl/min 95% Methanol was used as a spray solvent with a 10psi nebulizing gas. For both sources the images were acquired at 2, 5 and 10hz.

### Preliminary data (results)

Images have been acquired on a multi-reflecting QToF at 2, 5 and 10hz utilizing both source styles, demonstrating a mass resolution of >200,000 and mass accuracy of <500 pbb was maintained across a mass range of m/z100-2400. The mass resolution is provided by a ToF with multi-reflecting technology housing a ~50m flight path within a 1m flight tube. Gridless planar mirrors are used to minimize ion losses and maintain good signal intensity.

The MALDI and DESI sources, designed specifically for imaging applications incorporate several features to improve imaging quality and ease of use.

The MALDI source utilizes a dual attenuator laser system, allowing a large (software controlled) focusing range (>200µm - <15µm) ensuring the laser beam diameter is adjustable to the desired pixel size. This minimizes under/over sampling and associated effects, helping to maximise the signal intensity. Data has been generated exploring the effect of laser tuning (focus and attenuation) for a number of different pixel sizes (<15µm - 200µm) as a demonstration of the source capabilities.

The DESI source boasts a high-performance sprayer capable of delivering a solvent beam diameter of <20µm, and heated transfer line allowing for molecule specific tuning of temperature (ambient - 450°C) to maximize sensitivity for a given species.

Due to the orthogonal nature of the instrument, the performance - with regard to mass accuracy and mass resolution - is demonstrated to be independent of source (MALDI / DESI).

### Please explain why your abstract is innovative for mass spectrometry?

A multi-reflecting QToF capable of high mass (>200,000 FWHM) and Image (<15µm) resolution at a scan speed of 10hz, incorporating both MALDI and DESI sources on a single platform.

### Co-authors:

*Emmanuelle Claude, Waters Corporation*

*Dale Cooper-Shephard, Waters Corporation*

*Martin Palmer, Waters Corporation*

Poster number: **IM-PA-098**

## **MICROGRID TECHNOLOGY FOR ROBUST HIGH LATERAL RESOLUTION IMAGING DOWN TO THE (SUB)CELLULAR LEVEL**

Abstract ID: **898**

**Presenting author: Michael Easterling, Bruker Scientific LLC**

### **Introduction**

MALDI imaging is a powerful technique to map biomolecules in tissue. To create a spatially resolved ion image, most MALDI instruments move the sample using an x-y stage relative to a stationary laser beam, to create a mass spectrum for every pixel. However, approaching spatial resolution of only a few micrometers, over wide travel range, poses a challenge for the mechanical accuracy of most stages, affecting the quality of the MALDI images.

Here, we introduce microGRID, a new instrument design which combines both stage and laser beam positioning to eliminate imaging artifacts down to about 5  $\mu\text{m}$ . In combination with MALDI-2, this new technique enables highly sensitive imaging at high spatial resolution without compromising on pixel fidelity.

### **Methods**

Optical encoders with sub-micron resolution were integrated into a regular stepper driven MALDI sample stage and monitor the actual position. Any deviation from the ideal raster is precisely detected by the encoders and sent to the adaptive smartbeam™ 3D laser optics for automatically on-the-fly correction and precisely irradiating the targeted pixel within  $\mu\text{m}$  accuracy.

Images were collected using smartbeam™ 3D systems with  $\sim 5 \mu\text{m}$  laser spot size at raster spacing of 5-20  $\mu\text{m}$ . We used various kinds of samples from single cells to complex tissues with CHCA, DHB and DHAP matrix-coating. Ablation craters were analyzed by high-resolving optical microscopy.

### **Preliminary data (results)**

The effect of our new microGRID technology was initially visualized by the laser beam positioning correction on thin matrix films. Further, artifact-free high-resolution imaging enabled by microGRID was demonstrated for different tissue types. For rat testis or brain samples, robust imaging at 5  $\mu\text{m}$  spatial resolution allows for visualizing small structures not distinguishable at higher pixel sizes. Hereby, the reduced sample material per pixel is delimiting the amount of ionizable analytes. This challenge regarding sensitivity is addressed by MALDI-2 post-ionization, boosting the ionization efficiency.

The drastically increase in accuracy and spatial resolution opens the field for the smallest unit of life – single eucaryotic cells. microGRID pushes imaging resolution to its outer limit and allows for the visualization of cellular fine structures.

### **Please explain why your abstract is innovative for mass spectrometry?**

microGRID allows for true high-lateral resolution MALDI-MSI down to 5  $\mu\text{m}$  pixel size. The combination with MALDI-2 allows for sensitive imaging down to (sub)cellular level.

### **Co-authors:**

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*Jens Hoehndorf, Bruker Daltonics GmbH & Co. KG*

Poster number: **IM-PA-099**

## **COMBINING FORCES OF TISSUE IMAGING, SHOTGUN AND NANOLC-MALDI ULTRA-HIGH-RESOLUTION-MASS SPECTROMETRY (UHR-MS): A UNIQUE WORKFLOW FOR UNTARGETED BIOMARKER DISCOVERY AT SANOFI, A STRATEGIC VIEW**

Abstract ID: **916**

**Presenting author: Beitz Wang, Department of DMPK, Sanofi**

### **Introduction**

Biomarker discovery provides the opportunity for a deeper insight on drug efficiency, adverse effects, and disease progression in drug research and development. Tissue mass spectrometry imaging (tMSI) is a robust and label-free technology that enables in-situ mapping of diverse analytes, including drugs, metabolites, lipids, peptides, and proteins. Due to its ability to retain spatial and histopathological relationship that is lost by classical LC-MS based methods, tMSI has become an attractive tool for biomarker discovery and for the evaluation of DMPK properties. Therefore, Sanofi tMSI group aims to evaluate and fuse the current standardized SpatialOmics strategy for biomarker discovery by tMSI with several MS platforms, including nanoLC-MALDI and shotgun UHR-MS, with an added value of validation and quantitation.

### **Methods**

In routine tMSI workflow, sectioned tissues were applied with specific matrix and analyzed by an MALDI UHR-MS. Subsequently, raw data were processed, and signals were annotated by an in-house database. After statistical analysis, several biomarker candidates were selected for further evaluation. In parallel, a shotgun UHR-MS approach for high throughput biomarker analysis and a nanoLC-MALDI UHR-MS method have been developed to validate the postulated biomarker candidates quantitatively and acquire analytes with specific properties.

### **Preliminary data (results)**

We have successfully applied our routine tMSI platform at Sanofi to identify potential biomarkers, evaluate target engagement and investigate mechanisms of toxicity in several R&D and preclinical safety projects, including tissue sections, cells, and spheroids samples. With further implementation of shotgun and nanoLC-MALDI UHR-MS platforms, we will be able to merge histopathology and different omics data acquired from solid and lipid biopsies, enabling an improved monitoring of disease progression and pharmacodynamics. Continuously advancing in bioinformatics and digitalization at Sanofi will enable us to efficiently analyze SpatialOmics and gain a thorough understanding of the pharmacological effects and/or adverse effects. Overall, our unique workflow of merging tMSI, shotgun UHR-MS and nanoLC-MALDI UHR-MS technologies for untargeted biomarker discovery will be implemented and show great value for filling the gaps in (early) safety assessment, putting 3R of animal warfare into actions and reducing the high attrition rate during drug research and development at Sanofi.

### **Please explain why your abstract is innovative for mass spectrometry?**

An unique workflow of merging tMSI, shotgun UHR-MS and nanoLC-MALDI UHR-MS technologies as SpatialOmics strategy for untargeted biomarker discovery; furthermore, enabling an improved monitoring of disease progression and pharmacodynamics.

### **Co-authors:**

*Caroline Chipeaux, Department of DMPK, Sanofi*

*Constanze Holz, Department of DMPK, Sanofi*

*Manfred Schudok, Department of DMPK, Sanofi*

*Jens Riedel, Department of DMPK, Sanofi*

Poster number: **IM-PA-100**

## **SPATIAL METABOLOMICS ON A NOVEL DUAL-FUNNEL LDI – ORBITRAP EXPLORIS FOR MATRIX- AND SURFACE-ASSISTED LASER DESORPTION IONIZATION MS IMAGING**

Abstract ID: **932**

**Presenting author: Christoph Bookmeyer, University Rovira i Virgili, Pere Virgili Institute for Health Research**

### **Introduction**

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) has developed into an indispensable tool for biomolecular analysis. By spatially mapping the chemical composition in tissue sections, metabolomic processes can be “caught in the act”, e.g., in cancer-affected organs. Numerous studies showed the successful application for lipids – for small molecules, however, the scheme is more challenging and strongly depends on the performance of the analytical instrumentation. The detection of these analytes is moreover hampered by strong overlapping matrix signals in the lower mass range. A way to majorly extend the range of detectable compounds is with Surface-assisted LDI (SALDI), which uses photoactive surfaces that feature specific ablation and ionization processes for, e.g., hydrophobic compounds, which renders SALDI a promising strategy for MSI of low-weight compounds.

### **Methods**

Here, we present the first adaptation of a novel dual-funnel LDI-MSI interface mounted on an Orbitrap-Exploris MS for versatile LDI-MSI with mass high resolution. Mammal tissue sections were covered with diaminonaphthalene (DAN) MALDI-matrix by thermal evaporation or by sputtering of gold nanolayers for surface-assisted LDI by sputtering. We optimized several parameters of the system (e.g., funnel voltage settings, in-source pressure, and laser energy and repetition rate) for high ion counts in the lowest mass range with bovine liver homogenate.

### **Preliminary data (results)**

We compared the metabolomics and lipidomics prospects of DAN matrix to the Au nanolayers on the example of mouse tissue sections at 20µm pixel sizes. To assess the degree of fragmentation and support the identification of endogenous compounds, we spiked metabolite standards into tissue homogenate and measured concentration ladders with the optimized parameter settings. With both approaches we yielded intense and partially complementary ion signals, with SALDI, however, without background signals omitting interferences with the detection of small molecules of interest.

### **Please explain why your abstract is innovative for mass spectrometry?**

First combination of the Spectrograph Injector with an Orbitrap Exploris; optimization for spatial metabolomics in the low mass range at high spatial and mass resolution aided by sputtered gold nano-layers.

### **Co-authors:**

*Toufik Mahamdi, University Rovira i Virgili*

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*Xavier Correig-Blanchar, University Rovira i Virgili, Pere Virgili Institute for Health Research, Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders*

*María García-Altare, University Rovira i Virgili, Spanish Biomedical Research Centre in Diabetes and Associated Metabolic Disorders*

Poster number: **IM-PA-101**

## **FROM LIPIDS AND DRUGS TO PROTEINS: IDENTIFYING CO-LOCALIZED MOLECULES ACROSS MULTIPLE CLASSES USING MALDI-HI-PLEX-IHC**

Abstract ID: **940**

**Presenting author: Joshua L. Fischer, Bruker**

### **Introduction**

Proteins provide unique challenges for imaging mass spectrometry (IMS), as the low abundance of these molecules makes them both difficult to detect and identify. Recently, Ambergen has developed MALDI-HiPLEX-IHC, a new immunohistological staining method that allows the visualization of proteins through photocleavable peptide mass tags attached to protein specific antibodies. The peptides are readily visualized with IMS, revealing the spatial distribution of the protein of interest. Here, we demonstrate the potential MALDI-HiPLEX-IHC with our proprietary software, SCiLS Lab, to provide insights into species colocalized with proteins as well the potential to couple the technology to mapping out drug delivery using a JQ1 dosed mouse model.

### **Methods**

Non-perfused whole brain tissues from mice dosed with 10 mg/kg JQ-1 i.v. were provided by St. Jude Children's Research Hospital (Memphis, TN). Coronal tissue sections (10  $\mu$ m thickness) were thaw-mounted to a Bruker Intellislide. Slides were spray coated with DHAP using a HTX M3+ sprayer system. Labeled JQ1 (99%, D<sub>2</sub>) was doped into the matrix as an internal/normalization standard. Lipid images were acquired on a Bruker timsTOF fleX (20  $\mu$ m spatial resolution, positive mode). Tissues slides were subjected to MALDI-HiPLEX-IHC, coated with DHB through sublimation, and imaged with the timsTOF fleX (20  $\mu$ m spatial resolution).

### **Preliminary data (results)**

Initial studies focused on providing lipid images (detailing regions such as the hypothalamus and corpus callosum) to be used to colocalize lipids with a neuronal suite of proteins targeted by the HiPLEX technology. The tissue sections at 2 and 0Bregma. A total of five antibodies were used in the HiPLEX process, and each of the expected mass tags were detected in the imaging experiment. The antibodies Histone H2A.X (HIST) and GLUT-1 showed unique distributions with little to no spatial overlap and were chosen as targets to co-localize with the lipids. GLUT-1 localized primarily in the corpus callosum, where HIST localized in every other region of the brain. Segmentation in SCiLS Lab was used to generate a region of the corpus callosum and a region for the brain excluding the corpus callosum in the original lipid image. These regions were used to determine molecular species co-localized to each region and were imported to Metaboscape 2022, which was used to provide tentative assignments based on the accurate mass of each lipid using an internal lipid library.

After normalization, JQ1 (457.14  $\pm$  0.0150 m/z) signal was found relatively localized in the ventricle. An expected result as the tissue was not perfused prior to collection. Most Interestingly, SCiLS segmentation was able to identify lipids co-localized with the drug in the ventricle. These results further demonstrate the utility of MALDI-HiPLEX in future drug studies, providing a compendium of the molecular constitution of such heterogeneous tissue while also observing drug-on-target.

### **Please explain why your abstract is innovative for mass spectrometry?**

First multimodal image workflow to co-localize lipid, drug and protein from the same tissue. Acquired by combining two serial imaging experiments including a wholly unique MALDI-HiPLEX-IHC sample approach.

### **Co-authors:**

*John J. Bowling, St. Jude Children's Hospital*

*P Jake Slavish, St. Jude Children's Hospital*

*Zoran Rankovic, St. Jude Children's Hospital*

*Mark J. Lim, Ambergen*

*Gargey Yagnik, Ambergen*

Poster number: **IM-PA-102**

## **RECENT METHODOLOGICAL DEVELOPMENTS AND APPLICATIONS OF MALDI-2 MASS SPECTROMETRY IMAGING**

Abstract ID: **956**

**Presenting author: Klaus Dreisewerd, University of Münster**

### **Introduction**

MALDI with laser-induced postionization (MALDI-2) can be used to boost the ion yields for numerous classes of analytes, such as phospho- and glycolipids, sterols, glycans, and various classes of pharmaceuticals. Because of the only small amounts of material available per pixel, MALDI-2 is particular useful for highly-resolved MALDI-MS imaging applications.

Since its introduction in 2014, our group has conducted numerous studies with the aim to better understand and potentially optimize the MALDI-2 process and, secondly, to identify applications in the life sciences where the method could have a particular high impact.

In my poster, I will summarize and discuss some of the most relevant recent MALDI-2 developments with a focus on potential new applications.

### **Methods**

Data were generated with different hybrid MALDI-2 mass spectrometers, all equipped with modified elevated pressure ion sources: two Synapt G2-S HDMS QTOFs (Waters), a Q Exactive plus Orbitrap (Thermo) with dual-ion funnel source (Spectrograph), and a timsTOF fleX MALDI-2 QTOF (Bruker). As postionization (PI) lasers, wavelength-tunable OPO-lasers and solid state Nd:YAG lasers (266 nm) with ~6-10 ns and 28 ps pulse duration and pulse repetition rates between 20-1000 Hz were utilized.

Next to the top-illumination geometry ( $\geq 5 \mu\text{m}$  pixel size), transmission-mode (t-)MALDI-2-MSI was used to achieve a ultrahigh resolution at 1-2 micrometers.

### **Preliminary data (results)**

In this overview poster, I will present selected examples of potentially important applications of the MALDI-2 and t-MALDI-2 technologies in the natural and life sciences. This will include the analysis of thin cryosections obtained from healthy and malignant tissues, that of cell cultures--here including a correlation with light microscopy and bioinformatic classification of chemical heterogeneities on the single-cell level--and the visualization of chemical messenger molecules in bacterial colonies/biofilms.

Since 2020, with the timsTOF fleX MALDI-2 (Bruker) the postionization technique is also commercially available. I will demonstrate the power of this instrument with selected examples and highlight how the additional ion mobility separation offers further potential for the characterization of highly complex sample systems.

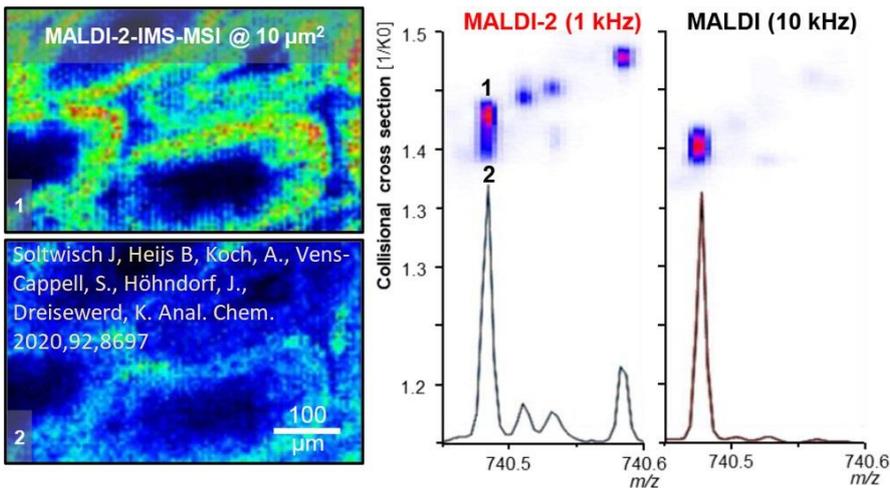
Finally, the "future" potentials of t-MALDI-2-MSI for obtaining potentially even a subcellular resolution for most eukaryotic cells and that of correlative approaches will be briefly discussed.

### **Please explain why your abstract is innovative for mass spectrometry?**

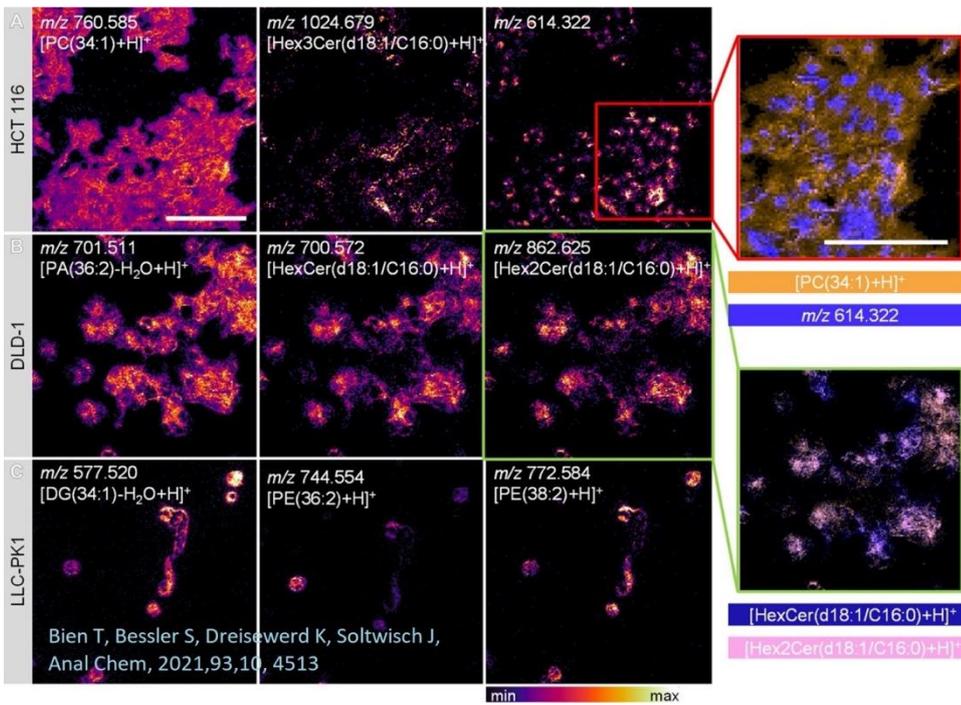
MALDI-2 and t-MALDI-2 MS imaging enable the visualization of numerous biomolecules from tissue sections, cell cultures, and bacterial biofilms with significantly improved analytical sensitivity at high spatial resolution.

POSTER SESSION A

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IMS-MSI of rat testis with the timsTOF flex MALDI-2



t-MALDI-2-MSI of cell cultures at 2 μm-pixel size

Poster number: **IM-PA-103**

## **STUDYING THE METALLOME AT SINGLE CELL LEVEL BY LA-ICP-TOFMS IMAGING - A PROOF OF PRINCIPLE STUDY IN MACROPHAGE SUBTYPES**

Abstract ID: **970**

**Presenting author: Anna Schoeberl, University of Vienna**

### **Introduction**

Understanding cell to cell interaction in the tumor microenvironment is key for finding new therapeutic avenues and requires analytical tools capable of providing single cell resolved information in a spatial context. We propose elemental single-cell analysis as essential tool for metal based anticancer drug development, allowing to study drug accumulation together with the cellular metallome in a quantitative manner. Our approach features LA-ICP-TOF-MS technology utilizing a low dispersion laser ablation setup with excellent spatial resolution down to 1  $\mu\text{m}$ . Fundamental *in vitro* experiments using a panel of immune cells upon incubation with the three clinical established platinum drugs show the power of this tool set.

### **Methods**

A high-throughput laser ablation-inductively coupled plasma-time-of-flight mass spectrometry (LA-ICP-TOFMS) workflow was implemented for quantitative single-cell analysis. Therefore, cytopins of THP-1 monocytes, and M0, M1 and M2 macrophages were produced allowing the concentration of cells on a small area with an even distribution to enable reliable single cell segmentation. Prior to cytopin preparation the cells were treated with the chemotherapeutic drugs cisplatin, oxaliplatin and carboplatin at a concentration of 10  $\mu\text{M}$  for 6h. Quantification was achieved via a gelatin-based microdroplet quantification strategy. In addition, the presented method was cross-validated by single-cell analysis in suspension and LA-ICP-TOFMS of chambered glass coverslips.

### **Preliminary data (results)**

The monocyte model, THP-1 and the monocyte-derived macrophages (M0, M1 and M2) showed varying uptake levels of the investigated chemotherapeutic drugs cisplatin, oxaliplatin and carboplatin. The relative differences of the uptake of the three platinum drugs were in agreement with results obtained for cancer cells. In addition to the varying platinum levels depending on the treatment, an effect of the cell type and polarization state of the macrophages on the uptake of the metallodrugs could be observed. These high variations allowed the separation of the cell types and the metallodrugs by statistical analysis. Furthermore, the metallome of monocytes and macrophages was assessed without treatment. Significant differences could be observed between the four investigated cell types, THP-1, M0, M1, and M2, and this pattern could be used to differentiate those cell types.

### **Please explain why your abstract is innovative for mass spectrometry?**

Bioimaging at the single-cell level allowed to study the uptake of metal-based drugs in monocytes and macrophages. The potential of the elemental pattern to distinguish cell types could be highlighted.

### **Co-authors:**

*Michael Gutmann, Medical University of Vienna*

*Sarah Theiner, University of Vienna*

*Walter Berger, Medical University of Vienna*

*Gunda Koellensperger, University of Vienna*

Poster number: **IM-PA-104**

## **SPATIALOMX AT ITS BEST: COMPARISON OF DIFFERENT STAINING PROCEDURES AND SLIDE TYPES**

Abstract ID: **997**

**Presenting author: Corinna Henkel, Bruker Daltonics GmbH & Co. KG**

### **Introduction**

SpatialOMx<sup>®</sup> on the timsTOF fleX provides high ID-rates from small sample amounts to combine regio-specificity from MALDI Imaging with PASEF empowered X-Omics. Here we present the SpatialOMx<sup>®</sup> workflow in combination with 4D-Proteomics<sup>™</sup>. Aim of this study was to find the optimal experimental setup to perform SpatialOMx<sup>®</sup> using MALDI Imaging in combination with Proteomics from the same tissue section. Defined tissue areas from 0.1 mm<sup>2</sup> to 1 mm<sup>2</sup> were excised from sections using laser microdissection (LMD) and two different types of slides (IntelliSlides<sup>™</sup> or PEN-slides) were compared with regards to their performance in the Omics experiment. Additionally, different staining procedures were compared to examine their compatibility with the follow-up proteomics experiments.

### **Methods**

Mouse liver sections (Fresh Frozen) were mounted on PEN membrane slides and IntelliSlides<sup>™</sup>. Slides were coated with DHB matrix (15mg/ml in 90%ACN, 10%H<sub>2</sub>O, 0.1%TFA) using the HTX TM-sprayer and measured on a timsTOF fleX (Bruker Daltonics). MALDI Imaging experiments were performed on lipid or peptide level. Slides were stained with H&E (Hematoxylin and Eosin), H alone or left unstained. Several areas with defined sizes (0.1 – 1mm<sup>2</sup>) were selected in SCiLS<sup>™</sup> Lab (Bruker Daltonics) and coordinates were transferred to the laser microdissection (LMD) 7000 device using the SCiLS Region Mapper. Afterwards 4D-Proteomics was performed.

### **Preliminary data (results)**

The SpatialOMx workflow on the timsTOF fleX provides the possibility to dig deeper into Omics information of areas defined by MALDI Imaging. Lipid or peptide imaging on the timsTOF fleX from mouse liver sections was used as a basis to define regions of interest (ROIs). Different area sizes (0.1 mm<sup>2</sup> – 1mm<sup>2</sup>) were selected in SCiLS Lab and transferred by the SCiLS Region Mapper for later excision using a Leica LMD7000 (Leica, Wetzlar, Germany). Proteomics results showed that the entire SpatialOMx workflow could be successfully performed independent of the slide type (PEN or IntelliSlides). 4D-Proteomics was conducted and the different amounts of proteins per area were compared between IntelliSlides and PEN slides. The impact of different staining methods on the protein ID rate was investigated. Lipid and peptide MALDI Imaging experiments upfront showed different effects on ID rates in the OMICS experiment. Additionally, the benefits and drawbacks of the two different LMD cutting methods (draw and cut for PEN slides and draw and scan for IntelliSlides) will be discussed and shown. Final results will present the best workflow in terms of slide selection (PEN or IntelliSlides), staining and recommended area sizes for the best SpatialOMx experiment.

### **Please explain why your abstract is innovative for mass spectrometry?**

The SpatialOMx workflow allows the combination of MALDI Imaging and in depth 4D- Proteomics experiments on one tissue section

### **Co-authors:**

*Signe Frost Frederiksen, Bruker Daltonics GmbH & Co. KG*  
*Nagarjuna Nagaraj, Bruker Daltonics GmbH & Co. KG*  
*Tobias Boskamp, Bruker Daltonics GmbH & Co. KG*  
*Michele Genangeli, Bruker Daltonics GmbH & Co. KG*  
*Janine Beckmann, Max Planck Institute for Marine Microbiology*  
*Manuel Liebeke, Max Planck Institute for Marine Microbiology*

Poster number: **IM-PA-105**

## **DEVELOPMENT OF A FULL MSI-LC-MS/MS WORKFLOW FOR LIPID LOCALIZATION, QUANTIFICATION AND IDENTIFICATION IN A PARKINSON'S DISEASE MODEL**

Abstract ID: **1020**

**Presenting author: Tim Hendriks, Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, The Netherlands**

### **Introduction**

Lipids are biomolecules known for their metabolic role. They are the main constituent of cellular membranes, part of protein anchors and function as signaling and transport molecule. Exploring new workflows that can identify lipids in their native state is crucial to understand the molecular mechanisms underlying diseases, such as Parkinson's Disease (PD). Present-day research focusses mainly on identifying biomarkers, such as proteins or lipids, that may be beneficial in the diagnosis of PD and in developing future interventions. Here we created a novel spatial lipidomics workflow that can detect, localize, identify and quantify lipids in a transgenic PD-mouse model on the same tissue section. On a single tissue section, we combine the spatial information from mass spectrometry imaging (MSI) with non-targeted lipidomics using LC-MS/MS.

### **Methods**

A transgenic mouse model carrying D409V-mutations in the glucocerebrosidase-gene (GBA1) is used. Alterations in glycosphingolipids-concentration was compared across brain sections from wildtype and heterozygous or homozygous GBA1-mutant mice. Tissues on indium-tin-oxide-slides were sprayed with an in-house-developed lipid-internal standard mix and 2,5-dihydroxybenzoic-acid. MALDI-2-MSI-experiments were performed on a TimsTOF system (Bruker) in positive-ionization mode and spatial-resolution of 30  $\mu\text{m}$ . Based on segmentation data (SCiLS (Bruker)), regions-of-interest were cut-out using laser-capture-microdissection on a LMD7000 (Leica). Extracted lipids were analyzed using Vanguish-Explorer480-Orbitrap (LC-MS/MS)(Thermo) in positive-ionization mode. Lipids were identified and quantified via LipidSearch (Thermo) and Lipostar (Molecular-Horizon)

### **Preliminary data (results)**

MALDI-2-MSI results normalized by means of the lipid internal standard mix show accumulation of certain hexosylceramide species. HexCer 36:1;2 and HexCer 42:2;2 accumulate in heterozygous (HT) and homozygous (HM) mutants of D409V mice brain compared to its wildtype (WT). The buildup is particularly seen in the hindbrain region, cerebellum and white matter fiber tracts. These accumulation regions appearing in MALDI-2 based segmentation analysis were exported to an optical image used for laser capture microdissection (LMD). After we optimized the laser settings and the laser coordinate system of the LMD, the system was able to cut out the regions of interest automatically from the same imaged tissue section.

On the cut-out region, LC-MS/MS based lipidomics was able to detect, quantify and identify lipid molecules in a non-targeted approach without needing chemical modification of lipids. In approximately 1mm<sup>2</sup> of tissue, the LMD-LC-MS/MS workflow was able to detect an average of 400 lipids in different classes such as e.g. glycerophosphocholines, glycerophosphoethanolamines, sphingomyelins and triacylglycerols. These results show the development and optimization of an automated lipidomics-MSI-LC/MS workflow on a one single tissue slide. This workflow opens opportunities in lipidomics research for Parkinson's disease.

### **Please explain why your abstract is innovative for mass spectrometry?**

Novel spatial lipidomics workflow that allows for detection, localization, quantification and identification on a single Indium-tin-oxide-slide.

### **Co-authors:**

*Michiel Vandenbosch, Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, The Netherlands*  
*Isabeau Vermeulen, Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, The Netherlands*

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*Shadrack Mutuku, Molecular Horizons and School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW Australia*

*Nathan Hatcher, Merck & Co., Inc, Kenilworth, NJ, USA*

*Shane Ellis, Molecular Horizons and School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, NSW Australia , Illawarra Health and Medical Research Institute, Wollongong, NSW, Australia*

*Ron Heeren, Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, The Netherlands*

*Eva Cuypers, Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, The Netherlands*

Poster number: **IM-PA-106**

## **DNA-BARCODED SIGNAL AMPLIFICATION FOR IMAGING MASS CYTOMETRY ENABLES SENSITIVE AND HIGHLY MULTIPLEX TISSUE IMAGING**

Abstract ID: **1033**

**Presenting author: Tsuyoshi Hosogane, University of Zurich**

### **Introduction**

Imaging mass cytometry (IMC) is a highly multiplexed, antibody-based imaging method that captures heterogeneous spatial protein expression patterns at subcellular resolution in complex biological samples. While the dynamic range of IMC is close to  $10^5$  the imaging of low-abundance proteins using IMC is challenging due to the instruments limit of detection. Standard amplification approaches that use isotope-labelled secondary antibodies cannot be implemented in a highly multiplexed fashion due to the low number of orthogonal host species for primary antibodies. Therefore, in order to extend high-multiplex IMC to low-abundance targets, a highly multiplex signal amplification approach is needed. Here we report the extension of IMC to ultralow-abundance markers through incorporation of the existing DNA-based multiplex signal amplification by exchange reaction (SABER).

### **Methods**

Each antibody targeting a marker of interest is tagged with a unique 42-mer bridge-DNA, and then hybridized to the 5' end of a single-stranded DNA concatemer. The concatemer carries multiple repeats of a 9-mer barcode sequence at the 3' end that is then further hybridized to a large number of metal isotope-modified complementary DNA strands (called 'imagers'). By using orthogonal bridge-DNA sequences and 9-mer barcode sequences for different antibodies, signals of multiple targets can be simultaneously amplified. Additional rounds of concatemer hybridisation can further enhance the amplification.

### **Preliminary data (results)**

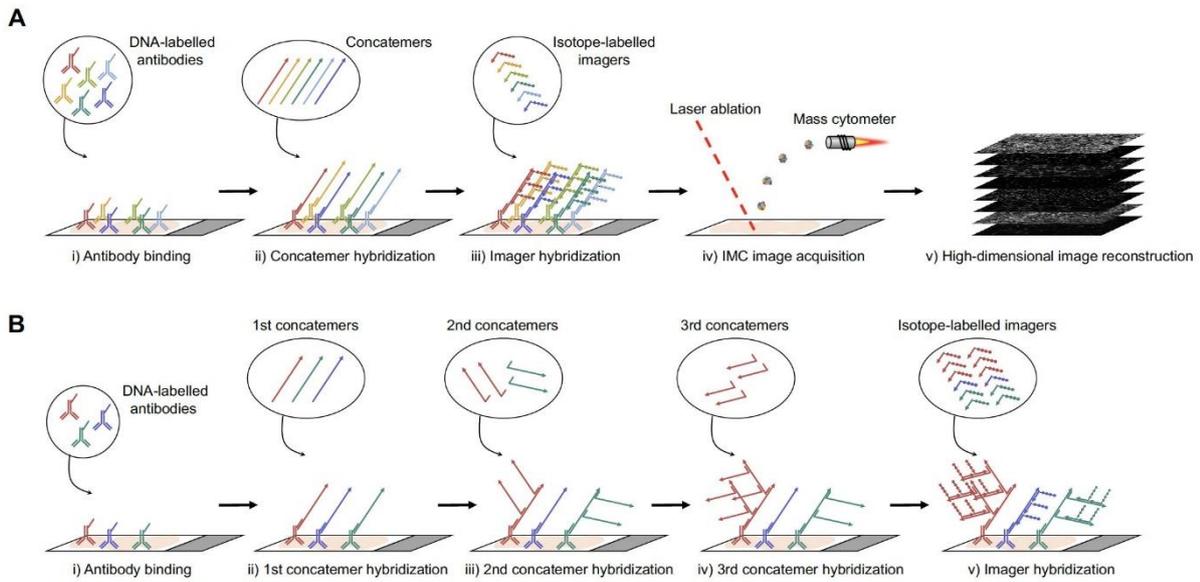
SABER-IMC amplified signal 0.7 to 4 fold with one round of amplification, 8 to 16 fold with two rounds of amplification, and 16 to 68 fold with three rounds of amplification with minimal reduction in signal-to-background ratio, enabling detection of multiple low-abundance immunomodulatory molecules in formalin-fixed, paraffin-embedded tissue sections. SABER-IMC enabled clear detection of low-abundance immunoregulatory molecules, such as CTLA4, and should prove useful for numerous other classes of low-abundance proteins, such as transcription factors, as well. We confirmed the specificity of the SABER-IMC signal in human cell lines and tonsil samples with known expression patterns. After optimisation of experimental conditions to eliminate nonspecific binding, SABER-IMC reproduced expected staining patterns and, for high-abundance markers where comparison was possible, showed similar staining patterns to standard IMC. The SABER-IMC workflow can be combined with the current IMC workflow, such that amplified and non-amplified signals for targets spanning a high abundance range can be simultaneously imaged. We applied 38-plex SABER-IMC to image the tumour-immune microenvironment in human melanoma. Single-cell protein expression data obtained using SABER-IMC allowed identification of immune cell phenotypes, such as exhausted and regulatory T cells, that are not detectable with existing multiplexed imaging methods. In summary, SABER amplification expands highly multiplexed IMC to low-abundance markers, enabling a wide range of analyses of complex biological tissues.

### **Please explain why your abstract is innovative for mass spectrometry?**

SABER-IMC provides a highly multiplex amplification platform for high-resolution tissue imaging, allowing the detection of low-abundance targets that are impossible or difficult to detect with the standard IMC setup.

**POSTER SESSION A**

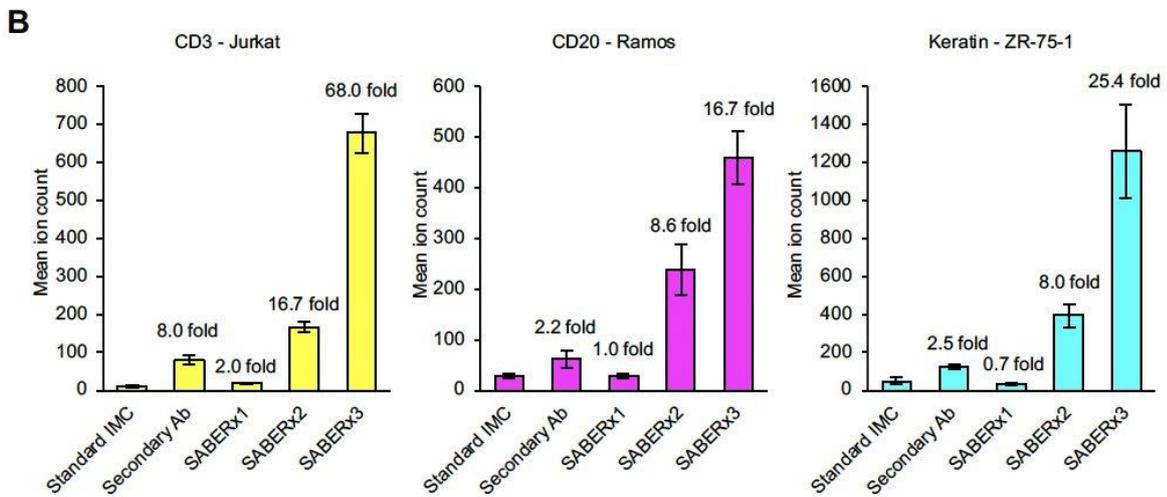
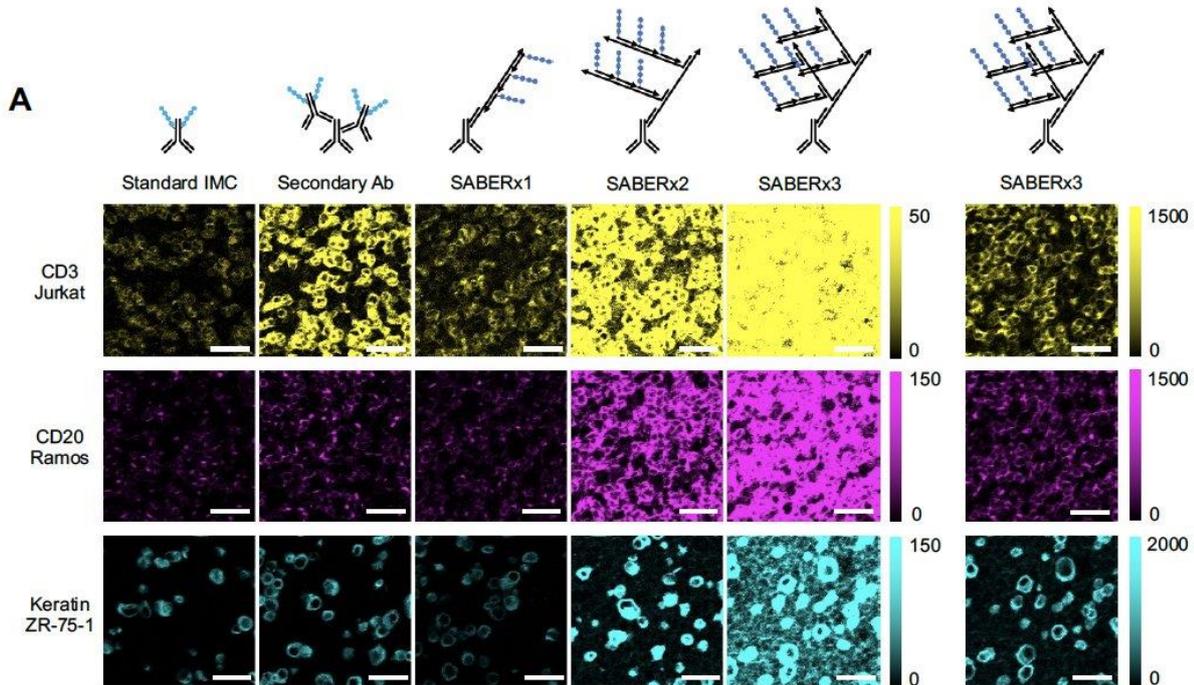
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**A**, General SABER-IMC workflow. **B**, Workflow for further signal amplification.

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Quantification of SABER-IMC signal amplification compared to standard IMC

## Session: Ion Spectroscopy, physical and chemical principles underlying MS (fundamentals)

Poster number: **IM-PA-107**

### **DERIVING ENERGETICS OF POLYMETALLIC COMPLEXES WITH ION MOBILITY MASS SPECTROMETRY**

Abstract ID: 29

**Presenting author: Niklas Geue, The University of Manchester**

#### **Introduction**

Motivated to explore the physics of polymetallic rings with promising magnetic properties, a family of anionic heterometallic macrocycles of the type  $[\text{Cr}_7\text{M}^{\text{II}}]^{-}$  ( $\text{M}^{\text{II}} = \text{Mn, Fe, Co, Ni, Cu, Zn, Cd}$ ) have been investigated over the last two decades by our groups. These rings have proven to be suitable hosts for a range of secondary ammonium cations, leading to the formation of hybrid organic-inorganic rotaxanes with potential applications in quantum information processing. To improve our understanding of their properties and dynamics, ion-mobility mass spectrometry was applied to characterise various rotaxane systems.

#### **Methods**

We investigated a series of [2]-rotaxanes of the general formula  $[\text{NH}_2\text{RR}'][\text{Cr}_7\text{M}^{\text{II}}\text{F}_8\text{Piv}_{16}]$  ( $\text{Piv} = \text{O}_2\text{C}^t\text{Bu}$ ) involving two different secondary ammonium cations (Thread, Ph and Am) surrounded by polymetallic rings with varying divalent metals (Figure 1). Energy-resolved collision-induced dissociation studies were performed to quantitatively investigate the systems' energetics. Additionally, ion-mobility mass spectrometry experiments were conducted to understand the detailed fragmentation mechanism of the isolated ring anions and rotaxane species.

#### **Preliminary data (results)**

The results show that three factors strongly influence the [2]-rotaxane stability: the structure of the secondary ammonium cation, the divalent metal  $\text{M}^{\text{II}}$  and the charge carrying species ( $\text{H}^+$  vs.  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cs}^+$ , Figure 2a). The most stable rotaxane was found with the most polar ammonium cation, exhibiting the strongest non-covalent bonds to the polymetallic core. Additionally,  $\text{Ni}^{\text{II}}$  enhances the ring stability significantly compared to other divalent metals, whereas the use of protons as charge carrier strongly destabilizes the system.

Ion-mobility mass spectrometry data showed that the opened fragment ions of the polymetallic rings exhibit a structurally rich set of conformers that can both be contracted and extended relative to the ring precursor ion, suggesting the occurrence of both horseshoe-like structures and rearranged smaller rings (Figure 2b).

#### **Please explain why your abstract is innovative for mass spectrometry?**

With this study, we take a first step in understanding the intrinsic dynamics of heterometallic ring systems and bridged the gap between their gas phase and solution phase behavior.

#### **Co-authors:**

*Lennart A. I. Ramakers, Wageningen University & Research*

*Tom S. Bennett, The University of Manchester*

*Grigore A. Timco, The University of Manchester*

*Eric J. L. McInnes, The University of Manchester*

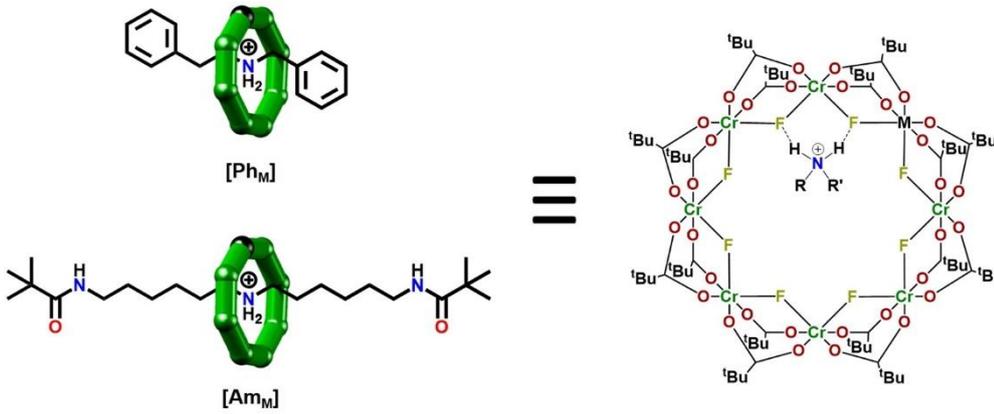
*Peter B. Armentrout, University of Utah*

*Richard E. P. Winpenny, The University of Manchester*

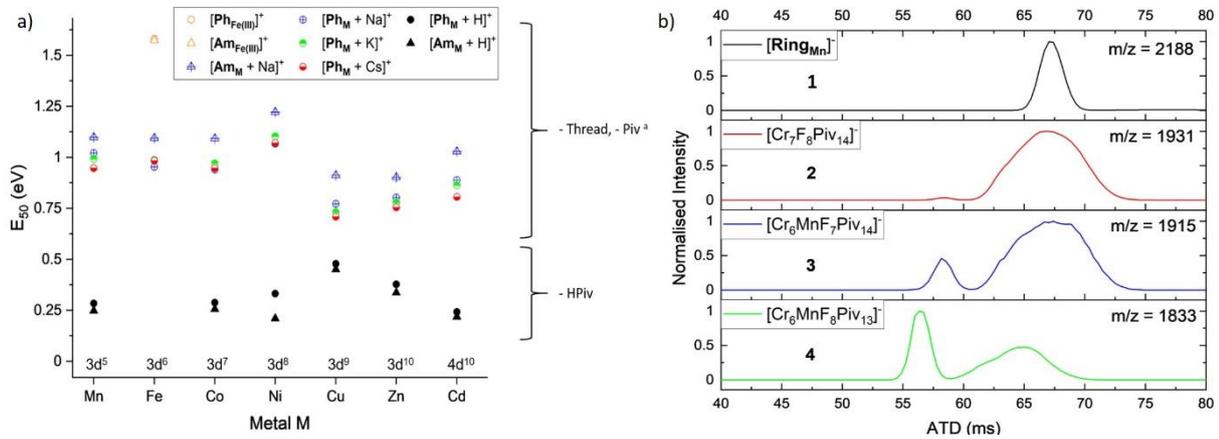
*Perdita E. Barran, The University of Manchester*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
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Schematic structure of the rotaxane families  $Ph_M$  and  $Am_M$ .



$E_{50}$  values (stability) and ATDs of ring and rotaxane ions.

Poster number: **IM-PA-108**

## **GAS PHASE INVESTIGATION OF CONCAVE-CONVEX INTERACTIONS BETWEEN C<sub>60</sub> AND SADDLE-SHAPED $\pi$ -EXTENDED PORPHYRINS**

Abstract ID: 74

**Presenting author: Johannes Oschwald, Physical Chemistry I, Friedrich-Alexander-University Erlangen Nuremberg**

### **Introduction**

Supramolecular complexes of C<sub>60</sub> fullerene guests and porphyrin hosts have been studied extensively and show the formation 1:1 complexes in ESI and MALDI MS. The formation of 2:1 complexes is uncommon.

### **Methods**

Here we report on the formation of supramolecular 1:1 and 2:1 complexes between C<sub>60</sub> and different  $\pi$ -extended porphyrins in ESI MS. Furthermore, the fragmentation energetics of 1:1 and 2:1 complexes are compared by energy-dependent collision-induced dissociation (CID) experiments which reveal a significant increase in binding energies compared to similar complexes with porphyrins.

### **Preliminary data (results)**

This improvement is achieved by maximizing the  $\pi$ - $\pi$ -interactions between host and guest by expanding the  $\pi$ -system of the porphyrin into a benzo- or naphthoporphyrin, which possess a saddle-shape with negative curvature. This structure features two concave cavities to which the convex C<sub>60</sub> fullerene can bind more efficiently compared to flat porphyrins. Additionally, this structure allows the addition of a second C<sub>60</sub> resulting in a 2:1 complex which is not observed for the corresponding flat porphyrins. Interestingly, in both the 1:1 and 2:1 complexes all C<sub>60</sub> molecules display essentially the same binding motive to the porphyrin, where the convex cavity of the porphyrin host follows the concave surface of the C<sub>60</sub> guest without causing any major distortion of the porphyrin. Whereas for flat porphyrins this distortion is known to be significant and is necessary in order to maximize the  $\pi$ - $\pi$ -interactions between C<sub>60</sub> and the host.

In addition, the influence of CH- $\pi$ -interactions was studied for benzo- or naphthoporphyrins by varying the functional groups in the meso-position of the porphyrin. A significant increase in stability is observed for bulkier substituents, taking up a conformation perpendicular to the porphyrin plane. Thus, the CH- $\pi$ -interactions are essential for the binding between C<sub>60</sub> guest and porphyrin host.

### **Please explain why your abstract is innovative for mass spectrometry?**

In conclusion, CID experiments are well suited to study the interactions between C<sub>60</sub> fullerene guests and porphyrin hosts.

### **Co-authors:**

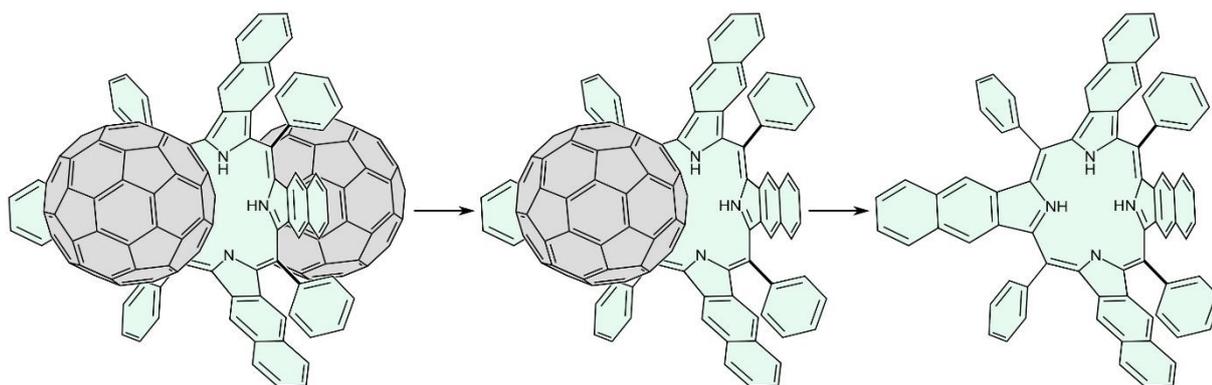
*Fabian Plass, Physical Chemistry I, Friedrich-Alexander-University Erlangen Nuremberg*  
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*Dirk M. Guldí, Physical Chemistry I, Friedrich-Alexander-University Erlangen Nuremberg*  
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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

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Poster number: **IM-PA-109**

## DISSOCIATION BEHAVIOUR OF DOUBLY FUSED N-HTA SCAFFOLDS IN THE GAS-PHASE STUDIED BY ESI-MS/MS

Abstract ID: **105**

**Presenting author: Marina Kinzelmann, Friedrich-Alexander-Universität Erlangen-Nürnberg**

### Introduction

Following on from an earlier study of monomeric N-heterotriangulene (N-HTA)<sup>[1]</sup>, we investigate the gas phase behaviour of a doubly fused N-HTA hexabenzocoronene (**B**) and its tetraphenylbenzene-linked precursor (**A**) with electrospray ionization mass spectrometry (ESI-MS).

### Methods

N-HTA derivatives are prone to undergo electrochemical oxidation during the ESI process. Thus, not only the molecular ions ( $M^+$ ) but also the dications ( $M^{2+}$ ) were observed in the mass spectra of compound **A** and **B**.

### Preliminary data (results)

We report on the energy-dependent collision-induced dissociation (CID) behaviour of the mono- and dication of **A** and **B**. Experiments show a stepwise fragmentation by successive loss of six methyl radicals from the dimethylmethylene bridges, three from each N-HTA subunit.

The breakdown graph of the molecular ion of the flexible molecule **A** shows the methyl losses in a highly regular energetic succession with only the first fragment being more resilient towards dissociation than the subsequent ones. The breakdown graph of the rigid molecule **B** displays a fairly irregular pattern, this suggests a dependence on the molecular structure specifically the ability of charge migration from one N-HTA unit to the other after the first three methyl losses.

The rigid structure of **B** also causes the N-HTA units to act independently in the dissociation behaviour of the dication. The loss of the first methyl radical from one triangulene unit is directly followed by the loss of a second methyl radical at the other unit; similar behaviour is observed for the loss of subsequent methyl radicals. For the flexible molecule **A**, an almost simultaneous fragmentation of one methyl radical is observed from each N-HTA unit, while the following losses appear at different collision energies.

[1] J. F. Hitzengerger, P. O. Dral, U. Meinhardt, T. Clark, W. Thiel, M. Kivala, T. Drewello, *Chempluschem* **2017**, *82*, 204–211.

### Please explain why your abstract is innovative for mass spectrometry?

We report on the energy-dependent collision-induced dissociation (CID) behaviour of the mono- and dication of a doubly fused N-HTA hexabenzocoronene (**B**) and its tetraphenylbenzene-linked precursor (**A**)

### Co-authors:

*Nina Fröhlich, Friedrich-Alexander-Universität Erlangen-Nürnberg*

*Frederik Gnannt, Friedrich-Alexander-Universität Erlangen-Nürnberg*

*Milan Kivala, Friedrich-Alexander-Universität Erlangen-Nürnberg, Ruprecht-Karls-Universität Heidelberg*

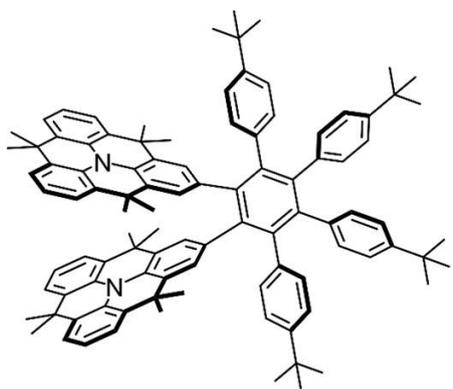
*Thomas Drewello, Friedrich-Alexander-Universität Erlangen-Nürnberg*

**POSTER SESSION A**

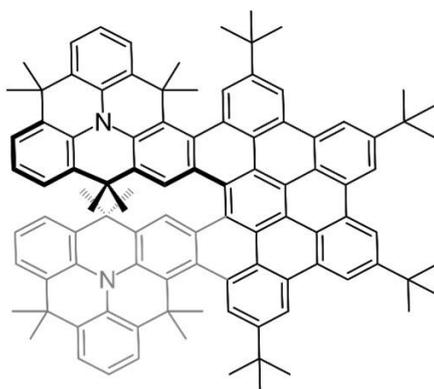
Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours



**A**



**B**

Poster number: **IM-PA-110**

## INVESTIGATION OF STRUCTURE-STABILIZING ELEMENTS IN PROTEINS BY ION MOBILITY MASS SPECTROMETRY AND COLLISION-INDUCED UNFOLDING

Abstract ID: 199

Presenting author: **Edwin De Pauw, Mass Spectrometry Laboratory, ULiege**

### Introduction

The shape of biomolecules in the gas phase results from a balance between Coulomb repulsion and stabilizing intramolecular interactions. Depending on the ionization mechanism, structural elements can be conserved. A proteolytic reactor, designed in the Laboratory, was coupled to ion mobility mass spectrometry to monitor the evolution of collision cross section of the residual parts of proteins subjected to mono enzymatic digestion.

### Methods

Upon the progressive loss of various peptides during digestion, the CCS of the remaining sequence of the protein was measured. It can either comply from the classical  $2/3$  power trend of the CCS-mass assumed for globular structures or diverge if strong interactions are present. In complement to the determination of their CCS, the residual structures have also been characterized using collision induced unfolding (CIU) to probe their respective resilience towards collision with a neutral gas. The resilience can be linked to the presence of structure stabilizing element(s) introducing a barrier for conformational changes.

### Preliminary data (results)

A model system bearing 2 disulfides bridges was first submitted to CIU under its oxidized and reduced form. Three proteins were analyzed:  $\beta$ -lactoglobulin (2 disulfide bridges), cytochrome c (heme) and calmodulin ( $\text{Ca}^{2+}$  coordinating cation). CCS were measured and CIU was performed on intact protein and their residual structures after limited digestion. TWCCS  $\text{N}_2 \rightarrow \text{He}$  of the studied species were plotted as a function of their masses and compared to two trend curves describing the CCS/mass relation: (1) a trend curve established by Ruotolo et al.  $\text{CCS} = 2.45 \times m^{2/3}$  a similar trend curve established in our laboratory using the trypsin digest of cytochrome c and  $\beta$ -lactoglobulin sprayed in non-denaturing conditions  $\text{CCS} = 2.39 \times m^{2/3}$  ( $m/z$  range 400 – 3000). One can consider that TWCCS  $\text{N}_2 \rightarrow \text{He}$  situated below or on the trend curve reflects the presence of more compact structures while those located above the trend curve are related to more extended species. Our study shows that proteins and residual structures bearing structure stabilizing elements such as disulfide bridges, heme or coordinating cation systematically present lower TWCCS  $\text{N}_2 \rightarrow \text{He}$  and a higher resilience towards CIU. The effect of the formation of strong complexes and study of other biomolecules is undergoing.

### Please explain why your abstract is innovative for mass spectrometry?

The online enzymatic reactor coupling with IMS allows to progressively disassemble proteins and verify the importance of the composing elements for the gas phase structure stability.

### Co-authors:

*Elodie Griffee, Mass Spectrometry Laboratory, ULiege*  
*Christopher Kune, Mass Spectrometry Laboratory, ULiege*  
*Cedric Delvaux, Mass Spectrometry Laboratory, ULiege*  
*Loic Quinton, Mass Spectrometry Laboratory, ULiege*  
*Andre Matagne, Laboratory of Enzymology and Protein Folding, ULiege*  
*Gabriel Mazzucchelli, Mass Spectrometry Laboratory, ULiege*  
*Johann Far, Mass Spectrometry Laboratory, ULiege*

Poster number: **IM-PA-111**

## TOWARDS ROUTINE INFRARED ION SPECTROSCOPY IN THE ANALYTICAL LABORATORY

Abstract ID: **233****Presenting author: Giel Berden, Radboud University**

### Introduction

A major challenge in analytical chemistry is identifying the full molecular structure of low-abundance small molecules. Compounds found at nanomolar concentrations can be detected by combining liquid chromatography and (tandem) mass spectrometry (LC-MS), but structural information is limited to retention times and (fragment) masses, which are often inconclusive. Infrared ion spectroscopy (IRIS) has emerged as a powerful analytical technique to elucidate molecular structure. IRIS combines the sensitivity of LC-MS with the structural information of gas-phase IR spectroscopy and has been successfully applied for the identification of low-abundance species in complex mixtures. Here we implement IRIS on commercial MS platforms using commercial turn-key infrared lasers and show that integration in analytical laboratories is now on the horizon.

### Methods

Infrared spectra of  $m/z$  isolated ions were recorded using several small commercial infrared lasers in combination with a Bruker AmaZon Speed quadrupole ion trap instrument. Spectra are recorded in the hydrogen stretching region (CH, OH, NH stretch vibrations) between 2900-3800  $\text{cm}^{-1}$ .

### Preliminary data (results)

To evaluate the performance of the small IR laser systems for IRIS in ion trap mass spectrometers, we performed several experiments which will be discussed in this contribution. Additionally, several applications will be presented, including the following example of biomarker identification. A few years ago, sodiated N-acetylmannosamine ( $[\text{ManNAc}+\text{Na}]^+$ ,  $m/z$  244) was identified as a biomarker for the disease NANS-deficiency using untargeted LC-MS. It was necessary to use NMR techniques, requiring significantly larger sample volumes and having lower sensitivity than MS-based methods, to identify the biomarker elevated in patient samples as ManNAc and not GlcNAc and GalNAc, which have the same exact mass and CID MS/MS fragmentation patterns. Here, we show that the three sodiated sugars can be distinguished based on their infrared spectra recorded in the 3400-3700  $\text{cm}^{-1}$  spectral range with the table top IRIS setup. Additionally, we discuss the use of IRIS set-ups in the identification of drug metabolites, synthetic drug (novel psychoactive substances) isomers, and micro-pollutants in water.

### Please explain why your abstract is innovative for mass spectrometry?

Infrared ion spectroscopy using small, commercially available, turn-key lasers for use in analytical laboratories with limited laser technology expertise.

### Co-authors:

*Rianne van Outersterp, Radboud University**Jonathan Martens, Radboud University**Filip Cuyckens, Janssen R&D**Jos Oomens, Radboud University*

Poster number: **IM-PA-112**

## **COVALENT MODIFICATION OF LYSINES: IMPACT OF MODIFICATIONS AND THEIR MASS ON ION MOBILITY AND COLLISIONAL INDUCED UNFOLDING OF PROTEINS**

Abstract ID: **330**

**Presenting author: Thomas Tilmant, Mass Spectrometry Laboratory-ULiège**

### **Introduction**

Mass spectrometry coupled with ion mobility (IM-MS) is now an established tool for ion shape analysis. However, the use of ESI-MS implies the transfer of proteins from the solution to the gas phase, that can lead to structural changes. Complementarily, collision induced unfolding (CIU) enables to monitor progressive protein unfolding in the gas phase upon a gradual increase of the ion activation prior to mobility. The preservation of elements of structure upon ionization is linked to the presence of stabilizing non-covalent interactions. Here we report an approach based on chemical modifications of interacting moieties to probe their contribution to gas-phase protein structures.

### **Methods**

We focused on the role of lysines. In solution, lysines are often essential in the stabilization of protein structures because their  $\epsilon$ -amino group can form hydrogen bonds and salt bridges. We chemically disrupted this property by selectively modifying their side chains with NHS ester derivatives. Model proteins such as Cytochrome c or Ubiquitin have been modified with NHS ester derivatives of various polarity, mass, and volume. The CCS of labeled and non-labeled proteins are compared. IM-MS and CIU measurements were performed on a traveling wave ion mobility mass spectrometer (Synapt G2-Si).

### **Preliminary data (results)**

Lysine modifications show minimal impact on the CCS, limited to the expected increase due to raising the number of added labels. However, the influence of labeling shows up clearly during CIU experiments. The collision energy required to observe extended conformations is a function of the increasing number of labels. In several cases, we observed the loss of the initial extended conformations, observed with the unlabeled proteins. Disrupting lysine interactions induces a "compaction effect" during CIU, meaning that lysines play an active role in trapping the extended conformations in the gas phase. Secondly, increasing the size of the label added to the proteins amplifies this compaction effect. Thus, it is possible to play on the amount of modification or on the size to obtain a similar behavior in the gas phase for different labels. For example, in the case of cytochrome c the effect observed during CIU experiment is nearly similar for 7 lysine acetylations (+ 42 Da) or 3 groups of 194 Da. This approach based on modification of lysines can provide insights into their role in gas-phase behavior of protein. We are currently investigating other approaches to improve our understanding of this phenomenon.

### **Please explain why your abstract is innovative for mass spectrometry?**

Probing non-covalent interactions contribution on gas-phase protein structures using chemical modifications. The polarity, mass and volume of the modification can modulate the protein behavior in the gas phase.

### **Co-authors:**

*Martin Greffe, Mass Spectrometry Laboratory-ULiège*  
*Christopher Kune, Mass Spectrometry Laboratory-ULiège*  
*Edwin De Pauw, Mass Spectrometry Laboratory-ULiège*  
*Loïc Quinton, Mass Spectrometry Laboratory-ULiège*

Poster number: **IM-PA-113**

## DEVELOPMENT OF A NEW ELECTROSPRAY IONIZATION TIME-OF-FLIGHT MASS SPECTROMETER FOR CHIROPTICAL STUDIES

Abstract ID: **480**Presenting author: **Peter Krüger, Radboud University**

### Introduction

Enantiomers can exhibit different bioactivity and therefore their distinction plays a central role in medicine and pharmacy.<sup>[1]</sup> Conventional enantiosensitive methods such as absorption circular dichroism spectroscopy offer only limited sensitivity and selectivity. As a consequence, new techniques which can be coupled to mass spectrometry have been developed for chiral discrimination. Among these, the photoelectron circular dichroism (PECD) technique is based on forward-backward asymmetries in the photoelectron angular distribution upon photoionization with circularly polarized light.<sup>[2]</sup> So far, the applicability of this powerful gas phase technique was severely limited regarding non-volatile samples. This limitation is overcome by utilizing an electrospray ionization source. The photodetachment of electrons from electrosprayed anions by circularly polarized laser pulses enables the distinction of enantiomers based on the PECD.<sup>[3]</sup>

### Methods

Anions are generated by electrospray ionization (ESI) and can be accumulated inside an octupole ion trap before they are guided into a chirality spectrometer. Photodetachment is then enforced using circularly polarized ns laser pulses, which are generated by means of a quarter wave plate from the third harmonic of a Nd:YAG laser (355 nm). Created photoelectrons are projected onto a microchannel plate detector with two half circle anodes for quantification of forward-backward asymmetries relative to the laser propagation axis. Ions are analyzed inside a linear time-of-flight mass spectrometer (TOF-MS).

### Preliminary data (results)

The development of a new enantiosensitive mass spectrometer is reported. A ESI source was coupled to a linear TOF-MS and a direction-resolved electron detection unit via a multipole ion trap. The viability of the setup for chirality analysis of electrosprayed anions is demonstrated in proof-of-principle experiments on two amino acids: 3,4-Dihydroxyphenylalanine (DOPA) and glutamic acid (GLU). The photodetachment of monoanions  $[M-H]^-$  was investigated in the case of DOPA, while a distribution of solvated dianions  $[(M-2H)(CH_3CN)_x(H_2O)_y]^{2-}$  was studied for GLU. In either case, a large chiral response of several percent was observed in the photoelectron angular distribution, allowing an unambiguous distinction between the respective L- and D-enantiomers.<sup>[3]</sup> In contrast, it was not possible to distinguish between the enantiomeric pairs based on the circular dichroism in anion yield differences. The presented new ESI-PECD technique offers several advantages over classical approaches based on the photoionization of neutrals. The charged nature of the analyte enables precursor ion mass selection. Additionally, the employed single photon detachment scheme can be expected to be applicable to a wide range of samples, which is important for analyzing multicomponent mixtures. Simultaneously, the technical requirements for light sources are greatly reduced since relatively low photon energies and intensities are sufficient.

### References

[1] L. A. Nguyen, H. He, C. Pham-Huy, *Int. J. Biomed. Sci.* **2006**, *2*, 85.

[2] B. Ritchie, *Phys. Rev. A* **1976**, *13*, 1411.

[3] P. Krüger, K.-M. Weitzel, *Angew. Chem. Int. Ed.* **2021**, *60*, 17861.

### Please explain why your abstract is innovative for mass spectrometry?

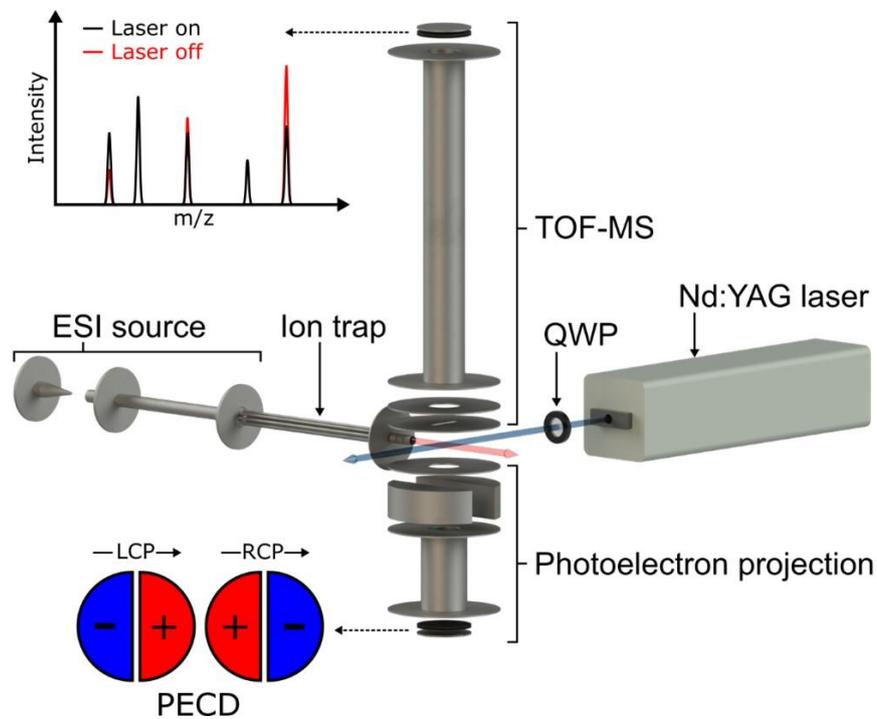
For the first time, it was demonstrated that enantiomers of non-volatile samples can be distinguished by combining the highly enantiosensitive PECD technique with ESI-TOF mass spectrometry.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
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Tuesday 30 August 2022 from 14:00 to 15:30 hours

**Co-authors:**

*Karl-Michael Weitzel, Philipps-Universität*



Novel electro spray ionization time-of-flight mass spectrometer for chiroptical studies.<sup>[3]</sup>

Poster number: **IM-PA-114**

## **METASTABLES AS A CAUSE FOR BASELINESHIFT IN QUADRUPOLE MASS ANALYZERS**

Abstract ID: **501**

**Presenting author: Markus Langner, Bergische Universität Wuppertal**

### **Introduction**

Quadrupole mass filters are commonly used as high- or band pass filters, the latter in most cases with unit mass resolution. The former mode ("RF only") generally transports ions to a second mass selective device (e.g. a TOF) with the option to suppress abundant (low-mass) ions and thus to increase the overall dynamic range of the tandem system. Increasing the ion current produced in an ion source is generally a means of increasing the amount of analyte ions of interest reaching the mass analyzer. Our studies suggest though that metastable species, also generated in the ion source, can reach the detector and lead to a false positive signal, shifting the baseline and thus reducing signal to noise ratio as a result.

### **Methods**

A commercially available RGA system QMG 422 (Inficon, Bad Ragaz, Switzerland) consisting of a closed electron ionization source, quadrupole filter (20 cm rod length), SEM as well as Faraday cup is used for all studies. A needle valve directly mounted upstream of the ion source is used to directly add gas mixtures. For comparison studies with a mock-up system, a commercial stainless steel 4-way cross was fitted with an electron ionization source, custom electrode stacks for ion steering, and a Faraday cup as well as a SEM for detection.

### **Preliminary data (results)**

Results obtained earlier in our lab motivated the present study. A still unexplained observation is that using an RGA in filter mode the baseline lifts by orders of magnitude at ion currents reaching the  $\mu\text{A}$  range. Comparison between experimental data obtained with air as sample gas and simulations show that the detectors still register signals, even when the mass filter is operating at much higher RF voltages than the respective cut off voltage of the present species. At small ion currents (up-to several nA), the simulations are in good accordance with the experiment regarding the cut off voltage, while at elevated ion currents the experiments begin to show different behavior as in the simulation, where the ions are still cut off at the respected cut off voltage. This behavior is speculated to be the result of the production of metastable species in the electron ionization source. This hypothesis requires though that the metastable species passing the quadrupole decompose to a significant extent between filter and detector to appear in the mass spectrum. There are several routes for metastable species to generate an electrical detector current, thus leading to false positive signal. I) Meitner-Auger-processes may cause emission of electrons from the metal surface ii) ionizing reactions after the mass filter may occur iii) emitted photons from relaxation could lead to emission of electrons due to the photoelectric effect. These cases are experimentally targeted and the results are discussed.

### **Please explain why your abstract is innovative for mass spectrometry?**

Systematic experiments regarding the impact of metastable species on the detector output of quadrupole mass filters and reaction mechanisms of metastable species.

### **Co-authors:**

*Lena Mokross, Bergische Universität Wuppertal*  
*Thorsten Benter, Bergische Universität Wuppertal*  
*Hendrik Kersten, Bergische Universität Wuppertal*

Poster number: IM-PA-115

## STUDYING THE KEY INTERMEDIATE OF RNA AUTOHYDROLYSIS BY CRYOGENIC GAS-PHASE INFRARED SPECTROSCOPY

Abstract ID: 514

**Presenting author:** Kim Greis, Fritz Haber Institute of the Max Planck Society, Freie Universität Berlin, Yale University

### Introduction

The structural characterization of reactive intermediates is particularly challenging. Their high reactivity leads to short lifetimes and impedes their isolation. As a result, only a few methods have been available in the past to study those highly reactive species, such as matrix isolation spectroscopy or NMR spectroscopy in super acids. Here, the structure of the investigated species can be distorted by the interaction with the matrix or the super acid. Recently, we established a workflow to characterize reactive intermediates in isolation by transferring them into the gas phase and probing their structure by cryogenic infrared spectroscopy in helium nanodroplets. In this presentation we use this technique to study the intermediate of RNA autohydrolysis (Figure 1).

### Methods

RNA fragments are generated by electrospray ionization and isolated in the “clean-room” environment of a mass spectrometer. The ions are accumulated in an ion trap and picked up by superfluid helium droplets (0.4 K). The doped droplets are excited by the infrared beam of a free-electron laser. Resonant excitation of vibrational modes leads to the release of the ions from the droplets, which are detected by a time-of-flight mass spectrometer. The ion count as a function of the photon wavenumber provides an infrared spectrum. The resulting infrared pattern yields the ion’s structure by comparison with calculated patterns of candidate structures.

### Preliminary data (results)

Fragmentation of deprotonated RNA dinucleotides leads to a variety of fragments (Figure 2). The  $m/z$  of  $c$ -fragments corresponds to that of the intermediate of RNA autohydrolysis. Here we probed its structure using cryogenic infrared spectroscopy in helium nanodroplets to test if it is identical to the intermediate of RNA autohydrolysis.

The infrared spectra of  $c$ -fragments of ApA, GpG, UpG, and CpG dinucleotides were recorded and compared to harmonic and anharmonic frequencies computed at the PBE0+D3/def2-TZVPP level of theory. The comparison suggests that the  $c$ -fragments are identical to the intermediate of RNA autohydrolysis. To further substantiate the claims, the infrared spectra of commercially available deprotonated 2',3'- and 3',5'-cyclic nucleotide phosphates were recorded for adenosine and cytidine phosphates. The results confirm the identity of the  $c$ -fragments to the 2',3'-cyclic nucleotide phosphates and rule out the formation of 3',5'-cyclic nucleotide phosphates or linear fragments. Hence, the  $c$ -fragments are identical to the intermediate of RNA autohydrolysis.

The spectral signature of CpG  $c$ -fragments is much more prominent than those of the other  $c$ -fragments and those of the 2',3'- and 3',5'-cCMPs. When the latter are activated in the ion-source, their infrared signature expands as well. This indicates that activation leads to tautomerization of the nucleobase, cytosine, which has previously been reported for the condensed phase. The findings are rationalized by calculations of transition states that suggest that the process might be catalyzed by water in the source region.

### Please explain why your abstract is innovative for mass spectrometry?

We developed a methodology to investigate the structure of the RNA fragments in the gas phase. This provides unprecedented insights on the fragmentation mechanisms.

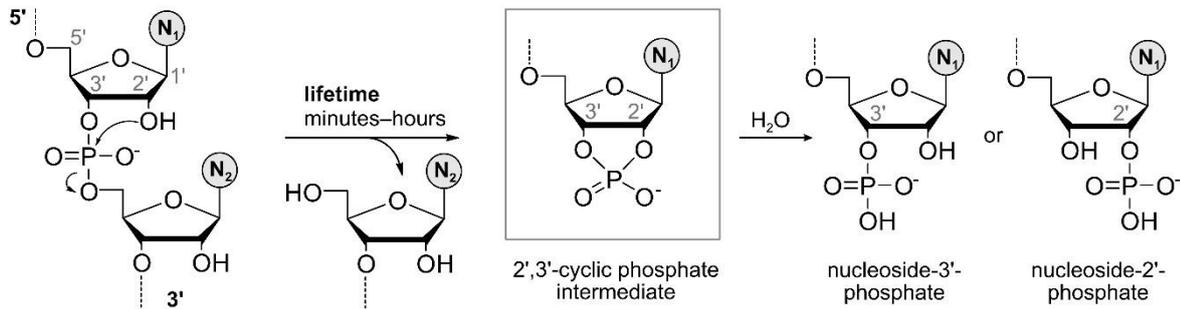
### Co-authors:

Carla Kirschbaum, Fritz Haber Institute of the Max Planck Society, Freie Universität Berlin  
Gerard Meijer, Fritz Haber Institute of the Max Planck Society

## POSTER SESSION A

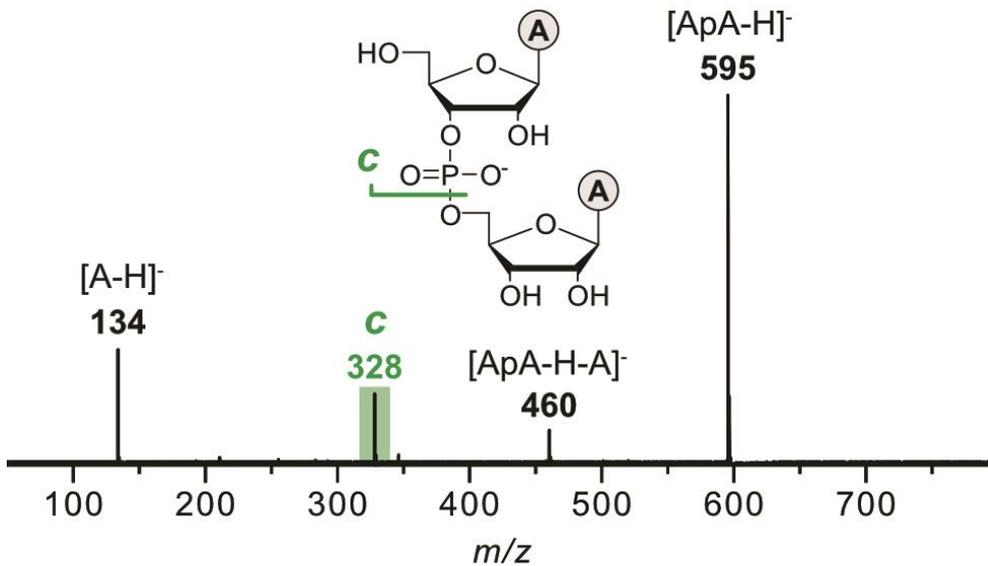
Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Gert von Helden, Fritz Haber Institute of the Max Planck Society  
Kevin Pagel, Freie Universität Berlin, Fritz Haber Institute of the Max Planck Society



Autohydrolysis reaction of RNA with its intermediate, a pentacyclic phosphate.

### MS/MS of ApA



Fragmentation of ApA dinucleotides leads to a variety of fragments.

Poster number: **IM-PA-116**

## **MASS SPECTROMIC PRODUCT STUDY IN THE INTERACTION OF SELECTED METAL SURFACES WITH H<sub>2</sub> PLASMA GENERATED SPECIES**

Abstract ID: **516**

**Presenting author: Joshua Rieger, University of Wuppertal**

### **Introduction**

Over the past decades, plasmas have gained considerable interest in diverse research and development areas. Generally, they are utilized to initiate chemical and physical processes through the interaction between plasma constituents and solid, liquid, or gaseous matter, resulting in a multitude of subsequent reaction pathways. This includes the interaction of high-energy hydrogen plasmas in the presence of metals, for example tin, leading to the formation of corresponding metal hydrides. The initial step in the hydride formation process is a heterogeneous gas/surface reaction of electronically excited H atoms and metal atoms M, successively forming H-M bonds. The volatile metal hydrides eventually desorb and may then adsorb on other (metal) surfaces upon decomposing back to the metal and hydrogen.

### **Methods**

Two different low-pressure hydrogen RF plasmas are used to generate the metal hydrides. For detection of neutral and ionic metal hydride species, the plasma chamber is directly coupled to different mass spectrometers that detect the ions directly or ionize neutral species by EI or both (MAT95XP double-focusing sector field MS, Thermo Finnigan; LTOF time-of-flight MS, Tofwerk AG; CTOF time-of-flight MS, Tofwerk AG). Elemental metals samples are located directly in the RF plasma region and are exposed to the reactive plasma species. For reference, stannane (SnH<sub>4</sub>) was synthesized and analyzed by MS.

### **Preliminary data (results)**

Mass spectrometric analysis of hydrogen plasma experiments with elemental tin or other pure metals show besides the Sn<sup>+</sup> (in general M<sup>+</sup>) also ionic tin hydrides of the type SnH<sub>x</sub><sup>+</sup>. The initial step in the hydride formation process is a heterogeneous gas/surface reaction of electronically excited H atoms with surface metal atoms, successively forming H-M bonds. The volatile metal hydrides eventually desorb and may then adsorb on other surfaces upon decomposing back to the metal and hydrogen. The rich isotopic pattern of Sn is fully reproduced in the recorded mass spectra and thus used for unequivocal compound identification. Since the mass spectrum of the ionic species and ionized neutral plasma generated species are virtually identical to the EI reference spectrum of the synthesized stannane, it is confirmed that the detection of all SnH<sub>x</sub><sup>+</sup> species in the mass spectra originate from neutral SnH<sub>4</sub> via parent ion fragmentation. A shift of the signals to lower or higher metal hydrides by changing the plasma conditions provides important information on the prevailing mechanisms. The thermodynamic instability of neutral metal hydrides and their spontaneous dissociation into the elements is well known. Surface deposited tin can be converted back to volatile tin hydrides by reactive hydrogen (plasma) species but not by molecular hydrogen. It is expected that the currently used experimental setups will lead to a much better understanding of the chemical behavior of tin and other relevant metal surfaces in the presence of reactive hydrogen species.

### **Please explain why your abstract is innovative for mass spectrometry?**

Detection of ionic and neutral products generated in the interaction of hydrogen RF plasma effluents and group IV compounds and discussion of product formation mechanisms.

### **Co-authors:**

*Hendrik Kersten, University of Wuppertal*

*Thorsten Benter, University of Wuppertal*

Poster number: IM-PA-117

## MOLECULAR DYNAMIC SIMULATION OF ESI DROPLET FRAGMENTATION IN MS VACUUM STAGES

Abstract ID: 527

Presenting author: Leonie Grashoff, Bergische Universität Wuppertal

### Introduction

Recent research has shown that vacuum systems of commercial mass spectrometers (MS) can be penetrated deeply by charged liquid droplets generated by the ionization process in the Electrospray Ionization (ESI). A large fraction of the bare ions detected in the mass analyzer is suspected to originate from those droplets. They must therefore withstand significantly reduced background pressure and potentially strong electric fields present within the inlet- and ion transfer-system. To get a better understanding of the ion generation process in ESI we use classical molecular dynamics (MD) simulations to create a microscopic model of the collision induced internal temperature increase and fragmentation dynamics of liquid droplets at such conditions.

### Methods

Moltemplate is used for particle ensemble set up, while the classical MD simulations are performed with the open source code LAMMPS. Simulated liquid nanodroplets of various composition, with circa 1000 individual molecules, are set up and heated up with a numerical thermostat function with different heating rates. The heating leads to evaporation and fragmentation processes and resulting fragmentation patterns are analyzed with cluster analysis methods and simulated fragment mass spectra are calculated from the MD simulation results. The visualization and simulation of the trajectory analysis is carried out using the program Ovito in combination with specially developed Python programs.

### Preliminary data (results)

It is possible with the available computing resources to perform whole series of individual simulations. The number of observed fragmentation events in a single run is comparably low, since in every simulation run only a single initial droplet is considered. Though this can be bypassed with multiple reproductions of such simulations with randomized initial conditions allowing to acquire a sufficient statistical set of reaction events to infer simulated mass spectra and probabilities for individual evaporation / fragmentation steps. Preliminary simulations show that the chosen approach is feasible and the simulated fragment mass spectra calculated from the simulations match qualitatively with experimental results from ion trap instruments in which the fragmentation of large charged aggregates (most probably charged nanodroplets) captured in the mass analyzer is observed. It is also shown that the observed fragmentation patterns are heavily dependent on the internal droplet temperature and composition. High temperatures lead to higher fragmentation rates and smaller resulting fragments. The variation of the droplet composition shows significantly different droplet stability and the internal structuring of droplets: Water seems to strongly attract charges within the droplets while less polar compounds are found primarily in the surface layer of the droplets. It is planned to further extend the set of performed MD simulations with additional solvent / analyte combinations and different initial droplet sizes. Furthermore we will compile datasets which quantify the probabilities of the most common fragmentation / evaporation reactions from the simulations, which are required for more high level droplet trajectory models.

### Please explain why your abstract is innovative for mass spectrometry?

Systematic MD simulations of heating and fragmentation of charged liquid droplets from ESI under MS vacuum stage conditions

### Co-authors:

Clara Markert, Bergische Universität Wuppertal  
Walter Wißdorf, Bergische Universität Wuppertal  
Thorsten Benter, Bergische Universität Wuppertal

Poster number: IM-PA-118

## AN INVESTIGATION OF FRAGMENTATION MECHANISM ON NIFEDIPINE-TYPE NITRO-CONTAINING MOLECULES BY TANDEM MASS SPECTROMETRY AND ION MOBILITY SPECTROMETRY

Abstract ID: 528

Presenting author: Peiliang Han, Maastricht University

### Introduction

The fragmentation pathways of small molecules upon collision induced dissociation (CID) in QTOF tandem mass spectrometry (MS/MS) is widely applied to identify unknown molecular structures of synthesis byproducts and drug metabolites. Currently, the interpretation of MS/MS spectra for new drug-like molecules relies on the comparison with literature, frequently lacking full evidence. As an example, the even-electron rule, involved in most of literature, of fragmentation on  $[M+H]^+$  limits the explanation on radical fragments. Fragmentation of sodium adducts is even less discussed for structure elucidation studies. To have better understanding on sodium adducts and unusual fragments like radical cation, a set of Nifedipine-like nitro-containing molecules was investigated in detail. The MS/MS data were combined with information from ion mobility spectrometry experiments.

### Methods

All compounds were analyzed with a Synapt HDMS G2Si equipped with an electrospray interface. All commercially available target molecules were injected (continuous flow) at a  $10\mu\text{M}$  concentration. CID studies at a variety of collision energies combined with travelling wave ion mobility spectrometry, complemented with the theoretical calculation and experimental analysis of the collision cross section (CCS) were performed. Optimization of molecular geometry was performed on ORCA 5.1 with B3LYP functional and Def2-TZVP basis set. The optimized structure was applied to IMos software to calculate theoretical CCS value by trajectory method.

### Preliminary data (results)

Fragmentation investigations of both  $[M+H]^+$  and  $[M+Na]^+$  have been performed for Nifedipine, meta-Nifedipine, para-Nifedipine, Nisoldipine and Aranidipine. The fragments generated from  $[M+H]^+$  (mass 347) and  $[M+Na]^+$  (mass 369) of Nifedipine showed a significant difference with other two positional isomers but have similarity with Nisoldipine and Aranidipine, which indicated the significance of ortho-effect by nitro-group. The results of  $[M+H]^+$  indicated the formation of radical cations in agreement with previous research. For sodium adduct, meta and para isomers were stable under low collision energy. At high energy (20eV), the overall intensity of these two isomers were dropped dramatically due to the loss of charge. Nifedipine, Nisoldipine and Aranidipine molecules were easier to generate fragments at low energy and kept the intensity at high energy. Two mechanisms were proposed for this observation. The fragmentation at low energy involved the loss of water molecule (mass 351) and the subsequent radical loss of one acetate ester (mass 292) group. At high energy was supposed to lose NaOH. Deuterium labelled  $d_6$ -nifedipine and  $d_6$ -aranidipine were studied. The mass shifts on fragments resulted from deuterium were consistent proposed molecular structures. CCS values were calculated in order to provide additional evidence on gas-phase fragments. While, current stage, most of calculated CCS value had large deviation with experimental values. More geometries and conformations had to be tested.

### Please explain why your abstract is innovative for mass spectrometry?

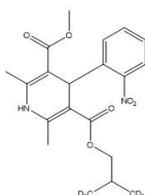
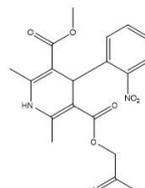
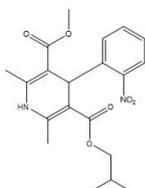
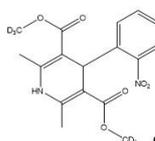
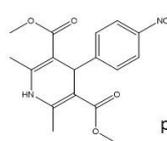
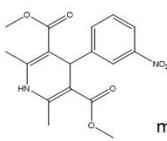
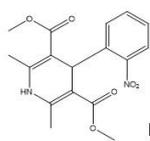
Studies of nifedipine-type molecules provide more insights on unusual fragments. Fragmentation of sodium adducts and assessments of CCS values will strengthen the potential of MS/MS for molecular structure elucidation.

### Co-authors:

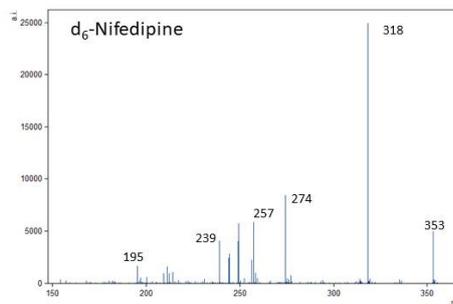
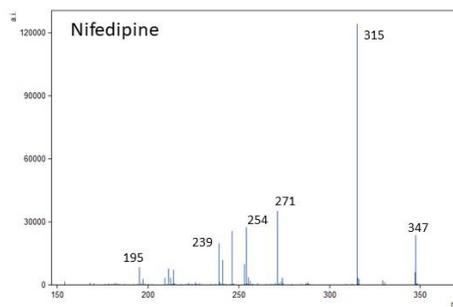
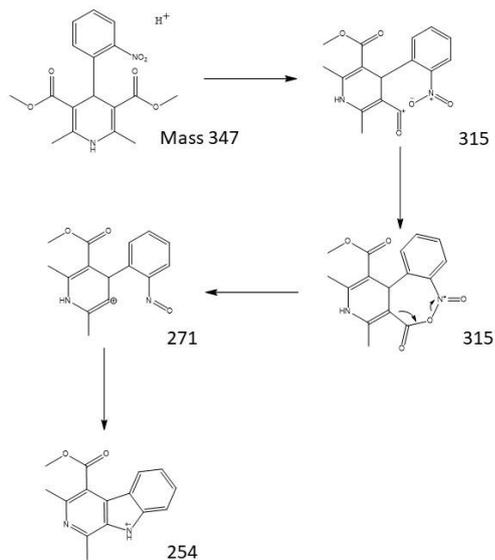
Maarten Honing, Maastricht University

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



MS/MS of [M+H]<sup>+</sup>



Poster number: **IM-PA-119**

## **EFFECTS OF ESI SOURCE PARAMETERS ON CHARGED DROPLETS OBSERVED IN API-MS SYSTEMS**

Abstract ID: **598**

**Presenting author: Laura Lehmann, University of Wuppertal**

### **Introduction**

Electrospray ionization (ESI) is one of the most important ionization methods in mass spectrometry (MS). An analyte solution is sprayed into an electric field, resulting in the formation of highly charged droplets containing the analyte. Experimental observations show that a significant fraction of the charged droplets generated by the ESI source can pass the entrance of the mass spectrometer due to their long lifetimes [1]. The ion source parameters, as well as the mass spectrometer settings, have a significant influence on the electrospray, the formation of the droplets and therefore also on the droplet particle ensemble aspirated into the mass spectrometer. We present the influence of these parameters on the droplets and their dynamics in the high vacuum region of atmospheric pressure MS.

### **Methods**

The experimental studies of the charged droplets are performed with a Bruker Daltonics amaZon ETD Quadrupol Ion Trap(QIT) with an Apollo ion source. Due to the high  $m/z$  ratios of the droplets, an isolation range between 1500 and 2500  $m/z$  was chosen in the QIT and their CID fragment spectra were systematically analyzed. The analytes used for these experiments are para-substituted benzylpyridinium ions (thermometer ions) and reserpine in different solvent systems. The solvents used are 1:1 mixtures of ACN, isopropanol or methanol and water with 0.1% formic acid.

### **Preliminary data (results)**

The operating parameters of the ion source and the accumulation time of the ion trap clearly influence the mass spectra of charged droplets. The effects of the nebulizer gas, dry gas, temperature and liquid flow of the syringe pump were varied systematically to study their effects on the aspirated charged droplets. Additionally, a variation of the accumulation time and the isolated mass range was performed. In all measurements a broad, reproducible double peak structure consistently occurred within the isolation window, often with signals of higher  $m/z$  values than the primarily isolated mass range. This finding is probably because the multiple charged droplets are presumably subject to charge losses which leads to higher  $m/z$ . When the storage time was increased, the abundance of the analyte ions increased and the double-peak signal became broader, which we attribute to fragmentation and evaporation processes of the captured droplets in the ion trap. The ion source parameters influence the release mechanism of the analytes from the droplets, which is indicated by extracted ion count chromatograms, which indicates a changing stability of the trapped droplets. These results are clear evidence of the influence of ion source parameters on charged droplets from ESI aspirated into AP-mass spectrometers.

### **Please explain why your abstract is innovative for mass spectrometry?**

Observation of effects of ion source parameters on charged droplet ensembles from electrospray ionization in a quadrupolar ion trap.

### **Co-authors:**

*Clara Markert, University of Wuppertal*

*Walter Wißdorf, University of Wuppertal*

*Thorsten Benter, University of Wuppertal*

Poster number: IM-PA-120

## IDENTIFICATION OF DRUG METABOLITES WITH INFRARED ION SPECTROSCOPY – APPLICATION TO IN VITRO INCUBATIONS OF MIDAZOLAM

Abstract ID: 655

**Presenting author: Rianne van Outersterp, Radboud University, Institute for Molecules and Materials, FELIX Laboratory**

### Introduction

Understanding the metabolism of a drug compound, including full structure identification of each metabolite, is crucial in the drug discovery and development process. Liquid chromatography-mass spectrometry (LC-MS) is commonly used to detect metabolites in *in vivo* or *in vitro* matrices and metabolic reactions taking place are usually inferred from *m/z*-values of detected metabolites. Resolving the precise reaction site and thus reaction product structure, however, is difficult since isobaric alternatives are often not easily discriminated. Infrared ion spectroscopy (IRIS) can distinguish and identify highly similar isomers, but has been challenging to directly couple with liquid chromatography. We present a novel LC-IRIS approach and demonstrate identification of metabolites resulting from *in vitro* incubation of the drug midazolam.

### Methods

LC-MS and IRIS experiments were performed using a Bruker Elute HPLC system and Bruker AmaZon mass spectrometer coupled to the FELIX free electron laser. The time needed to record an IR spectrum far exceeds the width of a typical chromatographic peak. Therefore, we developed an interface between the chromatography system and ion trap using a switching valve that traps an analyte peak in a sample loop. The sampled peak is slowly infused into the ion trap, elongating the signal for IRIS experiments. Metabolites were identified by comparing the recorded IR spectra to theoretical or experimental reference spectra.

### Preliminary data (results)

We demonstrated LC-IRIS by focusing on the drug midazolam, which undergoes hydroxylation and glucuronation reactions in human metabolism. After incubation of the drug in human hepatocytes, we detected three metabolites, which were recognized (by their *m/z*-value) as midazolam-glucuronide, OH-midazolam and OH-midazolam-glucuronide, but the site of the metabolic transformations could not be determined.

The chromatographic peak widths were approximately 10 seconds while IRIS requires >10 minutes of ion signal. The developed LC-IRIS interface elongated the peaks into >30 minutes of ion signal, which allowed recording an IR spectrum of each metabolite. Based on comparison to computational reference spectra generated using density functional theory, we determined the hydroxylation-site of the OH-midazolam metabolite and the glucuronation-site of the midazolam-glucuronide. This was confirmed by recording experimental spectra of directly infused reference standards.

Reference standards of OH-midazolam-glucuronides were not available. For this compound, we recorded an additional IR spectrum of the glucuronide-loss fragment generated by collision-induced dissociation. This spectrum was identical to the IR spectrum of OH-midazolam, confirming the position of the OH-group in the precursor ion and the metabolic link between the two metabolites. Subsequently, we identified the glucuronation site by comparison to theoretical reference spectra.

The results demonstrate the potential of LC-IRIS in pharmaceutical studies: using LC-IRIS we are able to generate an *in vitro* or *in vivo* drug metabolism scheme with advanced structure identification. This approach has minimal sample requirements compared to NMR spectroscopy and only requires reference standards for final, definitive structural confirmation.

### Please explain why your abstract is innovative for mass spectrometry?

Novel integrated LC-IRIS method for the sensitive and reference-free identification of drug metabolites in drug metabolism studies.

## POSTER SESSION A

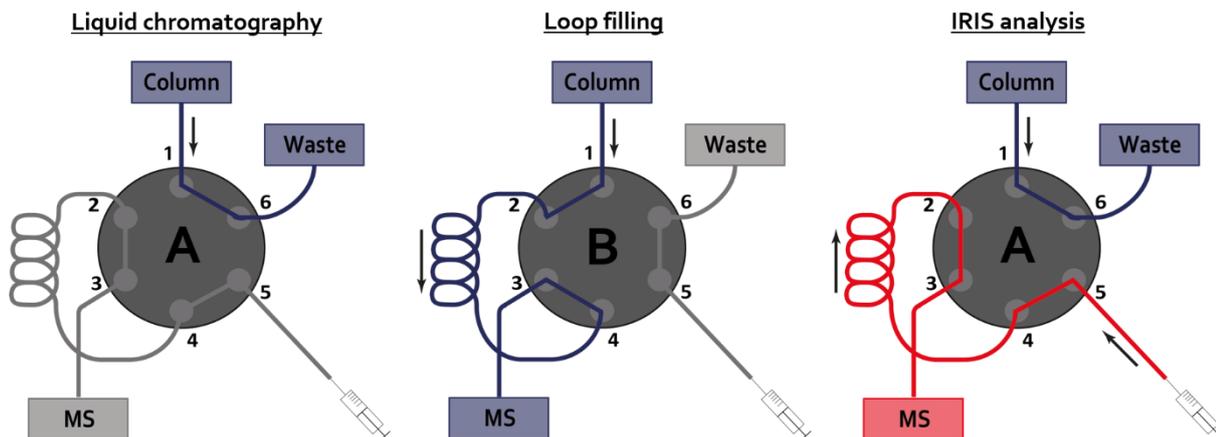
Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

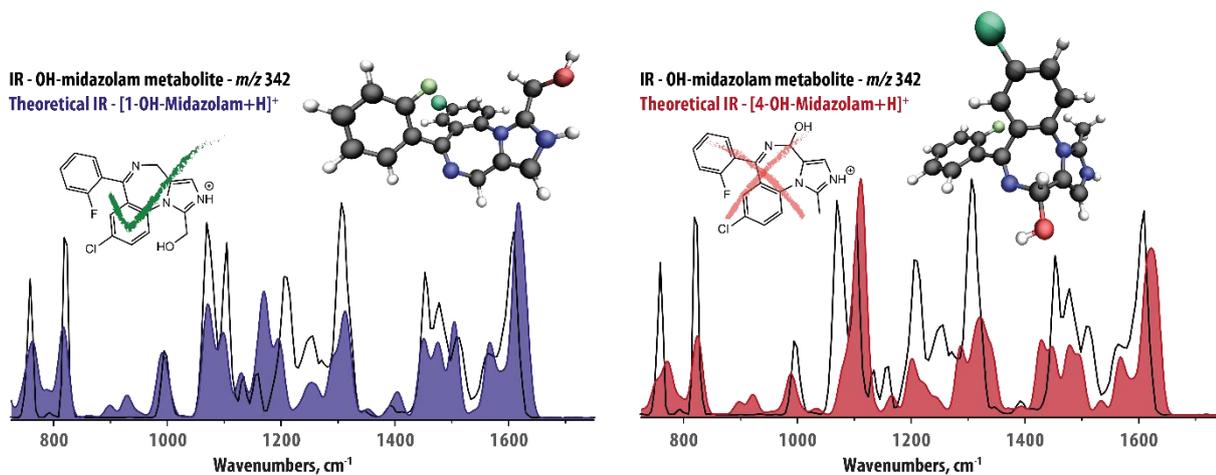
Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Co-authors:

Jitse Oosterhout, Radboud University, Institute for Molecules and Materials, FELIX Laboratory  
Jonathan Martens, Radboud University, Institute for Molecules and Materials, FELIX Laboratory  
Giel Berden, Radboud University, Institute for Molecules and Materials, FELIX Laboratory  
Filip Cuyckens, Drug Metabolism & Pharmacokinetics, Jansen R&D  
Jos Oomens, Radboud University, Institute for Molecules and Materials, FELIX Laboratory



A new LC-IRIS interface elongates ion signals, allowing IRIS analysis.



Drug metabolites are identified by matching to theoretical reference spectra.

Poster number: **IM-PA-121**

## **STRUCTURAL ELUCIDATION IN MALDI MASS SPECTROMETRY IMAGING WITH INFRARED ION SPECTROSCOPY**

Abstract ID: **657**

**Presenting author: Jelle Schuurman, Radboud University - HFML-FELIX**

### **Introduction**

Mass spectrometry imaging (MSI) is a powerful technique used to study the spatial distribution of molecules in samples. An interesting and recent application of MSI is in the study of spatially resolved metabolomics which aims to generate an improved understanding of metabolic diseases. Even though MSI is mass-selective and can be highly sensitive, distinguishing the spatial patterns of isomers and identifying previously unknown  $m/z$ -features remains challenging for this technique. InfraRed Ion Spectroscopy (IRIS) is an increasingly popular mass spectrometry-based method that provides an orthogonal, spectroscopic basis for structural characterization of ions detected in the mass spectrometer. Here we present very recent work which combines MSI and IRIS for spectroscopic characterization of spatially resolved ions.

### **Methods**

Matrix-assisted laser desorption ionization (MALDI) is performed on a FT-ICR MS (7T SolariX, Bruker), connected to the Free-Electron Laser for infrared eXperiments "FELIX" for spectroscopy experiments. We use a mouse model where a group of control mice (wildtype) and knockout mice are compared. Brain tissues were snap frozen after collection and stored at  $-80\text{ }^{\circ}\text{C}$  before being sectioned for MSI analysis. Matrix application is performed according to the current state-of-the-art. Data analysis is done by SCiLS and an in-house developed algorithm for feature identification, where novel features correlating with the knockout group are targeted for spectroscopic characterization using IRIS.

### **Preliminary data (results)**

It is physically impossible to record an entire IR spectrum for every single pixel in the MSI experiment, as IRIS requires one mass spectrum for every frequency point in the IR spectrum. Instead, we implement either an imaging- or a spectroscopy-favoured experiment. The first experiment uses isomer-selective photo-dissociation to distinguish the presence of a particular isomer using known vibrational resonances unique to that isomer. The very short time scale of the laser pulse allows the MSI experiment to continue as it would under common tandem MS conditions. The second experiment aims to measure an IR spectrum by exploring a region of the sample where the  $m/z$  of interest is present rather than considering only that  $m/z$  from a single pixel. Instead of using a fixed, selective frequency, the frequency can be tuned between pixels to generate an IR spectrum of the  $m/z$  of interest over tens or hundreds of pixels. To demonstrate the technique, we apply combined MSI+IRIS to study the metabolic disease pyridoxine dependent epilepsy (PDE), aiming to generate spatially resolved detection of metabolites associated with this disease in a mouse model. Our preliminary MSI data contains several known as well as a handful of unknown metabolites ( $m/z$ -features) that correlate with our knockout group. Using this example, we will highlight how IRIS can be combined with MSI and used to confirm the molecular structures of detected  $m/z$ -features in MSI experiments as well as to elucidate the structures of previously unidentified  $m/z$ -features.

### **Please explain why your abstract is innovative for mass spectrometry?**

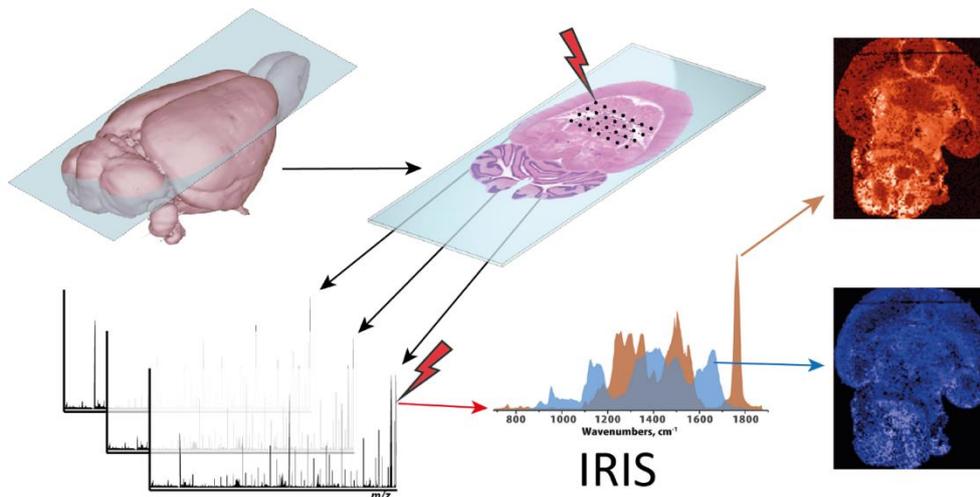
This is the first application of structural molecular identification by IRIS in MALDI-MSI.

### **Co-authors:**

*Pieter Kooijman, Radboud University - HFML-FELIX*  
*Jonathan Martens, Radboud University - HFML-FELIX*  
*Jos Oomens, Radboud University - HFML-FELIX*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Overview of mass spectrometry imaging combined with infrared ion spectroscopy.

Poster number: **IM-PA-122**

## **KINETICS AND THERMODYNAMICS INSIGHTS IN CONFORMATIONAL RELAXATION FROM TRAP AND RELEASE TANDEM-IMS MEASUREMENTS**

Abstract ID: **671**

**Presenting author: Fabien Chirot, Institut Lumière Matière, UMR5306 Université de Lyon, Université Claude Bernard Lyon 1, CNRS**

### **Introduction**

A classical route to the characterization of reaction kinetics and to the characterization of transition states consists in temperature dependent kinetics measurements. The evolution of reaction rates as a function of temperature can then be exploited to derive the relative enthalpy and entropy of the transition state. This procedure was largely exploited in the gas phase, especially to investigate ion fragmentation kinetics in thermalized ion traps. We recently demonstrated that this procedure can also be applied to characterize isomerization processes, based on tandem-IMS measurements, yielding detailed insight in the conformational landscape of the investigated system.

### **Methods**

We used a homemade dual-drift tube instruments in which IMS selected ions can be stored for a controlled duration at a controlled temperature before analysis by IMS-MS. The temperature of the ion trap can be controlled from room temperature to 250°C and processes with half-lives ranging from few milliseconds to several seconds can be investigated.

### **Preliminary data (results)**

Our trap and release procedure was applied to different classes of molecular species, displaying different types of conformational changes, ranging from "simple" cis-trans isomerization to large scale structural reorganization. Figure 1 provides an example of arrival time distributions (ATDs) recorded after trapping the cis form of an azobenzene derivative for different times at 180°C. The Eyring plot derived from such measurements as a function of temperature is shown in Figure 2. We discuss the type of information that can be derived from such measurements as a function of the complexity of the system, of the number of conformational states involved, and on their relative stability. We also compare our results to more classical collision-induced isomerization experiments (often denoted collision induced unfolding, or CIU, in the case of proteins).

### **Please explain why your abstract is innovative for mass spectrometry?**

We demonstrate the versatility of trap and release IMS measurements to study a broad range of conformational changes across a broad range of temperatures.

### **Co-authors:**

*Aurélien Le Fèvre, Institut des Sciences Analytiques, UMR5280 Université de Lyon, Université Claude Bernard Lyon 1, CNRS*

*Benjamin Tassignon, Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry (CISMa), University of Mons*

*Thomas Robert, Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry (CISMa), University of Mons*

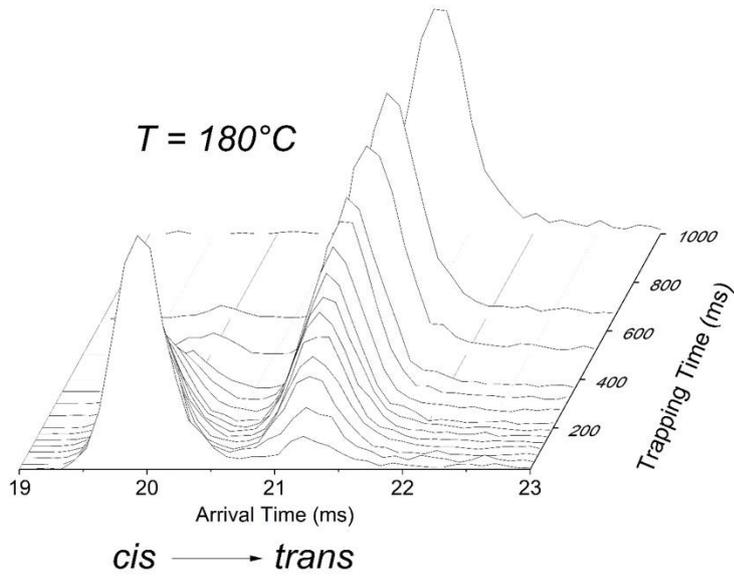
*Clothilde Comby-Zerbino, Institut Lumière Matière, UMR5306 Université de Lyon, Université Claude Bernard Lyon 1, CNRS*

*Julien DeWinter, Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry (CISMa), University of Mons*

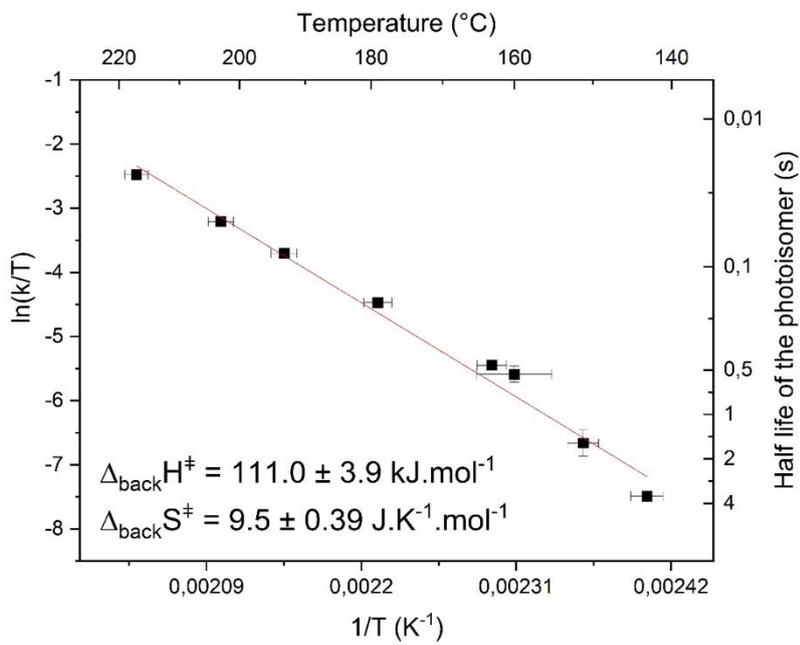
*Pascal Gerbaux, Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry (CISMa), University of Mons*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



ATDs showing the back isomerization of an azobenzene derivative.



Eyring's plot for the back isomerization of an azobenzene derivative.

Poster number: **IM-PA-123**

## STRUCTURAL CHARACTERIATION BASED ON ISOTOPIC SHIFTS IN HIGHRESOLUTION ION MOBILITY/MASS SPECTROMETRY SPACE

Abstract ID: 736

Presenting author: **Alexandre A. Shvartsburg, Wichita State University, Wichita State University**

### Introduction

Since the discovery of isotopes by mass spectrometry, the two fields were intertwined. The omnipresence of isotopes has motivated the technology development in MS hardware and software, enabled novel analyses in diverse areas, and broadly penetrated the MS applications. As most elements have multiple stable isotopes, nearly all compounds are isotopologic mixtures. Their MS envelopes with sufficient resolution tell the exact molecular composition, but nothing about the ion geometry. The isotopic effects in ion mobility spectrometry (IMS), including linear IMS and differential IMS (FAIMS), have surfaced recently with improved instrumental resolving power. As the ion motion in gases (unlike vacuum) depends on the internal mass distribution, the shifts between the isotopologues and isotopomers in IMS space carry structural information and can distinguish isomers.

### Methods

A planar-gap FAIMS unit operating at ambient pressure is coupled to the Thermo LTQ XL ion trap or LTQ Velos/Orbitrap Elite mass spectrometers directly or via a custom electrodynamic ion funnel interface. A bisinusoidal asymmetric waveform has frequency of 1 MHz and peak amplitude up to 6 kV - the highest employed in FAIMS. Binary gas mixtures comprising H<sub>2</sub>, He, N<sub>2</sub>, O<sub>2</sub>, or CO<sub>2</sub> are formulated by digital flowmeters and pumped to the unit at 3 L/min. Shifts of compensation voltage (CV) are measured between the isotopologic peaks selected by MS and integrated for precise CV definition.

### Preliminary data (results)

We started from haloanilines with up to three Cl, Br, or I atoms in varied ring positions and He/CO<sub>2</sub> buffers (with up to 60% He to avoid arc breakdown). The isotopic shifts for both heavy halogens (<sup>37</sup>Cl or <sup>81</sup>Br) and <sup>13</sup>C were substantial, increasing upon He addition as CVs were magnified by the non-Blanc phenomena. While species differed in the halogen position only, the three isomers with one Cl or Br were distinguishable by the <sup>13</sup>C but not halogen shifts. With multiple Cl or Br, both shifts were informative and all six isomers (including those co-eluted in FAIMS) were delineated on a 2-D map. The normally additive shifts allowed multiplexing (e.g., averaging the <sup>13</sup>C shifts in dichloroanilines containing <sup>35</sup>Cl<sub>2</sub>, <sup>35</sup>Cl<sup>37</sup>Cl, or <sup>37</sup>Cl<sub>2</sub>) to reduce the random error. With single I isotope, the <sup>13</sup>C<sub>2</sub> shift in iodoanilines was readily extracted and proved structurally informative.

The approach extends beyond halogenated species, 1+ ions, or <sup>13</sup>C shifts. We disentangled the three phthalic acid isomers as anions by the <sup>13</sup>C and <sup>18</sup>O shifts. New buffers including produce separations apart from those with He/CO<sub>2</sub> mixtures. Such orthogonality across gas compositions expands the dimensionality of shift matrices, greatly augmenting the method specificity. Resolution of most nominally isobaric isotopologues (e.g., the haloanilines with <sup>13</sup>C versus <sup>15</sup>N or phthalic acids with <sup>13</sup>C<sub>2</sub> versus <sup>18</sup>O) by the Orbitrap MS platform adds further shifts for fingerprint structural characterization. The additivity of shifts and ensuing multiplexing capability remain of key fundamental and practical importance.

### Please explain why your abstract is innovative for mass spectrometry?

New high-definition FAIMS/Orbitrap MS platform disentangling nominally isobaric isotopologues and use of novel gas buffers take the isotopic shift approach to molecular structure elucidation to the next level

### Co-authors:

*Pratima Pathak,*

Poster number: IM-PA-124

## OPTIMIZATION OF MATRIX SPRAYING ON OLEDs TO INCREASE THE IONIZATION IN TOF-SIMS FOR TANDEM MS APPLICATIONS.

Abstract ID: 756

Presenting author: Pierre Hirchenhahn, Université Grenoble Alpes, CEA-Léti

### Introduction

ToF-SIMS is an excellent tool for surface chemical characterization, and has been recently adapted to perform tandem MS measurements. Therefore the ionization of heavier fragments becomes crucial, especially when analyzing biological or polymer samples. The use of matrices is one way to increase the ionization, hence the intensity, of the fragments of interest. The applicability of matrices commonly used for MALDI has already been demonstrated as efficient for biological samples, but their use for OLED materials has never been reported. The goal is to optimize the use of matrices for OLED materials in ToF-SIMS measurements, which will help to better understand the ageing of these devices with a view to increasing device lifetime.

### Methods

Alq<sub>3</sub> surfaces and six other typical OLED materials – Ir(mppy)<sub>3</sub>, HATCN, F<sub>4</sub>TCNQ, NPB, STTB and TCTA – were sprayed with four different matrices: α-Cyano-4-hydroxycinnamic acid (CHCA), 2,3- dihydrobenzoic acid (DHB), N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDC), and the solvent used for spraying a mixture of water and acetonitrile 50% v/v. Three different passes of spraying were made: 6 passes, 2 passes, 1 passes and no passes. The surfaces were analyzed in ToF-SIMS using a PHI nanoTOF 2, by acquiring spectra, profiles and large area images.

### Preliminary data (results)

Firstly, the characteristic fragments of the different matrices studied were identified to make sure they do not interfere with the main fragments of the molecules of interest. Then the intensity of the ions of those molecule were thoroughly investigated. It appears that there is an optimum of matrix concentration around two passes of spraying for all the matrices as well as the solvent sprayed samples. The behavior of the intensity of the molecules differs between positive and negative modes. For the molecules that are more easily identified in the positive mode (Alq<sub>3</sub>, Ir(mppy)<sub>3</sub>, NPB, STTB and TCTA), the intensities of their fragments are at the highest when only the solvent is sprayed. For these molecules, no clear pattern of intensities was found in the negative mode. F<sub>4</sub>TCNQ and HATCN are more easily identified in the negative mode. In the same manner, for these two molecules the spraying of the solvent appears as the best solution to obtain higher intensities for the characteristic ions. Looking at the images and the profiles acquired, the surfaces undergo significant changes in topography and crystallinity when sprayed with matrices (see Figure 1). These changes are less visible on the samples sprayed with only the solvent. Therefore, the solvent gives the best results both in terms of ionization enhancement and in terms of minimal sample modification.

### Please explain why your abstract is innovative for mass spectrometry?

The use of MALDI matrices, or just the solvent, can be interesting in some cases to enhance ionization during TOF-SIMS analysis of OLED materials.

### Co-authors:

Claire Guyot, Université Grenoble Alpes, CEA-Léti

Tony Maindron, Université Grenoble Alpes, CEA-Léti

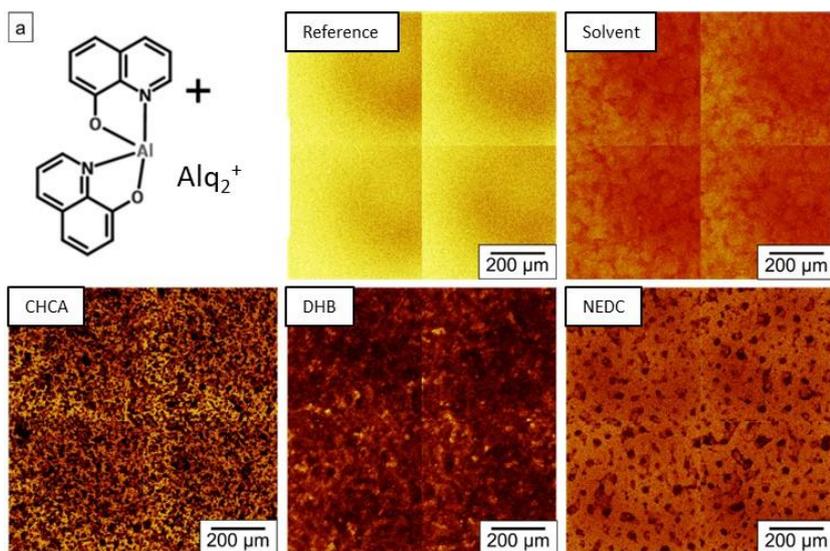
Benoît Gilquin, Université Grenoble Alpes, CEA-Léti

Greg Fisher, Physical Electronics

Jean-Paul Barnes, Université Grenoble Alpes, CEA-Léti

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



(a) Alq<sub>2</sub><sup>+</sup> ion, images of the different matrices sprayed.

Poster number: **IM-PA-125**

## **EFFECT OF DIFFERENT DOPANTS ON THE SENSITIVITY OF ION MOBILITY-MASS SPECTROMETRY TOWARD AUXIN PLANT HORMONES**

Abstract ID: 776

**Presenting author: Vahideh Ilbeigi, Comenius university**

### **Introduction**

Auxins are an important class of plant hormones or phytohormones which play key roles in plant growth and control the form and shape of the plant, root initiation, seed dispersal, fruit growth and development, and induces cell division and cell elongation. Hence, different methods mainly chromatographic techniques have been used for the determination of auxins in different parts of plants. In this work, ion mobility-time of flight mass spectrometry (IMS-TOFMS) is used for the determination of three different auxins, individually and in mixtures. The ionization mechanism and ionization efficiency of auxins in both the positive and negative modes of an atmospheric pressure chemical ionization-corona discharge (APCI) are investigated both experimentally and theoretically by density functional theory (DFT).

### **Methods**

Three auxins including indole-3-acetic acid (IAA), Indole-3-propionic acid (IPA), and Indole-3-butyric acid (IBA) were investigated (Sigma-Aldrich). Standard solutions were prepared in water and studied by direct injection into the IMS-TOFMS. The normal reactant ions in APCI-CD ion source are  $H^+(H_2O)_n$  for positive mode and  $CO_4^-$  and  $O_2^-$  in negative mode. Ammonia, chloroform, and bromoform were used as dopant to produce  $NH_4^+$ ,  $Cl^-$ , and  $Br^-$ , respectively. B3LYP functional was used for the structural optimization and calculations of the thermodynamic data for all ionization reactions. Calculations were performed with Gaussian 09 software.

### **Preliminary data (results)**

Because of the structural difference of the auxins IAA, IPA, and IBA they exhibit different acidity and basicity trend. Hence, their ionization mechanism in APCI-CD is different. Mass spectra showed that IAA with the highest acidity is mainly ionized by deprotonation to form  $[IAA-H]^-$  in the negative mode while IPA and IBA are ionized by  $O_2^-$  attachment and formation of adduct anion  $[M+O_2]^-$ . In the presence of chloroform and bromoform dopants where  $Cl^-$  and  $Br^-$  are the reactant ions, auxins are ionized by halide attachment and formation of  $[M+Cl]^-$  and  $[M+Br]^-$  (Figure 1). In the positive mode, as proton affinities (PA) of all three auxins are higher than PA of  $H_2O$  ( $691 \text{ kJ mol}^{-1}$ ), ionization proceeds via protonation and formation of  $[M+H]^+$ . However, in the presence of  $NH_4^+$  as a reactant ion, IAA is ionized only via ammonium attachment while IPA and IBA form both  $[M+H]^+$  and  $[M+NH_4]^+$  ions. IMS was used for simultaneous measurement of a mixture of three auxins. IM-spectra showed that normal positive mode is the optimum condition for separation of the auxins with minimum peak overlapping. The sensitivity of IMS toward auxins for each ionization pathway was assessed and it was found that  $NH_3$  and chloroform dopants not only simplify the IMS and MS spectra but also enhance the sensitivity. Methylation of auxins improved the sensitivity of the method by about one order.

### **Please explain why your abstract is innovative for mass spectrometry?**

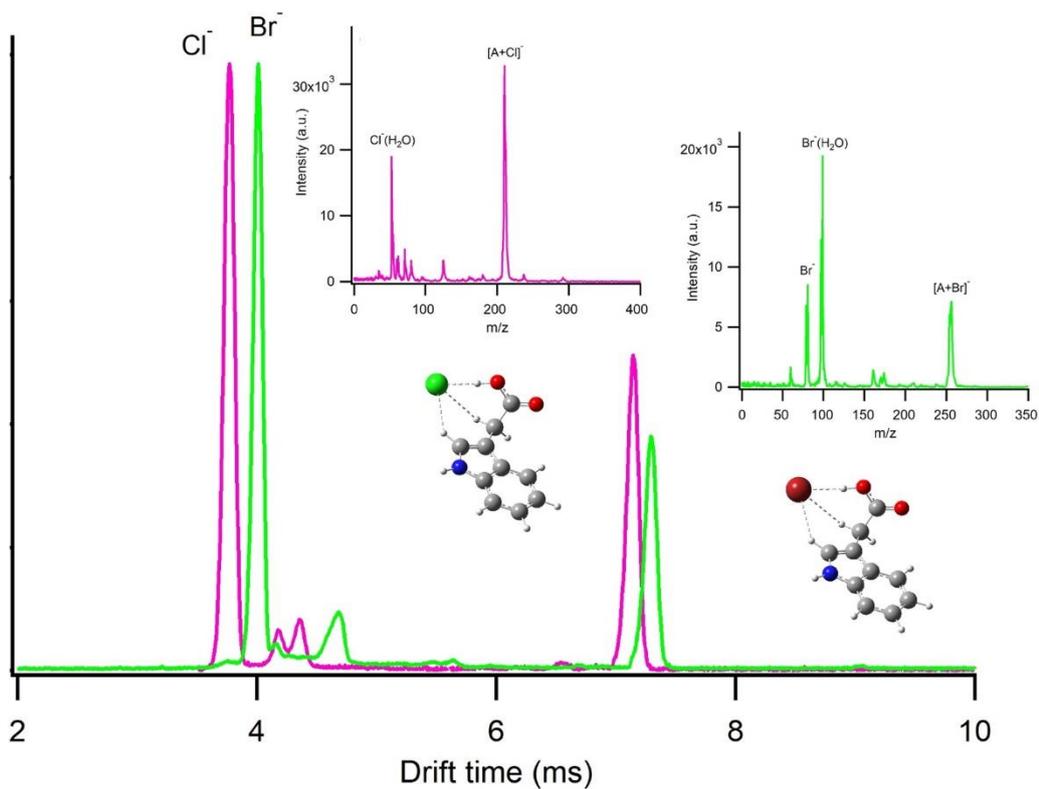
Three auxin plant hormones were measured by IM-TOFMS. Dopants were used to change the ionization mechanism and consequently, the sensitivity.

### **Co-authors:**

*Stefan Matejczik, Comenius university*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Ion mobility and mass spectra of IAA in negative mode

Poster number: **IM-PA-126**

## DETECTION OF THE RARE GALACTOFURANOSE SUGAR IN LICHEN POLYSACCHARIDES USING MS-IR SPECTROSCOPY

Abstract ID: **831**

**Presenting author:** Ozgur Yeni, University of Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière

### Introduction

Carbohydrates characterization is a major challenge of mass spectrometry because of the presence of numerous closely related isomeric structures (monosaccharide content, anomery, regiochemistry and stereochemistry). This complexity calls for the development of dedicated MS approaches and instrumentation.

The ALGAIMS project focuses on a particular type of isomery: the ring size of galactose. Galactose is an abundant monosaccharide essentially found in pyranose form (6- membered ring). Rare species (bacteria, fungi, lichen...) are capable of synthesizing the furanose form (5-membered ring) and its presence seems to correlate with remarkable biological resistance properties. The use of MS-based methods able to detect the galactose ring-size will allow to better understand its biological impact.

The interdisciplinary consortium ALGAIMS brings together carbohydrate chemists, lichen biologists, analytical chemists and molecular physicists.

### Methods

The coupling of mass spectrometry and infrared ion spectroscopy (MS-IR) is particularly promising for the distinction of isomers.<sup>1</sup> The scheme IRMPD (Infrared multiple photon dissociation) is a MS-IR method implemented in the ALGAIMS project in different analytical workflows (MS-MS-IRMPD, LC-MS-IR).

The method was first tested on synthetic models. A database containing their IRMPD fingerprints was created. Then, the methodology has been validated on galactofuranose-containing lichen oligosaccharides.

[1] B. Schindler *et al.*, *Nat Commun*, 2017, **8**, 1–7.

### Preliminary data (results)

Different monosaccharide and disaccharide models which may be found in lichen polysaccharides have been synthesized. Their IRMPD fingerprints in the 3  $\mu\text{m}$  range will be presented.<sup>1,2</sup> A database with obtained IRMPD spectra was created. These first results confirm that IRMPD can resolve the ring-size of galactose. Furthermore, MS-MS-IRMPD data suggest that the ring-size information is conserved upon MS-MS fragmentation. Thus, IRMPD spectroscopy has the potential to detect galactofuranose in larger oligosaccharides.

Our biologist collaborators have picked lichen and extracted their polysaccharides, which were then depolymerized to obtain oligosaccharides. Separative methods were added to the MS-IR workflow to deal with natural compounds complexity. Then, previously developed IR method will be applied on the natural oligosaccharides with a complete analytical workflow LC-MS-MS-IR in order to detect galactofuranose.

Furthermore, computational studies were carried out to interpret acquired IRMPD spectra. The results allow a better understanding of galactofuranose conformational preferences.

[1] J. S. Ho *et al.*, *J. Am. Chem. Soc.*, 2021, **143**, 10509–10513.

[2] B. Favreau *et al.*, *J. Org. Chem.*, 2021, **86**, 6390–6405.

**Please explain why your abstract is innovative for mass spectrometry?**

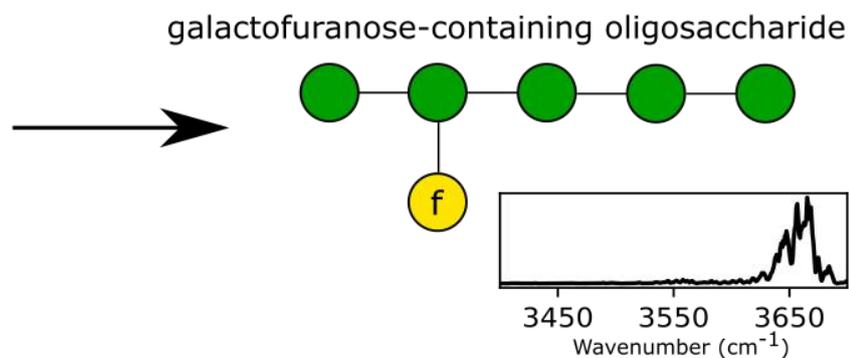
The development of a complete analytical approach to detect galactofuranose in natural lichen samples with the mass spectrometer at the core of the set-up

**Co-authors:**

*Isabelle Compagnon, University of Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière*



Lichen



Detection of galactofuranose in lichen polysaccharide using MS-IR spectroscopy

Poster number: **IM-PA-127**

## INTEGRATION OF A VARIABLE SOFT SPHERE COLLISION MODEL INTO THE IDSIMF ION TRAJECTORY SIMULATION FRAMEWORK

Abstract ID: **852**

**Presenting author: Sanna Benter, University of Wuppertal**

### Introduction

In ion mobility spectrometry (IMS) ions are separated by their characteristic motion through a gas filled drift tube under the influence of a static electric field. Former publications presented the simulation framework IDSimF (Ion Dynamics Simulation Framework), which allows ion trajectory simulations in electric fields with consideration of ion-ion interactions in large particle ensembles. IDSimF currently includes a hard sphere collision model to describe the collisional interaction between charged particles and neutral background gas. This work presents the implementation of a variable soft sphere (VSS) collision model in IDSimF. The results obtained are compared with those calculated from a variable hard sphere (VHS) model and with experimental results.

### Methods

The soft sphere model is implemented into the open-source framework IDSimF (<https://github.com/IPAMS/IDSimF>). IDSimF is implemented in C++ and follows a framework approach inspired by OpenFOAM. As of now IDSimF uses a hard sphere collision model and a statistical diffusion simulation (SDS) model to simulate the interaction with background gas. The modularized library structure of IDSimF allows a straightforward implementation of a variable soft sphere collision model. The model has already been implemented and tested in Python 3 and is currently translated to C++ and embedded in IDSimF.

### Preliminary data (results)

The VSS model is an extension of the VHS model. The VSS model uses the angular-scattering parameter  $\alpha$ , which introduces a softness of the colliding spheres. The interaction distance of a pair of particles within a collision is calculated via  $b = d \cos^{\alpha}(\chi/2)$ . Impact parameters greater than the maximum interaction distance  $b_{max}$  lead to no interaction of the particles. Thus, the maximum interaction distance  $b_{max}$  calculated with the VSS model is not only determined by the variable diameter  $d$ , as it is in the VHS model. Setting the angular-scattering parameter  $\alpha$  to 1, leads to hard sphere collisions and therefore converts the VSS model to a VHS model.

By using the parameter  $\alpha$ , it is possible to describe the deflection angle within a high velocity collision more accurately. Within high velocity collisions, the distance between colliding particles becomes very small, leading to a smaller deflection angle compared to isotropic hard sphere collisions. Such high velocity collisions particularly occur with strong electric acceleration of ions at high field conditions. Preliminary simulations indicate increased accuracy of VSS for IMS simulations at high field conditions without notable numerical costs.

VHS and VSS models are systematically compared at varying pressures, electric fields and temperatures in terms of computation time and comparability to experimental data.

### Please explain why your abstract is innovative for mass spectrometry?

IDSimF allows ion trajectory simulations in electric fields. The implementation of a VSS model allows a significantly more accurate calculation of ion trajectories under high field conditions.

### Co-authors:

*Walter Wissdorf, University of Wuppertal*  
*Hendrik Kersten, University of Wuppertal*  
*Thorsten Benter, University of Wuppertal*

Poster number: **IM-PA-128**

## **IMPLEMENTATION OF A MOLECULAR DYNAMICS COLLISION MODEL FOR DETERMINATION OF ION MOBILITIES IN THE ION DYNAMICS SIMULATION FRAMEWORK (IDSIMF)**

Abstract ID: **855**

**Presenting author: Michelle Rajkovic, University of Wuppertal**

### **Introduction**

Ion-mobility spectrometry (IMS) allows to separate gas phase ions, traveling through an electric field in a gas filled drift tube, based on their individual drift velocities. There are several software packages (e.g. MOBCAL and IMoS) available that are capable of accurately calculating ion mobilities for molecules in a vanishing electric field. However, in none of those frameworks the electric field is variable. The simulation framework IDSimF offers an IMS application to simulate ion trajectories with background gas interactions in arbitrary electric fields. Currently only hard-sphere or statistical diffusion collision models are implemented, which cannot accurately predict ion mobilities at low pressure and high fields. Therefore this work aims to implement a molecular dynamics based approach to model collisions similar to ideas found in other frameworks.

### **Methods**

IDSimF (<https://github.com/IPAMS/IDSimF>) is an open-source framework implemented in C++ and particularly influenced by the open source fluid dynamics framework OpenFOAM. The framework IDSimF currently contains several self-contained applications for ion trajectory simulations in e.g. ion mobility devices or quadrupole collisions cells. The additional molecular dynamics collision model, which can easily be used in several applications given by the modular structure of the framework, was integrated into IDSimF. Currently the newly implemented collision model is being tested in IMS applications, especially for low pressure (20 mbar) and higher field strength conditions (10 to 70 Td).

### **Preliminary data (results)**

The molecular dynamics (MD) collision model is based on a trajectory method, in which both the ion and the background gas are modeled explicitly as a rigid body. For this, the collision model uses a secondary trajectory integrator (either Leapfrog or Runge-Kutta-Fehlberg) embedded into the overarching ion trajectory simulation, where the gas particle is not calculated explicitly. The idea is to solve the Newton equation of motion, where the acceleration experienced by both particles results from the force acting on each other. Thereby, the force field is given by a combination of the 12-6 Lennard Jones potential, to model the close range repulsion between a pair of non-bonding particles, and an ion-induced dipole moment potential, representing long-range attraction.

First studies of the MD model show a better agreement with experimentally obtained ion mobilities for small ions and high electric fields compared to e.g. the hard-sphere model. While divergence between simulation and experiment is observable for lower field strengths in the range of 10 to 20 Td, the so far obtained results confirm that the MD model is able to describe the collision behavior inside IMS significantly better than simple hard sphere collisions.

### **Please explain why your abstract is innovative for mass spectrometry?**

Implementation of a molecular dynamics based collision model into IDSimF which accurately describes ion mobilities, particularly at high electric fields.

### **Co-authors:**

*Walter Wissdorf, University of Wuppertal*

*Hendrik Kersten, University of Wuppertal*

*Thorsten Benter, University of Wuppertal*

Poster number: IM-PA-129

## FORMATION AND STABILITY OF HOST-GUEST COMPLEXES BETWEEN CYCLOPARAPHENYLENES AND CROWN ETHERS

Abstract ID: 905

**Presenting author:** Lei Ye, Physical Chemistry I, Department of Chemistry and Pharmacy, Friedrich-Alexander Universität Erlangen-Nürnberg

### Introduction

Cycloparaphenylenes (CPPs) are strained ring molecules with only  $sp^2$ -hybridized carbon atoms. They have been widely reported as ideal supramolecular hosts for fullerenes<sup>[1, 2]</sup>, CPPs<sup>[1, 2]</sup> and polycyclic aromatic hydrocarbons (PAHs)<sup>[3]</sup>.

### Methods

In this study, host-guest complexes of crown ethers and their benzannulated derivatives with [n]CPPs ( $n = 8-12$ ) as host were investigated by electrospray mass spectrometry.

### Preliminary data (results)

The mass spectra show that host-guest complexes between CPPs and crown ethers are only formed when the diameter difference between host and guest is in the range of 4.3-7.1 Å. Energy-dependent collision experiments reveal that 15-crown-5 and 18-crown-6 form the most stable complexes with [10]- and [11]CPP, respectively. These complexes are characterized by a diameter difference between host and guest of roughly 5.8 Å. Complexes with benzannulated crown ethers as guests were considerably less stable. These larger crown ethers weaken the CH... $\pi$  interactions between host and guest due to a size mismatch. Our results suggest that, crown ethers are a novel promising class of guest molecules for CPPs, featuring noncovalent bonding based on CH... $\pi$  interactions.

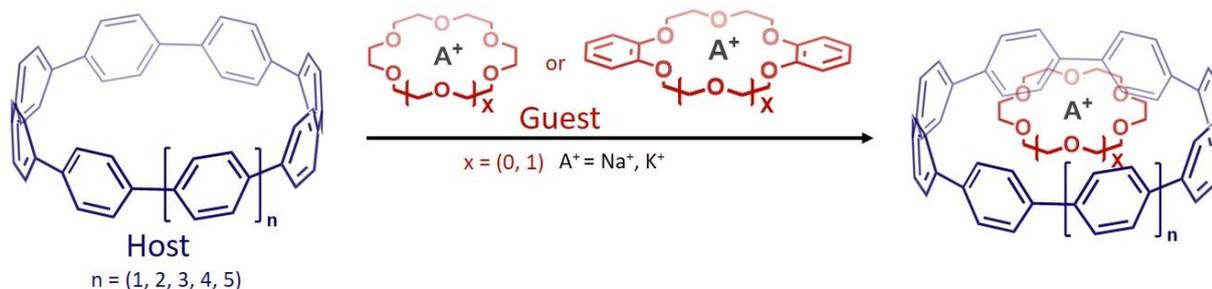
### Please explain why your abstract is innovative for mass spectrometry?

In conclusion, energy dispersive mass spectrometry is a great tool for studying non-covalent supramolecular aggregates.

### Co-authors:

Markus Freiberger, Physical Chemistry I, Department of Chemistry and Pharmacy, Friedrich-Alexander Universität Erlangen-Nürnberg

Thomas Drewello, Physical Chemistry I, Department of Chemistry and Pharmacy, Friedrich-Alexander Universität Erlangen-Nürnberg



### POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

#### References:

- [1] Xu Y., von Delius M., *Angewandte Chemie International Edition*, **2020**, 59(2), 559-573.
- [2] Minameyer MB., Xu Y., Frühwald S., Görling A., von Delius M., Drewello T., *Chemistry–A European Journal*, **2020**, 26(40), 8729-8741.
- [3] Kwon H., Carson B., *Nano Research*, **2022**, 1-11.

Poster number: **IM-PA-130**

## **COLLISION CROSS SECTIONS OF POLYOXOMETALATE ANIONS AND DETERMINATION OF LENNARD-JONES INTERACTION PARAMETERS OF MO AND W IN HE AND N<sub>2</sub>**

Abstract ID: **938**

**Presenting author: H el ene Lavanant, Normandie Univ, UNIROUEN, INSA Rouen, CNRS, COBRA**

### **Introduction**

Polyoxometalates (POM) are a class of oxo-clusters with diverse properties that form compact and negatively charged structures constituted of multiple polyhedra {MO<sub>x</sub>} sharing O atoms (M is Mo<sup>VI</sup> or W<sup>VI</sup>).

Ion mobility values and collision cross sections (CCS) were determined in helium and nitrogen for anions and clusters derived from the four typical structural motifs of POM, Lindqvist (or hexametalates), decametalates, Keggin and Dawson ions comprising either Mo<sup>VI</sup> or W<sup>VI</sup> atoms (Figure 1). To compare with theoretically derived CCS values, we used Lindqvist and Keggin ions to carry out collision cross section calculations with the trajectory method and sought Lennard Jones parameters for Mo<sup>VI</sup> or W<sup>VI</sup> interactions with He and N<sub>2</sub> adapted to our experimental values.

### **Methods**

Drift tube ion mobility mass spectrometry (DTIMS-MS) was performed with an Agilent 6560 IMS QTOF using helium (He) or nitrogen (N<sub>2</sub>) as buffer gas. Several sets of parameters were used to probe the influence of different experimental conditions on ion mobility and CCS determination.

Crystal structures from the Cambridge Crystallographic Data Centre (CCDC) were used in density functional theory (DFT) calculations with Gaussian09. The dispersion-corrected range-separated hybrid  $\omega$ B97XD exchange-correlation functional and 6-31G(d) basis set were used and atomic partial charges fitted to the electrostatic static potential were collected. Cross section calculations were carried out using MOBICAL and IMOS 1.10.

### **Preliminary data (results)**

The collision cross sections of bare Lindqvist and Keggin polyoxometalates anions with Mo and W atoms were determined and found to be reproducible with different experimental settings before and after the ion mobility cell.

As the geometry of the ion, more than its mass, determines the collision cross section with a given drift gas molecule, we found both Lindqvist ions Mo<sub>6</sub>O<sub>19</sub><sup>2-</sup> and W<sub>6</sub>O<sub>19</sub><sup>2-</sup> had <sup>DT</sup>CCS<sub>He</sub> values of 103 ± 2 Å<sup>2</sup>, and both Keggin ions PMo<sub>12</sub>O<sub>40</sub><sup>3-</sup> and PW<sub>12</sub>O<sub>40</sub><sup>3-</sup> had a <sup>DT</sup>CCS<sub>He</sub> values of 170 ± 2 Å<sup>2</sup>. Similarly, ion mobility experiments in N<sub>2</sub> led to <sup>DT</sup>CCS<sub>N<sub>2</sub></sub> values of 223 ± 2 Å<sup>2</sup> and 339 ± 4 Å<sup>2</sup> for Lindqvist and Keggin anions, respectively. The fact that POMs of similar structures and very different masses exhibited similar CCS values both in He and N<sub>2</sub>, although expected, is uncommon in ion mobility experiments. The existence of such ions of different masses and well-defined rigid structures could constitute attractive calibrants or model systems.

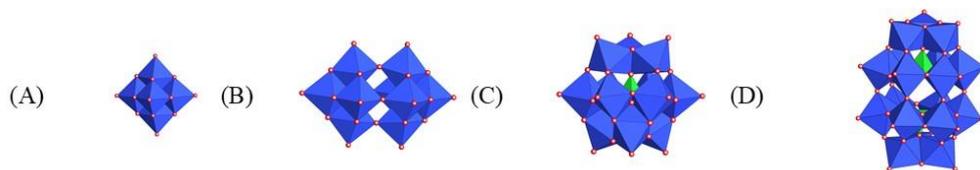
Using the optimized structures and partial charges determined from density functional theory calculations, followed by CCS calculations with the trajectory method using MOBICAL and IMOS 1.10 with the LJ optimizer, we determined Lennard Jones 6-12 potential parameters  $\epsilon$ ,  $\sigma$  of 5.60 meV, 3.50 Å and 3.75 meV, 4.40 Å for both Mo and W atoms interacting with He and N<sub>2</sub>, respectively. These parameters reproduced the CCS of polyoxometalates within 2% accuracy.

### **Please explain why your abstract is innovative for mass spectrometry?**

Molecular modelling completes the experimental determination with drift tube ion mobility mass spectrometry of the collision cross sections of polyoxometalates : highly compact and negatively charged rigid structures.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



(a) Lindqvist, (b) Decatungstate, (c) Keggin, (d) Dawson POM

Poster number: **IM-PA-131**

## **GAS-PHASE FRAGMENTATION OF METALLO-SUPRAMOLECULAR AGGREGATES**

Abstract ID: **966**

**Presenting author: Marianne Engeser, University of Bonn**

### **Introduction**

Self-assembly has led to numerous fascinating supramolecular structures and functional architectures based on an impressive variety of subcomponents. One way to design self-assembly processes uses the metallo-supramolecular approach based on well-chosen organic ligands interconnecting two or more metal centers. There are impressive examples in literature of highly selective self-assembly processes of multicomponent mixtures even leading to complex heterometallic assemblies. Mass spectrometry has become an indispensable tool to determine the stoichiometry of self-assembled aggregates. This task however is often very challenging as very soft ionization conditions are needed to avoid in-source fragmentation, although analysis of the detected fragments can also confirm the structure of the aggregate in fortunate cases.

### **Methods**

Accurate mass spectra were recorded with a Bruker Apex IV FT-ICR, a Bruker micrOTOF-Q Q/TOF instrument, or an Orbitrap XL mass spectrometer. Electrospray ion source parameters were individually tuned to soft ionization conditions to avoid in-source fragmentation for best ion abundance of the non-covalently bound aggregates. A series of ions in different charge states was obtained in each case by stripping off weakly coordinating counter ions. Ions or fragments in different charge states were mass-selected and subjected to CID.

### **Preliminary data (results)**

It is tempting to use induced fragmentation in the gas phase to deduce the structure of metallo-supramolecular aggregates in analogy to the well-established routes of structure elucidation of covalent compounds by mass spectrometry. However, the combination of rather weak non-covalent bonds that form the aggregate and strong Coulomb interactions between the charged metal centers and between metals and counter anions can result in unforeseen fragmentation pathways and rearrangements that easily can be misleading in structure determination. Thus, a much broader experimental basis is currently needed to better understand the gas-phase fragmentation patterns of metallo-supramolecular aggregates and establish fragmentation rules that are useful for structure elucidation of metallo-supramolecular aggregates by mass spectrometric means.

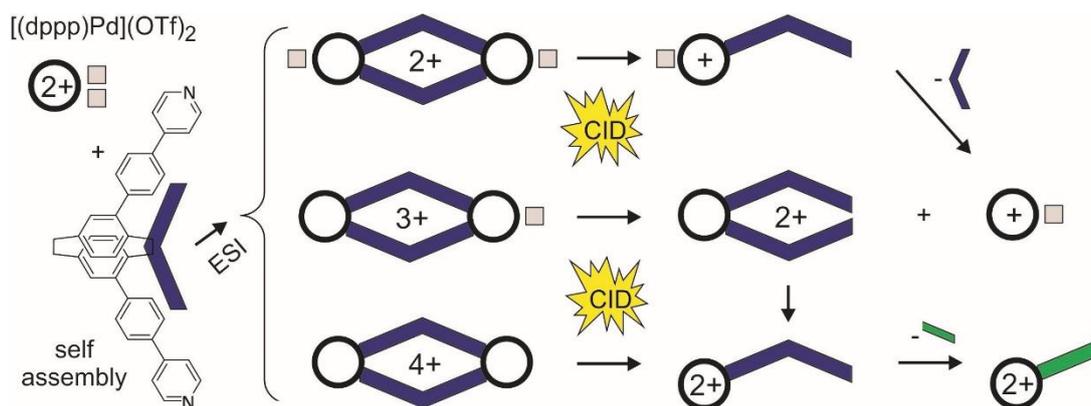
The poster presents results from gas-phase fragmentations of different metallo-supramolecular aggregates including squares of the classic Stang-type and heterobimetallic ones that exhibit much more complex fragmentation patterns which strongly depend on the ligand length and thus aggregate size. Metallo-supramolecular rhombs formed out of six metal centers (2x Pt/Pd, 4x Au) showed a very general and simple fragmentation scheme, whereas rhombs and helicates formed from chiral ligands based on a pseudo-meta-difunctionalized [2.2]paracyclophane skeleton exhibited a more peculiar fragmentation behavior which even includes rupture of the ligand backbone while non-covalent bonds stay intact.

### **Please explain why your abstract is innovative for mass spectrometry?**

Gas-phase fragmentation patterns of metallo-supramolecular aggregates are elucidated by tandem mass spectrometry.

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Poster number: IM-PA-132

## MASS SPECTROMETRY AS AN ALTERNATIVE TO NMR SPECTROSCOPY FOR SOLUTION STUDIES

Abstract ID: 995

Presenting author: Quentin Duez, Radboud University

### Introduction

In the recent years, mass spectrometry provided invaluable information on the structure and reactivity of isolated supramolecular assemblies. Especially, the coupling of ion mobility to mass spectrometry enabled the monitoring of isomerization reactions such as threading of (pseudo-)rotaxanes, or progressive guest ingestion in a host cavity. However, the difficulty to correlate the abundance of ions in gas phase with their concentration in solution prevents quantitative measurements such as determination of association constants. The *delayed reactant labelling* (DRL) method has been developed to bridge the gap between solution- and gas phase and thereby discard artifacts related to ESI processes. Here, we demonstrate that DRL can quantitatively assess the solution equilibria in a mixture of pyridine and macrocyclic porphyrin cage compounds (**Figure 1**).

### Methods

ESI-MS experiments were performed with a timsToF instrument (Bruker, Germany). A stirring solution of porphyrin cage complex with 1.4 equiv. pyridine was continuously infused in the mass spectrometer, leading to the detection of  $[M(III)C + Pyridine]^+$ . After one minute of delay, 1.4 equiv. pyridine-D5 is injected in the solution. For species in steady-state, the fitting the decrease of  $[M(III)C + Pyridine]^+$  relative to  $[M(III)C + Pyridine-D5]^+$  yields the host-guest dissociation rate constant  $k_{-1}$  (**Figure 2**).

### Preliminary data (results)

First, the  $k_{-1}$  determined by DRL for the dissociation of adducts between the macrocyclic porphyrin cage with a Co(III) centre and a pyridine ligand were benchmarked against selective exchange NMR spectroscopy (EXSY). Rate constants agreed well between DRL and NMR, suggesting that DRL represents accurately the dissociation of adducts between porphyrin cages and guests in solution.

Based on these results, we carried out DRL experiments at various temperatures and used the measured rate constants to build Eyring plots, enabling the determination of thermodynamic parameters for the dissociation of cage-pyridine adducts. A positive activation entropy  $\Delta S^\ddagger$  was obtained, implying a dissociative mechanism for pyridine exchange. Since a chloride counterion is coordinated to the metal in the CoC-Cl porphyrin cage, we investigated the effect of a non-coordinating counterion, such as  $PF_6^-$ , on the activation parameters. In this case, DRL experiments performed on CoC- $PF_6$  revealed a negative activation entropy, indicating that the guest exchange mechanism is strongly affected by the presence of a coordinating counterion.

Finally, the DRL approach was employed to probe the dissociation kinetics of porphyrin cages with a paramagnetic Mn(III) centre, MnC-Cl, for which NMR data are difficult to interpret. Dissociation kinetics were too fast to be accurately determined by our approach, but revealed nonetheless that the binding of pyridine ligands to the Mn(III) centre is weaker than for Co(III).

### Please explain why your abstract is innovative for mass spectrometry?

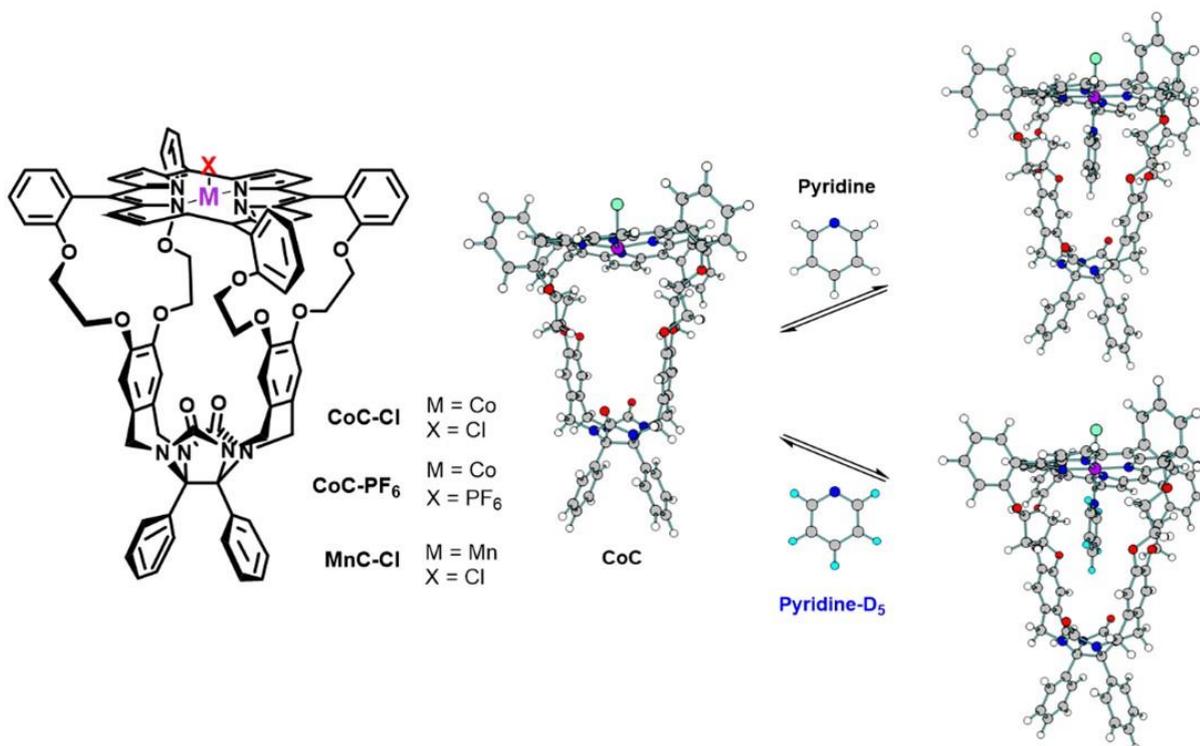
Rate constants were determined for the dissociation of a pyridine ligand from a porphyrin cage from mass spectrometry experiments, and enabled to gather insights on ligand exchange in solution.

### Co-authors:

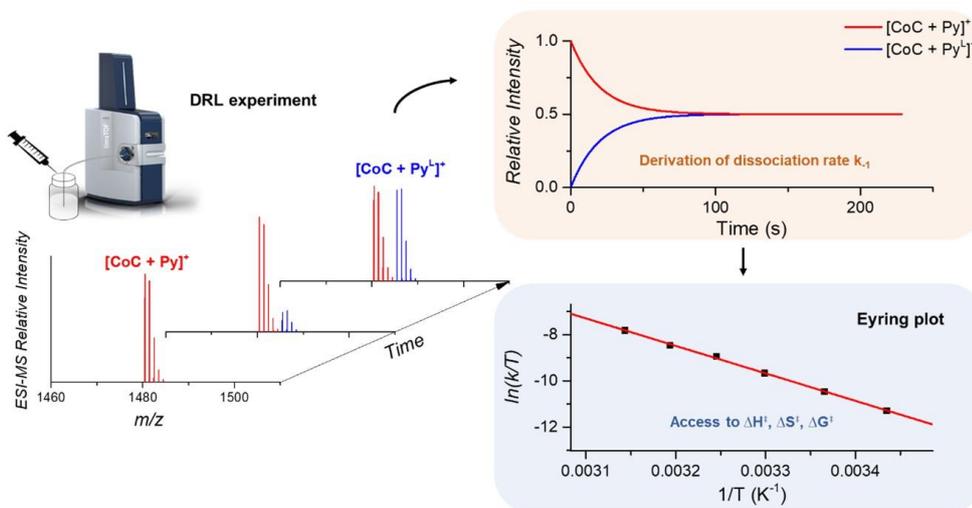
Jana Roithová, Radboud University

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
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 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Equilibria investigated in this work.



Workflow proposed to assess dissociation parameters for cage-pyridine adducts.

## Session: Ionization technologies

Poster number: **IM-PA-133**

### **APCI, APPI, APLI, AND LTP: UNCOMMON IONISATION METHODS FOR GC-MS**

Abstract ID: **39**

**Presenting author: Oliver J. Schmitz, Applied Analytical Chemistry, University of Duisburg-Essen**

#### **Introduction**

Although the coupling of GC/MS with atmospheric pressure ionisation (API) was reported as early as the 1970s, interest in coupling GC with atmospheric pressure ion sources has increased in the last decade. The requirement for a "soft" ion source to obtain highly diagnostic molecular ions is desirable, in contrast to "hard" ionisation techniques such as electron ionisation (EI) in conventional GC/MS, which fragments the molecule to a high degree.

#### **Methods**

Here, the ion sources we have developed for atmospheric pressure chemical ionisation (APCI), atmospheric pressure photoionisation (APPI), atmospheric pressure laser ionisation (APLI) and low-temperature plasma (LTP) for coupling with GC-MS are presented, compared with each other and the advantages and disadvantages of these analytical platforms are carefully discussed.

#### **Preliminary data (results)**

A closed APCI ion source leads to a better repeatability in comparison with an open APCI ion source.

APLI is the most sensitive ion source for the analysis of aromatic compounds such as PAHs, and GC-APLI-MS shows a more as 1000-fold better sensitivity as GC-EI-MS. The derivatization of non-aromatic compounds with an aromatic ionization marker increases the field of applications.

LTP allows the ionization of a wide range of compounds and highly electronegative compounds are preferably ionized by charge exchange reactions while the presence of  $-\text{NO}_2$ ,  $-\text{OH}$  and  $-\text{CO}$  groups enhance the formation of the protonated molecule.

#### **Please explain why your abstract is innovative for mass spectrometry?**

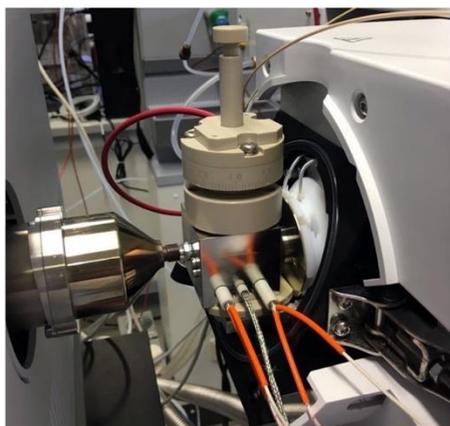
GC-LTP allows the ionization of a wider range of compounds than other API sources and GC-APLI shows an outstanding sensitivity and selectivity for PAHs.

#### **Co-authors:**

*Juan F. Ayala Cabrera, Applied Analytical Chemistry, University of Duisburg-Essen*

**POSTER SESSION A**

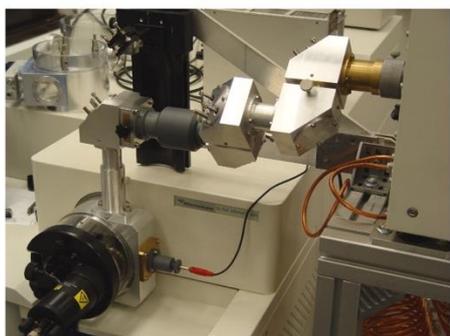
Monday 29 August 2022 from 11:30 to 13:00 hours  
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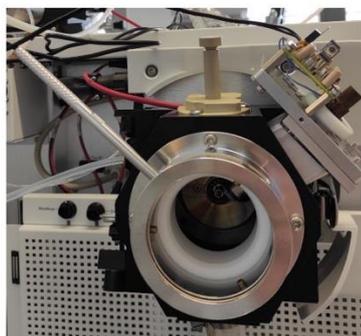
← APCI



LTP →



← APLI



APPI →

Various atmospheric-pressure ionization sources: APCI, APPI, APLI, and LTP

Poster number: **IM-PA-134**

## STRUCTURAL CHARACTERIZATION OF SUBSTANCE P USING GOLD NANOPARTICLES IN SALDI “IN-SOURCE DECAY” MASS SPECTROMETRY

Abstract ID: **118**Presenting author: **Gauthier Eppe, Mass Spectrometry Laboratory-ULiège**

### Introduction

Laser desorption/ionization mass spectrometry techniques (including MALDI and surface-assisted laser desorption/ionization, SALDI) are routinely used in analytical methods for the study of all kinds of biomolecules. In these techniques, the choice of the assisting material has now become vast, ranging from a large variety of organic matrices to nanoparticles (NPs) and other solid nanosubstrates. The selection of the assisting material appears not straightforward and the capabilities of novel assisting materials for the desorption/ionization for various (bio)molecules are poorly documented. This is typically the case of the SALDI nanosubstrates for which the potential for in-source fragmentation capabilities is seldom studied.

### Methods

In this work, gold nanoparticles (AuNPs) were used as assisting materials in SALDI FT-ICR mass spectrometry experiment. The “hardness” in terms of in-source decay (ISD) of the AuNPs was first evaluated based on the survival yield (SY) method, and compared to MALDI matrices. The SY of the AuNPs and the MALDI matrices were determined from the intensities of the parent and fragment benzylopyridinium (BP) thermometer ions:

$$SY = I_{\text{parent}} / (I_{\text{parent}} + I_{\text{fragment}})$$

### Preliminary data (results)

“Hard” assisting materials are characterized by a low SY (*i.e.* a high fragmentation yield), and therefore by a high energy transfer to the thermometer ions. In this study, the AuNPs were classified as “hard” assisting materials, able to transfer a high amount of energy to the analytes, inducing their fragmentation. Moreover, the SALDI mass spectra were characterized by a clean background, enabling matrix-free interference in the low *m/z* region. This property is especially useful for easily detecting the ISD fragments in this range, giving directly access to the sequencing of both extremities of any peptide. AuNPs were successfully tested in SALDI in-source decay (ISD) experiments using a dual ion source MALDI/ESI FT-ICR 9.4T Solarix XR mass spectrometer, in the positive ionization mode, for the structural characterization of the substance P peptide. Several types of ISD fragments (*a*-, *b*-, *c*-, *x*-, *y*- and *z*-ion series), originating from two main precursor ions (*i.e.* either  $[M + Na]^+$  or  $[M - H + Na]^+$ ), were detected in the SALDI mass spectra of the substance P, offering a great peptide sequencing coverage.

This study encourages to test other SALDI nanosubstrates in structural studies but also to undertake further fundamental investigations to understand not only the desorption and ionization processes but also the fragmentation mechanisms occurring in SALDI-ISD-MS.

### Please explain why your abstract is innovative for mass spectrometry?

Surface assisted laser desorption ionization - in source decay using gold nanoparticles as a tool for structural characterization and sequencing coverage of peptides

### Co-authors:

Wendy H Müller, Mass Spectrometry Laboratory-ULiège

Johann Far, Mass Spectrometry Laboratory-ULiège

Loïc Quinton, Mass Spectrometry Laboratory-ULiège

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Cedric Malherbe, Mass Spectrometry Laboratory-ULiège

Poster number: IM-PA-135

## FRAGMENTATION OF PRODUCT IONS IN HIGH KINETIC ENERGY ION MOBILITY SPECTROMETRY

Abstract ID: 201

**Presenting author: Christoph Schaefer, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology, 30167 Hannover, Germany**

### Introduction

In contrast to classical IMS that operate at atmospheric pressure (~1000 mbar), High Kinetic Energy Ion Mobility Spectrometers (HiKE-IMS) operate at reduced pressures of 10 – 60 mbar allowing for high reduced electric field strengths  $E/N$  of up to 120 Td, similar to Proton Transfer Reaction - Mass Spectrometers (PTR-MS). HiKE-IMS usually use corona discharge ionization sources providing the reactant ions  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ,  $\text{NO}^+(\text{H}_2\text{O})_m$ , and  $\text{O}_2^{++}(\text{H}_2\text{O})_p$  to ionize analytes in a reaction region before separating the ions by their ion mobility in a drift region. At high  $E/N$  prevailing in HiKE-IMS, the reactant ions reach high kinetic energies causing collision-induced cluster dissociation of hydrates formed at low  $E/N$ .

### Methods

In this work, we investigate the fragmentation of several alcohols and ketones in HiKE-IMS coupled with a time-of-flight mass spectrometer (TOF-MS). In contrast to PTR-MS, the reactant ions in HiKE-IMS are usually present simultaneously. Thus, product ion formation differs from that in classical IMS and PTR-MS.

### Preliminary data (results)

While hydrates of the hydronium ions  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$  are the dominant reactant ions in classical IMS, the bare  $\text{H}_3\text{O}^+$  ions are present at high  $E/N$  in HiKE-IMS, allowing for ionizing nonpolar substances with low proton affinity such as benzene. Furthermore, the operation at high  $E/N$  prevents the conversion of  $\text{NO}^+(\text{H}_2\text{O})_m$  and  $\text{O}_2^{++}(\text{H}_2\text{O})_p$  to  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$  allowing for ionizing analytes via new reaction pathways. In particular, ionization via charge transfer with  $\text{O}_2^{++}$  can lead to chemically induced fragmentation of some analytes due to the comparatively high ionization energy of  $\text{O}_2^{++}$ . In addition, the high kinetic energies of the ions at high  $E/N$  may lead to field-induced fragmentation. On the one hand, this fragmentation can complicate the interpretation of ion mobility spectra due to the formation of additional product ions. On the other hand, a unique fragmentation pattern can be used to reduce false positives. The results show that charge transfer with  $\text{O}_2^{++}$  and even proton transfer with  $\text{H}_3\text{O}^+$  can lead to fragmentation of the product ions. Beyond this chemically induced fragmentation, the investigated analytes also show field-induced fragmentation within the drift region. The ions form class-specific fragments, with the total number of formed fragments increasing with increasing ion size and branching.

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 318063177 and 390583968.

### Please explain why your abstract is innovative for mass spectrometry?

In contrast to classical IMS, analytes can fragment in HiKE-IMS due to the high  $E/N$ . The fragmentation of alcohols and ketones in HiKE-IMS-MS is investigated for the first time.

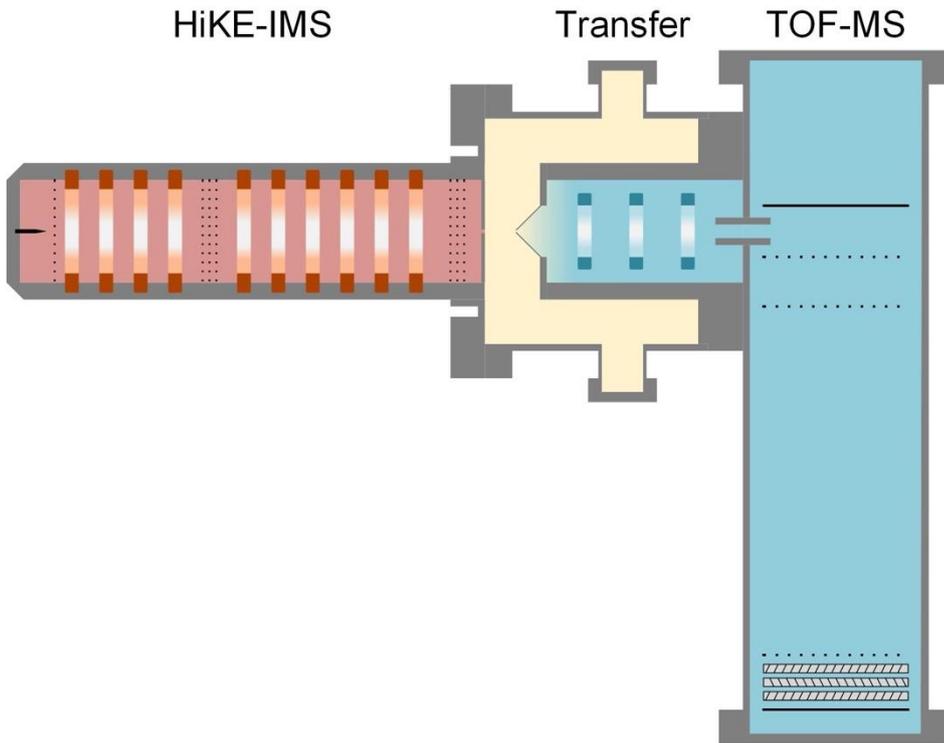
### Co-authors:

*Florian Schlottmann, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology, 30167 Hannover, Germany*

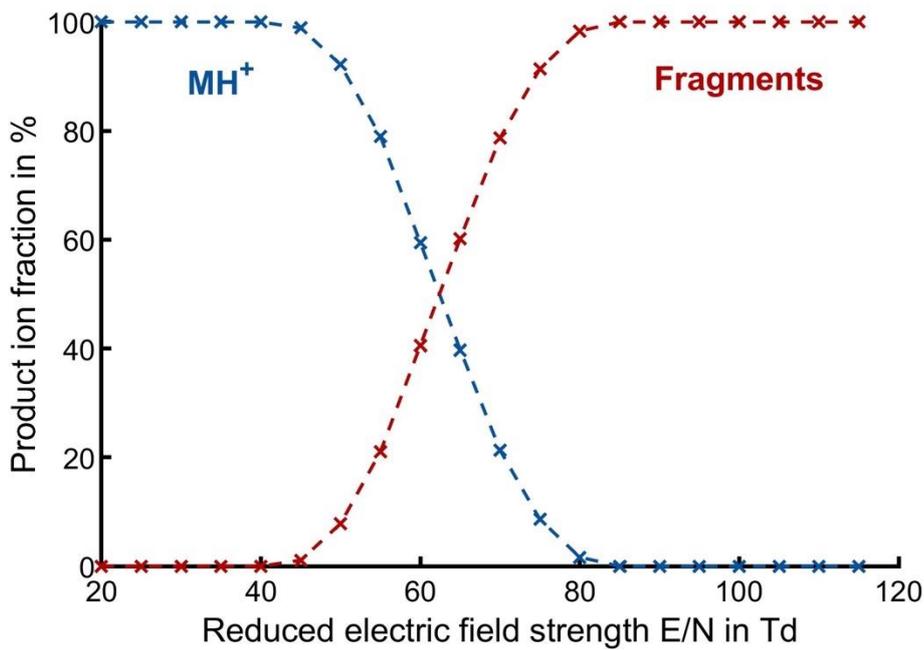
*Stefan Zimmermann, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology, 30167 Hannover, Germany*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Coupling of a HiKE-IMS with a TOF-MS.



Product ion fractions of 1-butanol depending on  $E/N$ .

Poster number: **IM-PA-136**

## **COUPLING DROPLET-BASED MICROFLUIDICS WITH ION MOBILITY-MASS SPECTROMETRY TO STUDY THE EARLY STEPS OF PROTEIN AGGREGATION**

Abstract ID: **222****Presenting author: Iuliia Stroganova, Vrije Universiteit Amsterdam, Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life Sciences**

### **Introduction**

Peptide and protein aggregation is one of the most important physical processes in the human body. The unavoidable build-up of aggregates leads to liquid-solid phase separation (LSPS) which is directly linked to age-related, neurodegenerative diseases such as Alzheimer's, Parkinson's, and prion diseases (M.G. Iadanza, Nat. Rev. Mol. Cell Biol. 2018). Besides LSPS, aberrant liquid-liquid phase separation (LLPS) can also play a role in disease and result in formation of stable assemblies, including so-called labile fibrils, hydrogels, and pathological amyloids (W.M. Babinchak, J. Mol. Biol. 2020). However, the molecular mechanism of protein aggregation behind these phase transitions is largely unknown. In order to be able to control and direct peptide aggregation, a full understanding of the early-steps of the peptide aggregation process is essential.

### **Methods**

Currently, we are developing a multi-dimensional setup, the photo-synapt, where we interface droplet-based microfluidic electrospray ionization (ESI) and ion mobility-mass spectrometry (IM-MS) with IR and UV photodissociation spectroscopy (Figure 1). This allows us to obtain both structural and kinetic information of the aggregation in a single experiment. Here, I focus on the coupling of the microfluidic source, which generates picoliter droplets, with the IM-MS. This unique combination will allow us to study aggregation in a more controlled environment, to probe both the structure and kinetics of the aggregated peptides in single droplets, and to unravel the effect of confinement on aggregation.

### **Preliminary data (results)**

Recently, we have realized the successful implementation of droplet-based microfluidic source (E.E. Kempa, Anal. Chem. 2020) coupled with two types of MS instruments from different vendors (Bruker, Waters). These vendors utilize different ESI sources depending on the point of application of high voltage (HV). ESI sources can be 'push' (HV is applied to the ESI needle, Waters) or 'pull' (HV is applied to mass spectrometer inlet, Bruker). Figure 2 shows our data from coupling our droplet source with the TIMS-TOF using such a 'pull' ESI source. Here, we focus on the tau protein segment Ac-PHF6-NH<sub>2</sub> which is associated with Alzheimer's disease (AD) and several other neurodegenerative diseases, known as "tauopathies". Both the mass spectra (MS) and the ion's mobility (IMS) can be measured in one single droplet (see Fig. 2 C and D, respectively).

The next step is to follow the effect of confinement and the kinetics of aggregation in droplets compared to bulk solution. This comprises generation of droplets, their storage for a desired amount of time and subsequent reinjection of droplets into MS instruments. Currently, we are optimizing experimental parameters to eliminate the effect of, for example, oil ion suppression in our mass spectra. Moreover, various droplet sizes and aggregation conditions will be examined. Ultimately, the coupling of our droplet-based source with IM-MS will be used to elucidate structural and kinetic evolution of heterogeneous oligomers which are formed during early steps of aggregation. Thereby focusing on both LSPS and LLPS processes that are associated with neurodegenerative diseases.

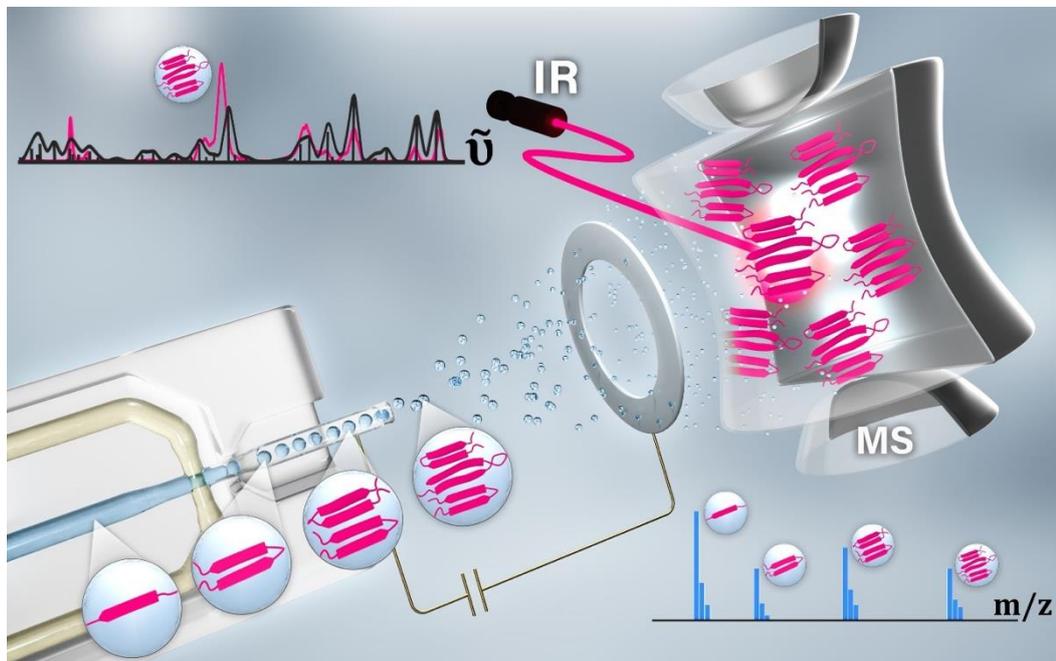
### **Please explain why your abstract is innovative for mass spectrometry?**

Coupling our droplet microfluidic source with ion mobility-mass spectrometry to study the aggregation mechanism of both LLPS and LSPS peptides and thereby investigate the effect of confinement on their kinetics.

### **Co-authors:**

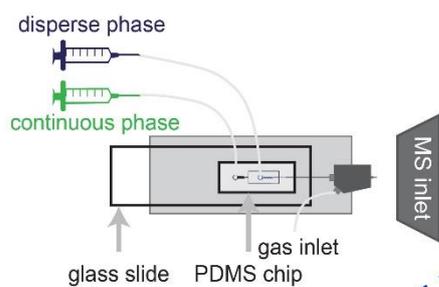
*Agathe Depraz Depland, Vrije Universiteit Amsterdam, Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life Sciences*

Aigars Piruska, Radboud University, Institute for Molecules and Materials  
 Wilhelm T. S. Huck, Radboud University, Institute for Molecules and Materials  
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 Molecular and Life Sciences

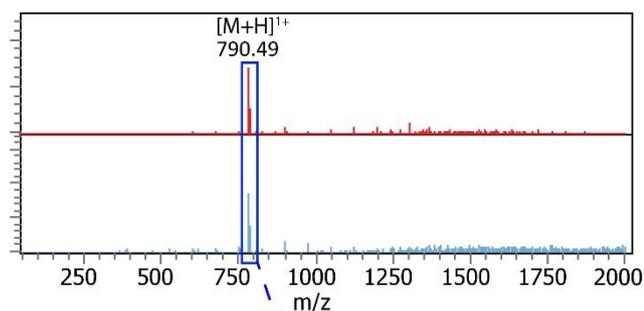


Schematic illustration of a microfluidic source coupled to MS.

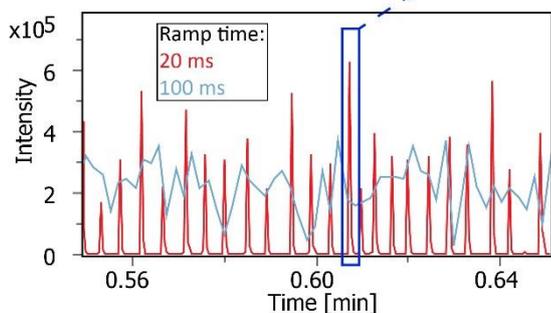
A) set-up



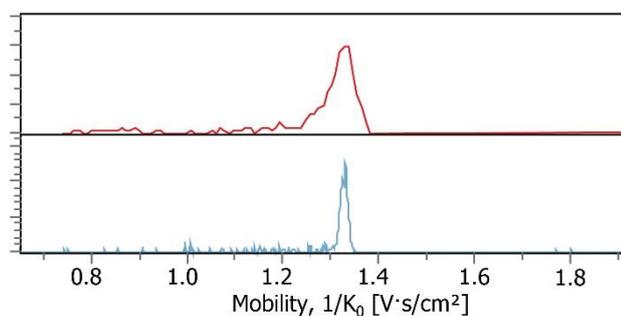
C) MS



B) TIC



D) IMS



Droplet set-up, Ion chromatograms, and Mass and Ion mobility spectra.

Poster number: **IM-PA-137**

## **INTELLIGENT INTERNAL RECALIBRATION**

Abstract ID: **248**

**Presenting author: Harry Taylor, Nottingham Trent University**

### **Introduction**

It is common practice to have to use internal mass recalibration to get the best possible mass accuracy. Then, this mass accuracy is used to assist in the process of peak assignment. For complex spectra, we commonly have to use one or more manual assignment steps in order to find and use the internal recalibration peaks – that is, if there are confidently known, positively identifiable peaks to use. But, even if such peaks are available in your data, if you have a large number of spectra to process, then this manual assignment step is both slow and can result in accidental errors. If you add in additional internal recalibration compounds to your sample then this can introduce other issues. Is there a better way?

### **Methods**

We have used a new, prototype Beacon source (Vibrat-Ion Ltd, UK) to undertake complex organic matter analyses on both FT-ICRMS and Orbitrap instruments. This novel ionisation source permits high confidence automated internal recalibration of complex organic matter spectra without additional peaks appearing in the final results. The results were processed using in-house developed LabVIEW software to read the resulting datafiles into the AutoLogis tool in AutoVectis for assignment.

### **Preliminary data (results)**

The Beacon source was mounted in place of the normal nano-micro spray sources on ICR, Orbitrap and TOF instruments. The performance of the system has been benchmarked using a previously well characterised (and delicious) Scotch whisky and the standard Suwannee River fulvic acid (SRFA) as exemplar complex organic matter samples, with the results compared to assignments undertaken using the normal manual methods of internal recalibration. As expected, there was no difference in the assignment lists between the two routes of internal recalibration. However, the workflow for processing the Beacon data was entirely automated whereas the normal route needed the extra manual step providing at least one internal recalibration peak. This advance then permits automated batch processing of large cohorts of spectra, whereas currently they have to be processed one at a time, with manual input. Additionally, the automated system indicates why it selected the internal recalibration peaks it did, which is very useful for retrospective audit purposes.

### **Please explain why your abstract is innovative for mass spectrometry?**

Novel ion source coupled to novel software for automated assignment of high resolution, high mass accuracy data.

### **Co-authors:**

*Mark Neal, Vibrat-Ion Ltd.*

*Logan Mackay, University of Edinburgh*

*Andrej Grgic, Maastricht University*

*Patrick Sears, University of Surrey*

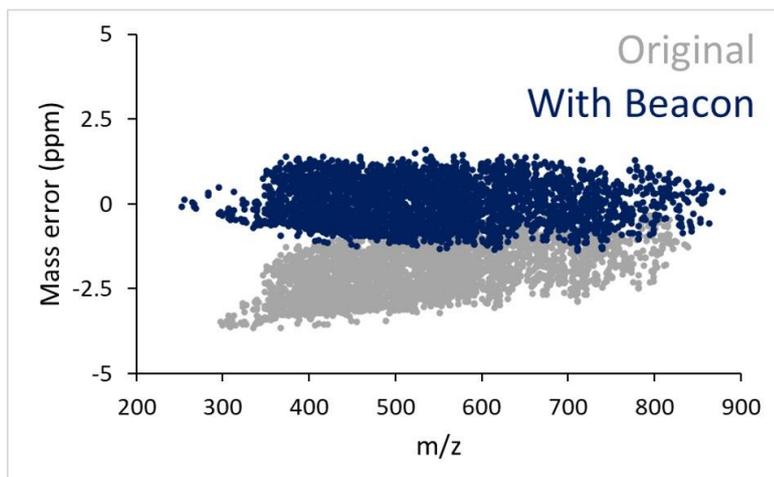
*Yury Tsybin, Spectroswiss Sarl*

*Ron Heeren, Maastricht University*

*David Kilgour, Nottingham Trent University*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Fulvic Acids data - effect of automated internal mass recalibration

Poster number: **IM-PA-138**

## **OPTIMIZATION OF HUMAN PLASMA PROTEOME HIGH-THROUGHPUT ANALYSIS USING FAIMS PRO INTERFACE**

Abstract ID: **293**

**Presenting author: Dana Hein, University Medical Center Mainz**

### **Introduction**

Due to the composition and associated properties of plasma, mass spectrometry-based proteomic analysis of human plasma is challenging. Compared to other tissues, the overall number of proteins is lower and their concentrations vary considerably. For these reasons, high-throughput analysis methods of plasma samples need to be constantly monitored and adapted in order to reach maximal proteome coverage.

### **Methods**

To achieve a high number of detected peptides and proteins in high-throughput analysis we decided to use the FAIMS Pro interface. FAIMS uses an asymmetric electric field to separate ions by their ion mobility. For optimal results, different voltages of the electrode and m/z filter combinations were tested. DIA-NN and MaxQuant were used for raw data analysis. DIA-NN employs neural networks and interference correction and is thus particularly well suited for high-throughput set up, allowing fast analysis and deep coverage of proteomes. MaxQuant is a quantitative proteomic software that can analyze large datasets and uses several labeling techniques.

### **Preliminary data (results)**

The number of identified peptides and proteins in human plasma obtained with FAIMS Pro interface differs by an order of magnitude compared to standard methods. For specific voltages, our data shows a significant increase of identified peptides and proteins compared to the standard method. We demonstrate that a meticulous selection of two or three FAIMS voltages results in significantly higher numbers of uniquely identified proteins and peptides. If paired with additional mass to charge filtering, the number of identified proteins and peptides drops slightly, while boosting the quality of findings.

### **Please explain why your abstract is innovative for mass spectrometry?**

With the FAIMS interface, an increase in the number of identified peptides and proteins in human plasma and thus a wider coverage of unique peptides and proteins can be achieved.

### **Co-authors:**

*Elena Kumm, University Medical Center Mainz*  
*Ute Distler, University Medical Center Mainz*  
*David Gomez-Zepeda, University Medical Center Mainz*  
*Mateusz Łącki, University Medical Center Mainz*  
*Stefan Tenzer, University Medical Center Mainz*

Poster number: **IM-PA-139**

## **A NOVEL EI SOURCE OPTIMIZED FOR USE WITH HYDROGEN CARRIER GAS IN GC/MS AND GC/MS/MS**

Abstract ID: **297**

**Presenting author: Remko Van Loon, Agilent Technologies**

### **Introduction**

Recent concerns with the price and availability of helium have led laboratories to look for alternative carrier gases for their GC/MS and GC/MS/MS systems. Among the problems encountered when converting to hydrogen carrier gas in GC/MS is that hydrogen is a reactive gas and may cause chemical reactions in the mass spectrometer electron ionization (EI) source. This can lead to disturbed ion ratios in the mass spectrum, spectral infidelity, and peak tailing. This presentation will discuss a novel EI source developed to address hydrogen-related issues in the EI source enabling improved performance with hydrogen carrier gas in GC/MS analysis. The benefits of using the novel EI source will be discussed for several GC/MS applications.

### **Methods**

A novel EI source was used in GC/MS and GC/MS/MS analysis with hydrogen carrier gas. GC parameters were optimized to enable the best analytical results. To evaluate system performance, several classes of compounds including SVOCs, PAHs, PCBs, phthalates, and pesticides were analyzed with GC/MS and GC/MS/MS using hydrogen as a carrier gas. The results achieved with the novel EI source were compared to those observed with the conventional EI source and the previously optimized for hydrogen carrier conventional EI source equipped with a 9 mm lens, which provided the best performance to date.

### **Preliminary data (results)**

The experimental results demonstrated:

- Good spectral fidelity for compounds susceptible to hydrogenation in the source resulting in higher library match scores observed against the NIST spectral library when compared to the conventional EI source (Fig. 1). This effect was especially pronounced for nitrobenzene and other nitro-compounds.
- Improved chromatographic peak shape, especially for late-eluting PAHs with a fast analysis method that is known to amplify any chromatographic peak tailing (Fig. 2)
- Dramatically improved the calibration performance for SVOCs with 50% more compounds meeting the EPA linearity criteria and significant (>50%) decrease in the number of compounds requiring a quadratic fit, when compared to the conventional EI source equipped with a 9 mm lens over a calibration range of 0.1-100 ppm
- Performed analysis of 74 SVOCs (EPA 8270 full mix) in 5.8 minutes using the novel EI source and hydrogen carrier gas with GC/MS/MS
- Demonstrated excellent sensitivity at the sub-ppb level for many analytes including PAHs and PCBs, exceeding that typically seen with hydrogen carrier gas with both GC/MS and GC/MS/MS
- Stable system performance for over 5,000 injections of a heavy soil extract without a need for EI source cleaning.

### **Please explain why your abstract is innovative for mass spectrometry?**

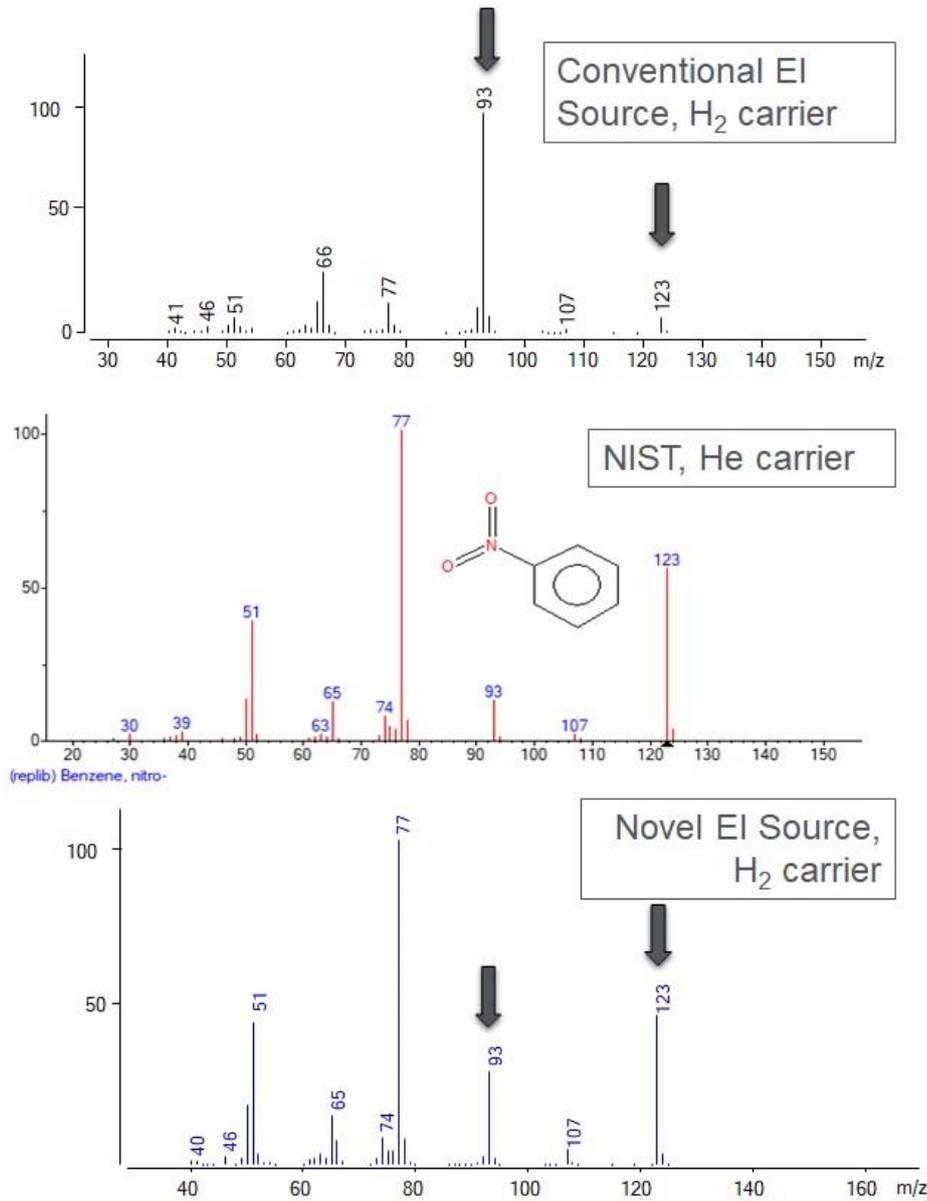
A novel EI source for GC/MS(/MS) was developed for use with hydrogen carrier gas. Its advantages are demonstrated for the analysis of SVOCs, PAHs, PCBs, phthalates, and pesticides.

### **Co-authors:**

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

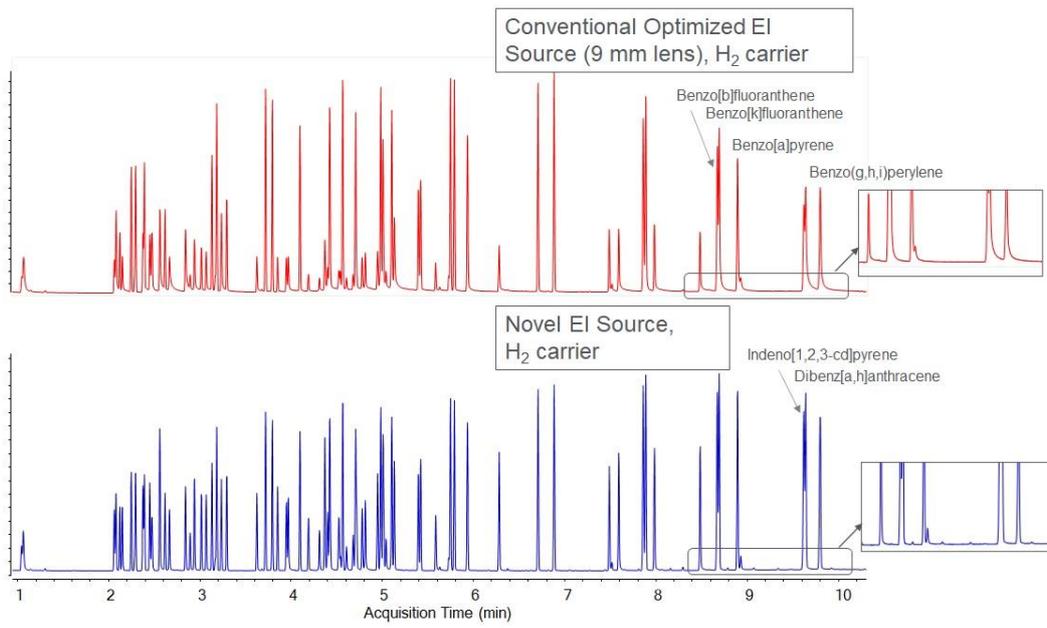
Anastasia Andrianova, Agilent Technologies  
Angela Smith-Henry, Agilent Technologies  
Amanda McQuay, Agilent Technologies  
Bruce Quimby, Agilent Technologies



Mass spectra of nitrobenzene compared to NIST

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



GC/MS chromatograms of SVOCs acquired with hydrogen carrier gas

Poster number: **IM-PA-140**

## **SIGNATURES OF LIQUID DROPLETS FROM ELECTROSPRAY IN THE MASS ANALYZER REGION OF AN COMMERCIAL ESI-TOF-MS**

Abstract ID: **487**

**Presenting author: Chris Heintz, University of Wuppertal**

### **Introduction**

Electrospray Ionization (ESI) is one of the most frequently used atmospheric pressure ionization (API) techniques in mass spectrometry (MS). An analyte-solution is sprayed in a strong electric field leading to the generation of charged droplets. It is a common assumption that the droplets fully evaporate within the ion source chamber bearing bare ions. However, a series of experiments shows that a significant quantity of large, charged droplets from the electrospray reaches the high vacuum region of an ESI-MS. Droplets penetrating deeply into the vacuum system potentially lead to a diminished analytical performance. Thus, it is crucial to understand the mechanisms leading to aspiration of large droplets into an ESI-MS to improve the quality of received mass spectra and lower the maintenance effort.

### **Methods**

Charged droplet signatures are investigated in a Bruker microTOF with an auxiliary ion detector (Secondary ion detector – SEM) located downstream the TOFs pulser region. By connecting the SEM output signal to an oscilloscope with appropriate input impedance, we were able to directly observe and analyze the appearance of charged droplet signatures in terms of intensive ion signal bursts. Analysis of the acquired burst oscillograms was performed with custom software. Furthermore, we recorded non-summed individual TOF spectra and analyzed them also with custom analysis programs. All measurements were done in dependence of ESI source parameters.

### **Preliminary data (results)**

The observed droplet signatures are visible in the oscillograms as very intensive and broad signal bursts with widths of approximately 50 to 100  $\mu$ s. Interaction with the TOF acceleration stage clearly show that the observed signals are temporally highly correlated bursts of individual ions rather than a massive, charged droplet: If the TOF pusher pulses while a signal burst appears, a sharply defined segment of the pusher's pulse length is "cut out" from the burst. The observed ion bursts have a common general shape but differ in width and integral. Broader signals seem to appear when parent droplets fission occurs earlier, while later fission leads to a narrower signal.

The frequency of the ion bursts appearance depends on the ESI source parameters. Critical parameters (e.g., the capillary voltage, nebulizer gas pressure and liquid flow) are generally connected the spray formation. Long-term measurements show that the droplet appearance frequency tend to decrease over time.

In another experimental series, we collected thousands of individual spectra and performed a statistical analysis. We identified a group of mass spectra with extraordinary high total signal intensity. A histogram of the signal intensity shows two different intensity distributions, one of the high intensity spectra and one of the common background spectra. These high intensity spectra are not observable with APCI and APPI, which is another strong indicator for aspirated droplets from ESI.

### **Please explain why your abstract is innovative for mass spectrometry?**

Characterization of charged droplet signatures in the analyzer region of an ESI-MS by direct observation of signal bursts and analysis of high frequency non-summed TOF spectra.

### **Co-authors:**

*Lisa Schnödewind, University of Wuppertal*

*Walter Wissdorf, University of Wuppertal*

*Hendrik Kersten, University of Wuppertal*

*Thorsten Benter, University of Wuppertal*

Poster number: **IM-PA-141**

## **3D PRINTED OFFLINE NANO-ELECTROSPRAY IONIZATION SOURCE FOR BRUKER MASS SPECTROMETERS**

Abstract ID: **493**

**Presenting author: Michael Götze, Freie Universität Berlin**

### **Introduction**

Nano-electrospray ionization (nESI) is a highly efficient and widely used technique for the ionization of biomolecules and the analysis of minute amounts of analytes. It is often used in conjunction with nano-flow liquid chromatography. An offline nESI source is convenient for the direct analysis of biomolecules and allows quick screening of sample quality, composition or the evaluation of reaction conditions. For Bruker instruments such as the Bruker timsTOF Pro, no such source is readily available. We provide an affordable, 3D-printable nESI source for Bruker instruments.

### **Methods**

We wanted to design a simple offline nESI source for Bruker instruments. We reversibly modified a Bruker ionBooster ESI-source and adapted a 3D printed, adjustable holder for palladium-coated glass capillaries. The glass capillaries are electrically connected to the source housing to reach ground level and provide a stable electrical connection to the instrument. The position of the needle can be adjusted in three directions to optimize signal intensity as well as signal stability.

### **Preliminary data (results)**

The source consists of eleven 3D printed parts, printed on a Prusa MK3S filament 3D-printer and an Elegoo Saturn resin 3D-printer. In addition, a threaded rod and guiding rods are used to move the coated glass-needle in and out of the source region. The source is attached in place of one of the three windows of a Bruker ionBooster ESI-source. The needle holder consists of a Swagelok connector that is held in place by the 3D printed adapter. The gas flow provided by the instrument to the ionBooster source is used as backing pressure for the nESI source.

High resolution IM-MS spectra were recorded for different biomolecules from purified standards as well as biological samples. We compared the ionization of different molecules using the 3D printed nano-ESI source and the ionBooster source.

The nESI source can be assembled by anyone with access to 3D printers. Some parts could even be machined from metal to further increase the stability of the device.

### **Please explain why your abstract is innovative for mass spectrometry?**

This 3D-printable nESI source provides an easily accessible device for the quick analysis of biomolecules on high-resolution Bruker mass spectrometers, with minor, reversible modifications to original Bruker hardware.

### **Co-authors:**

*Lukasz Polewski, Freie Universität Berlin*

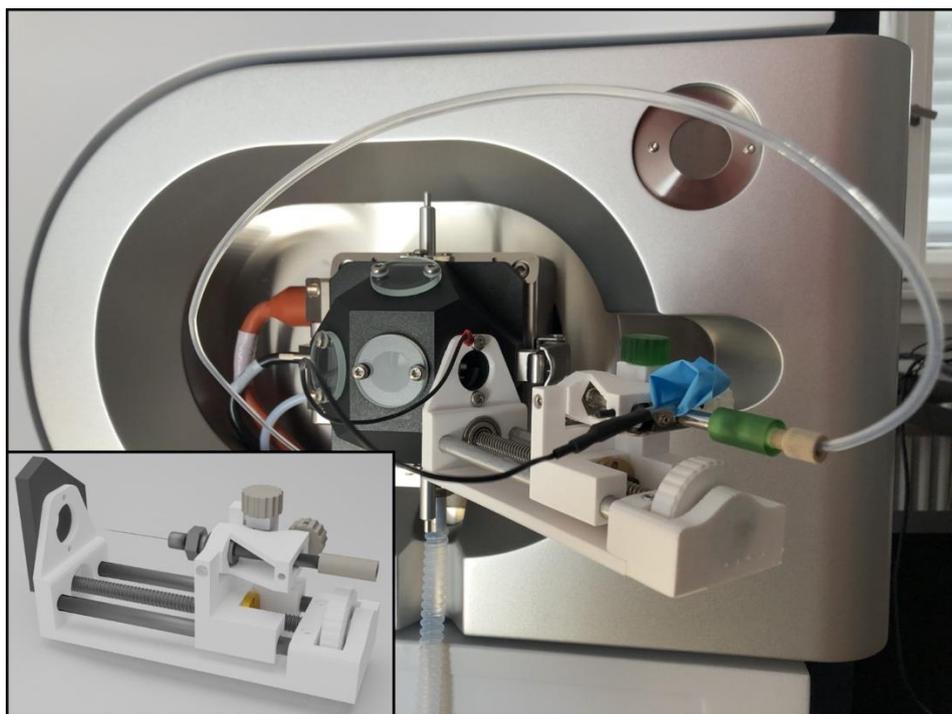
*Kevin Pagel, Freie Universität Berlin*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

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Render and picture of the 3D printable nESI source.

Poster number: **IM-PA-142**

## SOLVENT EFFECT AND COMPETITIVE REACTIONS IN AN ATMOSPHERIC PRESSURE CHEMICAL IONIZATION ION SOURCE

Abstract ID: **497**

**Presenting author: Younes Valadbeigi, University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Institute of Analytical Chemistry, Muthgasse 18, 1190 Vienna, Austria**

### Introduction

Atmospheric pressure chemical ionization (APCI) is one of the most important interfaces used for analytical LC-MS applications. APCI ion source designs for LC-MS use heating vaporizers and nebulizers to enable direct infusion of analyte solutions. A key feature of APCI is that ionization of the solvents themselves leads to formation of new ion species that influence the ionization of the analytes. To take the effect of solvent into account, proton affinities (PA) and ionization energies (IE) of solvents have been considered in previous studies. However, no correlation between IE values of the solvents and formation of radical cation ( $M^+$ ) has been established. Here, we aim to interpret the complexity of solvent effect on the APCI based on competitive reactions using a high-resolution MS platform.

### Methods

A range of benzene derivatives, PAHs, phenyl ketones and polyenes were investigated. These were prepared in typical analytical grade solvents relevant for LC-MS (methanol, acetonitrile, water, chloroform) and studied via direct infusion mass spectrometry. An Agilent 6560 IM-QTOF mass spectrometer equipped with APCI ion source was used for all measurements. Density functional theory (DFT) with the B3LYP functional was used for the structural optimization of molecules. Thermodynamic properties including proton affinities (PA), gas phase basicities (GB),  $\Delta H$  and  $\Delta G$  of deprotonation reactions were derived. Ionization energies (IE) were calculated using G4MP2 method. All calculations were performed with Gaussian 16 software.

### Preliminary data (results)

From the nature of the product ions, it is proposed that, other than  $(H_2O)_nH^+$ , another RI, probably  $NO^+$ , participates in the ionization of the analytes. Hence, the ionization mechanism is not only based on protonation of the analytes and ionization may occur via other pathways such as charge transfer and hydride abstraction.

Although a given ionization path is thermodynamically possible, it can be suppressed by a more favourable ionization pathway. Hence, by using the absolute thermodynamic data of a compound including PA and IE, we cannot determine the exact ionization mechanism and other paths should be considered and compared.

Based on the results obtained in this work, solvents influence the ionization mechanism in two ways. First, the solvent and the analyte compete for ionization. For example, acetonitrile with higher PA than methanol can reduce the protonation of the analyte while analytes are more easily protonated in methanol (Figure 1). Similarly, the presence of acetonitrile diminishes the protonation pathway and enhances ionization via charge transfer and hydride abstraction. Second, the ions produced from the ionization of solvents can act as new RIs to ionize the analyte and change the ionization mechanism. This was observed to be most important for the use of chloroform which produces  $CHCl_2^+$  to ionize the analytes via electrophilic substitution.

### Please explain why your abstract is innovative for mass spectrometry?

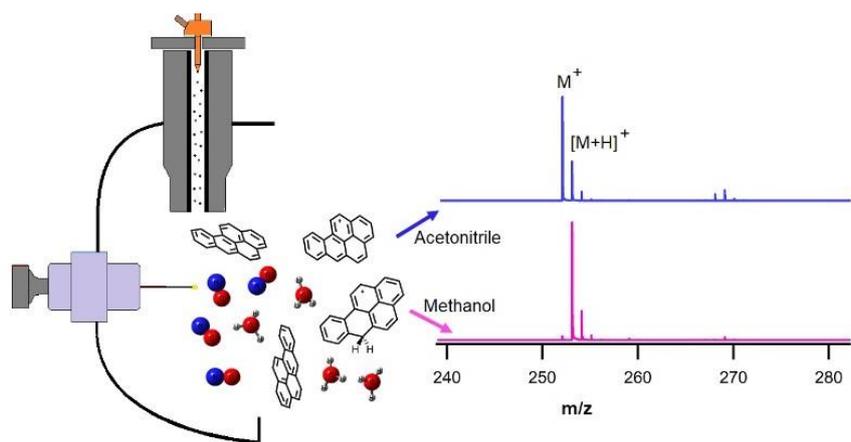
A comprehensive mechanism based on competitive reactions was proposed for a commercial APCI ion source used in many LC-MS instruments for analytical applications.

### Co-authors:

*Tim Causon, University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Institute of Analytical Chemistry, Muthgasse 18, 1190 Vienna, Austria*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



APCI MS of benzo[a]pyrene in acetonitrile and methanol

Poster number: **IM-PA-143**

## **CONTROL OF METAL ADDUCT FORMATION IN ELECTROSPRAY AND ITS BENEFITS FOR ELECTRON INDUCED DISSOCIATION AND DIFFERENTIAL MOBILITY SPECTROMETRY**

Abstract ID: **524**

**Presenting author: David Ruskic, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva**

### **Introduction**

The presence of alkali metal adducts  $[M+xA]x+$  in an MS spectrum is very often omitted due to low signal intensity, poor fragmentation in CID, and difficult annotation. Their origin is often attributed to the trace presence of the alkali metals in the mobile phase, and hence, poor reproducibility as their concentration is always changing. This is why the analyte identification using collision-induced dissociation in ESI-LC-MS analysis is predominantly based on the protonated  $[M+xH]x+$  species. However, more recently electron-induced dissociation (EID) has shown interesting benefits of using alkali metal adducts with information-rich fragmentation similar to EI. Therefore, approaches enabling better control of their formation in ESI are highly desirable regarding sensitivity and reproducibility.

### **Methods**

A 137 standard compounds (metabolites and pharmaceuticals) were mixed (~50 ng/ml) and separated on a reversed-phase column in gradient mode. Collision-induced and electron-induced spectra were acquired on a hybrid QqTOF (6600+ TripleTOF, Sciex) equipped with a prototype ExD fragmentation cell. Various metal salts were considered including (Na, K, L, Ca, Mg or Ba) at various concentrations, added on column or post-column.

### **Preliminary data (results)**

There are two widely used approaches to generate alkali-adduct in ESI analysis: i) addition of alkali-metal in the LC mobile phases, and ii) post-column addition by an external pump. With these approaches, it is difficult to switch between preferred ion species, protonated molecules, or metal adducts as manual system change is required. Both of these non-optimized setups suffer inefficiency in terms of system contamination and signal suppression and flexibility in terms of ease of operation. Our approach is based on controlling the addition of alkali metal salt (e.g. NaOAc, KOAc, LiCl) which enables to shift equilibriums from protonated  $[M+xH]x+$  to predominantly alkali-adduct forms  $[M+xA]x+$  for samples with corresponding alkali salt, whereas the samples injected without alkali salt showed predominated protonated species. The total number of species in the  $[M+Na]^+$  form increased from 2 to 54, and the  $[M+K]^+$  form increased from 1 to 37. More importantly, in a subsequent injection of the samples containing different types of counter metal ion, e.g. from  $Na^+$  to  $K^+$  or  $Li^+$ , the desired type of metal adduct was obtained without the presence of another metal adduct. Therefore, the ability to toggle between the desired mode of detection ( $[M+H]^+$  or  $[M+A]^+$ ) can be then used to take advantage of other analytical tools like DMS or ECD to gain additional selectivity or obtain orthogonal fragmentation information, respectively.

### **Please explain why your abstract is innovative for mass spectrometry?**

Control of metal adduct formation in ESI for differential mobility spectrometry and electron-induced dissociation.

### **Co-authors:**

*Yves Le Blanc, SCIEX, Concord, Ontario, Canada*

*G rard Hopfgartner, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva*

Poster number: **IM-PA-144**

## DETECTION OF EXPLOSIVES FROM VAPOUR PHASE UTILIZING MION INLET EQUIPPED API-TOF-MS

Abstract ID: **543**Presenting author: **Jyri Mikkilä, Karsa Oy**

### Introduction

With ever increasing need for transportation of goods all around the globe, there is also a need for fast and high throughput method for screening these packages and containers for explosives and other contraband. Gas phase detection of these compounds reduces the amount of labor and time taken by dismantling, sampling, and repackaging the shipments. Analysing the samples with an Api-ToF-MS (atmospheric pressure interface time-of-flight mass spectrometer) equipped with a MION inlet facilitates the fast sampling and analysis, but also provides sub-pg detection limits with a tunable selective chemical ionization scheme. Multiple reagent ions can also be used without mechanical changes to the instrument to screen for even larger set of targets.

### Methods

The used instrument consists of filter desorbing unit, chemical ionization (CI) source and Api-ToF-MS (Figure 1). The tested liquid standards of the selected compounds were injected on a metal mesh filter and then heated to 250°C. The vaporized sample is then carried with a sheath flow towards the charging region. Two chemical ionization sources were utilized in these measurements: straight radiation chemical ionization (SRCI) and Karsa MION. LOD injection runs were carried out with an autosampler, with each LOD measurement consisting of 58 injections of blanks and samples in three different concentrations.

### Preliminary data (results)

Most of the measured target compounds are detectable clustered with the added reagent ion bromide, but few of them are more prominent as deprotonated. The SRCI source could detect 7 out of the 11 tested targets, where as the MION could detect 9 out of 11. In addition to detecting more of the targets, the MION has an increased sensitivity in most of the shared detections and has LODs similar to and up-to two orders of magnitude lower than the SRCI with the used set of targets. As the MION source does not introduce neutral reagent into the sample flow, or irradiate the sample compounds with x-ray, secondary charging methods play a smaller role in the charging of the target compounds. This reduces the likelihood of deprotonation with the chosen reagent ion, thus reducing the LOD for compounds that are typically detected as deprotonated. All-in-all the LODs determined in these measurements are in the picogram to sub-picogram range for the detected compounds (Figure 2). The targets that could not be detected with bromide as the reagent ion, can in most cases be detected with other reagent ion chemistries and MION provides an excellent platform to do so, as the reagent can be switched with just turning electric fields on and off. Previous results indicate that nitrate, hydrated protons, ammonia, etc. can be used in the MION for both positive and negative polarity measurements to detect a vast number of compounds.

### Please explain why your abstract is innovative for mass spectrometry?

Soft chemical ionization mass spectrometry for high-throughput screening of threats. Tailorable chemical ionization inlet with switchable reagent for mass spectrometers. Selective ionization of explosive molecules.

### Co-authors:

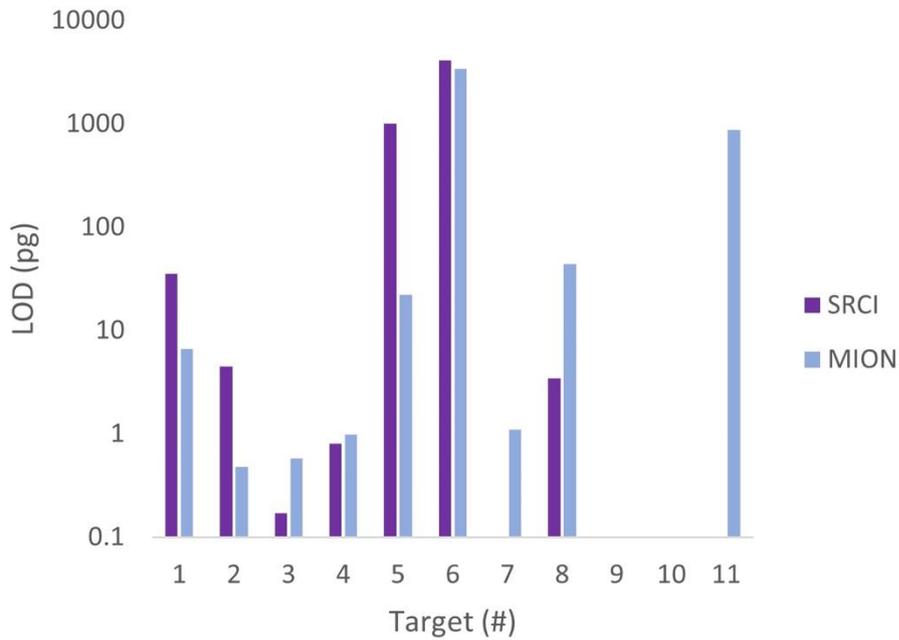
*Paxton Juuti, Karsa Oy*  
*Evgenia Iakovleva, Karsa Oy*  
*Juha Kangasluoma, Karsa Oy*  
*Jussi Kontro, Karsa Oy*  
*Nasib Naseri, Karsa Oy*  
*HJ Jost, Karsa Oy*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Instrumentation used for the LOD determination: MION-Api-ToF-MS with the autosampler.



Comparison of the LODs for SRCI and MION sources.

Poster number: **IM-PA-145**

## **PERMEATION TUBE ASSISTED REAGENT ION MANIPULATION ON A GC-EC-TOF FOR IMPROVED NON-TARGET ANALYSIS APPROACHES**

Abstract ID: **580**

**Presenting author: Sonja Klee, Tofwerk AG**

### **Introduction**

The main advantage using chemical ionization (CI) mass spectrometry (MS) is to obtain simplified mass spectra with ideally a dominant molecular or quasi molecular ion signal. Commonly, GC-MS analysis uses electron ionization (EI) where molecular ion information often is lost due to analyte fragmentation. CI was introduced as alternative to EI providing molecular ion information – essential evidence in non-target screening (NTS) approaches. However, classical CI mainly requires flammable gases e.g., methane and isobutane. Stored in compressed-gas cylinders the handling can become an issue for safety policies. Here we present an alternative medium pressure chemical ionization source using easily feasible reagents e.g., Nitrogen, water and ammonia offering a fast way of reagent ions manipulation and therefore molecular ion information without required hardware changes.

### **Methods**

A medium-pressure (mbar region), high temperature (>300°C), chemical ionization source used at the novel GC-EC-TOF (TOFWERK, Thun, Switzerland) is described. This ionization source utilizes a filament-free, hydrogen-based plasma source for  $H_3^+$  generation. Secondary reagent ions can be produced by adding  $N_2$  to the gas flow leading to the formation of  $N_2H^+/N_4H^+$  species. These reagent ions can get manipulated by doping the Nitrogen with another reagent e.g., water or ammonia via a heated perm tube setup. The ionization source performance and its impact for the outcome in NTS approaches are shown using material emission measurements.

### **Preliminary data (results)**

Figure 1 displays reagent ion distributions generated in the ionization source with pure nitrogen, and nitrogen doped with two different water concentrations and ammonia. The ion distribution shifts from very non-selective reagents, but strong gas phase acids e.g.,  $N_2H^+/N_4H^+$  to more selective but “softer” direct protonation reagents e.g.,  $H_3O^+$  and  $(H_2O)_2H^+$ . Additionally, a very “soft” ionization environment using  $NH_4^+$  for cluster ionization can be generated adding  $NH_3$ . The shift from one to another reagent can be carried out within 200 s and without any change of the system setup.

The ionization performance of the different reagent gases is shown using TD-GC-EC-TOFMS measurements of material emission samples. The CI source always is operated simultaneously to a 70 eV EI source using the EC-TOF. This way a direct comparison of ionization efficiencies compared to the non-selective EI process is visible.

Figure 2 shows the molecular ion yields detected using pure nitrogen and doped nitrogen as secondary reagent gas compared to the EI process for three different analytes. Whereas the EI does not provide any molecular ion information, CI molecular ion yields shift using different reagent ions according to their proton affinity and reaction excess energy, respectively.

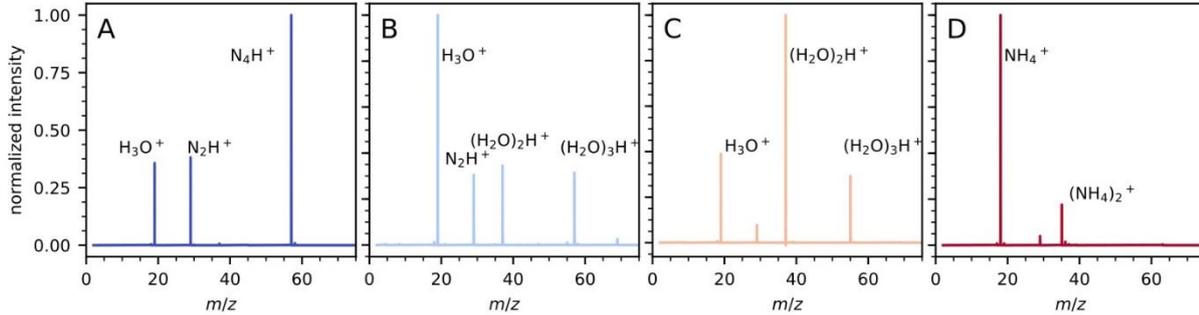
The generation of molecular ion information as well as the knowledge on the chemical ionization processes do provide important evidence for the compound identification in non-target screening approaches. Together with the structural information provided simultaneously in the EC-TOF this CI source feature provides a great increase of identification certainty.

### **Please explain why your abstract is innovative for mass spectrometry?**

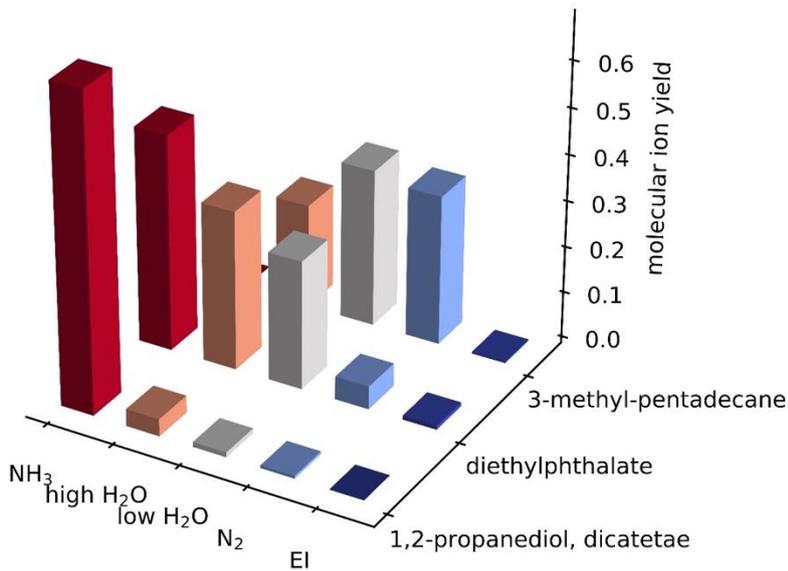
Fast reagent ion manipulation in a medium pressure chemical ionization source for GC-EC-TOFMS preventing hardware changes and the use of compressed- and flammable-gas cylinders.

**Co-authors:**

Steffen Bräkling, *Tofwerk AG, University of Wuppertal*  
 Thorsten Benter, *University of Wuppertal*  
 Hendrik Kersten, *University of Wuppertal*



Reagent-ion spectra of N<sub>2</sub> (A), H<sub>2</sub>O (B&C), NH<sub>3</sub> (D)



Molecular-ion-yields of 1,2-propanediol-diacetate, diethylphthalate and 3-methyl-pentadecane (CI&EI)

Poster number: **IM-PA-146**

## **INTACT LIFDI MOLECULAR-CATIONS AND -ANIONS OF REACTIVE COMPOUNDS**

Abstract ID: **670**

**Presenting author: Mathias Linden, Linden ChromaSpec GmbH**

### **Introduction**

Liquid-Injection Field Desorption Ionization (LIFDI), an improvement of Field Desorption (FD), supplies a solution of the analyte directly to the emitter in the ion source through a fused silica capillary. The capillary is aligned with the rear emitter whiskers. Dipping the other end in the solution is all the operator has to do ahead of each acquisition. Radical cations are formed by tunneling of an electron from the analyte molecule to the emitter due to the extremely high electric field at the tips of the emitter whiskers. The least bound electron is removed leaving a radical cation without excess internal energy. Or electrons can be attached to electron acceptor molecules forming radical anions.

### **Methods**

Sample handling is quick and easy at ambient and under inert conditions. Manipulating the probe and pumping the vacuum lock is required once the capillary or the emitter have to be changed. Only then the lens system has to be tuned. Spectra of many analytes can be acquired without re-focusing. Most organic solvents can be used including toluene. Rinsing the capillary and clean heating the emitter between samples does prevent carry over.

### **Preliminary data (results)**

Organic Light Emitting Diodes (OLEDs) and Organic Solar Cells (OSCs) use organic electron acceptor and donor compounds which have the merit of being lightweight, low cost and mechanically flexible. LIFDI spectra of such compounds, for instance perylene diimide based samples and others depict intact molecular radical ions. The electron acceptor compounds are efficient at forming singly and doubly charged radical anions whereas the donor compounds are better at giving singly and multiply charged radical cations under LIFDI conditions. The relative intensity of the doubly charged intact bi-radical molecular ions versus that of the singly charged radical ions can give some sort of in situ information on the efficiency of the electron attracting or donating properties. Highly reactive compounds which are susceptible to decomposition upon contact with oxygen or moisture may be a challenge to many ionization methods except for LIFDI. Septum capped sample vials with nitrogen or argon in the headspace above the sample solution guarantee that the LIFDI capillary aspirates inert gas ahead and after dipping it in the solution. LIFDI spectra exhibiting intact molecular ions will be presented of compounds which couldn't be characterized with other ionization methods.

### **Please explain why your abstract is innovative for mass spectrometry?**

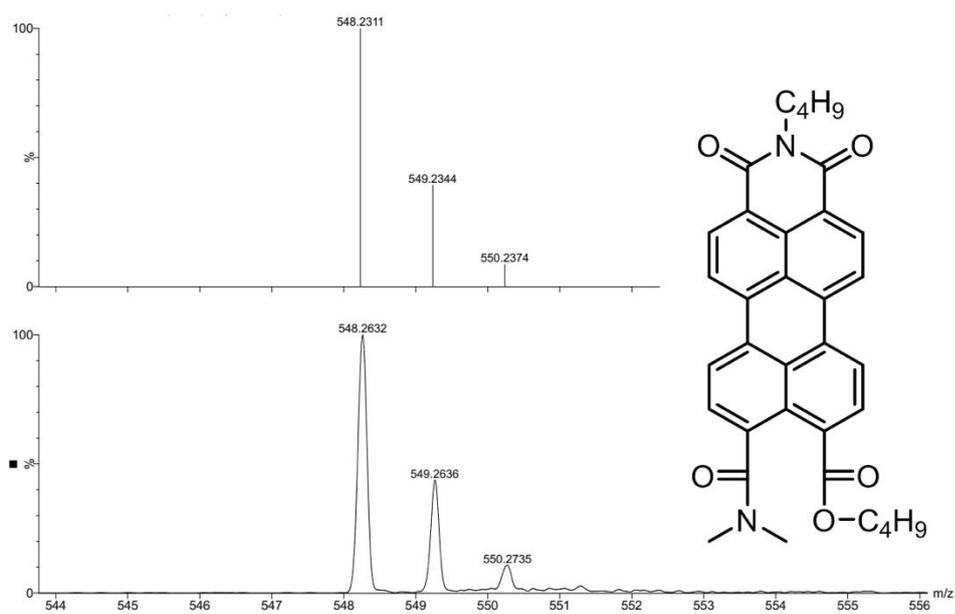
New doubly as well as singly charged intact radical molecular anions by means of LIFDI mass spectrometry are presented for the first time.

### **Co-authors:**

*H. Bernhard Linden, Linden ChromaSpec GmbH*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



LIFDI spectrum of C<sub>34</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub>, example of perylene based donor/acceptors

Poster number: **IM-PA-147**

## **MULTI-SCHEME CHEMICAL IONIZATION INLET (MION) FOR ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRY (CIMS) APPLICATIONS**

Abstract ID: **682**

**Presenting author: Jyri Mikkilä, Karsa**

### **Introduction**

Chemical ionisation mass spectrometry (CIMS) is a sensitive and selective analysis technique that enables detection of gas-phase molecular constituents at atmospheric pressure and at concentrations as low as  $10^{-5}$  cm<sup>-3</sup>. Different ionization chemistries ionize different compounds, and using several ionization schemes will give a comprehensive view on the composition of the sample, without losing the selectivity and extreme sensitivity of the chemical ionization method.

### **Methods**

We introduce the latest version of multi-scheme chemical ionization inlet (MION) capable of combining multiple ionization schemes in one instrument. The MION consists of one or more ion sources, from which the reagent ions are mixed in the sample without contaminating the sample with the neutral reagents. Switching between different reagent ions and furthermore polarities is performed in order of ~1s if the combined mass spectrometer is supporting fast polarity change. Instead of online gas phase measurements the MION can also be equipped with a filter desorber unit (Karsa Ltd.) allowing the analysis of filter samples and liquid microinjections.

### **Preliminary data (results)**

We studied the performance of the MION with several reagent ions including bromide (Br<sup>-</sup>), O<sub>2</sub><sup>-</sup>, bisulphate, Na<sup>+</sup>, H<sub>3</sub>O<sup>+</sup>. The capability of MION to switch between the ionization schemes within seconds was proven by connecting the inlet to ToF (L-TOF, Tofwerk AG) and Orbitrap (Velos pro, Thermo Fisher Scientific) mass spectrometers. Depending on the used reagent ion the total ion count per second was found to range from 100 000 to 1 000 000 which is at least an order of magnitude improvement compared to previous inlet designs for similar reagent ion schemes.

For the determination of sensitivity we connected MION with a desorber unit allowing known amount of sample from liquid microinjections to be evaporated into the gas stream. A variety of explosives and pesticides were chosen since they can be easily compared to previous results with similar chemical ionization schemes. The preliminary analysis shows LODs ranging from 100 fg to picograms which should translate to at least high ppq/s level after further analysis.

Our new MION inlet greatly expands the possibilities what can be done in the field of CIMS with one mass spectrometer. The fast switching and superb sensitivity allow for as many ionization schemes as the user finds practical, without losing much of the time resolution.

### **Please explain why your abstract is innovative for mass spectrometry?**

We have produced a new CIMS inlet, MION, capable of switching from one ionization scheme to another within seconds.

### **Co-authors:**

*Joona Mikkilä, Karsa*

*Matti Rissanen, Tampere University*

*Paxton Juuti, Karsa*

*Juha Kangasluoma, University of Helsinki*

Poster number: **IM-PA-148**

## PESTICIDES RESIDUE PRE-SCREENING USING KARSA MION INLET COUPLED TO MS WITH SELECTIVE CHEMICAL IONIZATION

Abstract ID: **684**

**Presenting author: Fariba Partovi, Aerosol Physics Laboratory, Physics Unit, Faculty of Engineering and Natural Sciences, Tampere University, Tampere, Finland, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland**

### Introduction

According to WHO, pesticides are substances or mixtures of substances intended for preventing, destroying or controlling plants from any pests, diseases or weeds, and humans from vector-borne diseases, such as malaria, dengue fever, and schistosomiasis. The increase of the pesticide usage to estimated 2.5 million tons annually worldwide since 1950, has created a number of public and environmental concerns. Currently there are no fast-screening methods for monitoring the levels of pesticides in food, and only a small percentage of all goods are being tested using the laboratory standardized methods. Here we propose that controlled chemical ionization could provide a rapid and selective means for detecting various pesticides containing different functional groups. The main aim was to determine the performance of selected ions toward each pesticide.

### Methods

The samples were analyzed using MION (Multi-Scheme Chemical Ionization) inlet with a filter desorbing unit (Karsa Ltd.) connected to ToF (H-TOF, Tofwerk AG) and Orbitrap (Velos pro, Thermo Fisher Scientific) mass spectrometers (Figure 1). The samples were injected as standard mixtures, purchased from LGC standards, or extract juice mixtures, from Finnish customs, Suvi Ojanperä. The samples were deposited on a filter kept at a constant temperature or subjected to a heating ramp after the injection. One measurement takes about 5 minutes, after which the next sample can be injected, facilitating a high throughput.

### Preliminary data (results)

We initiated the study by investigating a set of 10 representative pesticides imbedded in food samples and managed to detect 9 out of 10. After the initial study we progressed to analysing LC and GC pesticide mixtures, containing altogether 205 pesticides. At each measurement 1ng/µl of each pesticide was injected into the inlet. The target molecules are then detected as protonated or deprotonated, depending on the used polarity, or as an adduct when a reagent supply is used (water, dibromomethane and acetonylacetone were used as the reagent ion precursors). This method enables simultaneous detection of tens to hundreds of individual compounds per injection.

Altogether, with all the reagents in different modes, 115 pesticides were detected. This equals to 56 percent of all the pesticides in the mixtures including some of the most commonly used pesticides. We also studied the matrix effect by mixing two different pesticide standard solutions. The results were suggesting that at least in this target levels the existence of other targets does not affect the chemical ionization of individual pesticides. However, we will study the possible matrix effect in the upcoming work. Finally, we also measured actual extract and fruit samples and could detect the pesticides, which proves the potential of our fast pre-screening method.

These results show a promise in achieving a method devoid of sample preparation to rapidly pre-screen common food samples. Further advancements can be gained by studying the use of variety of reagent ions.

### Please explain why your abstract is innovative for mass spectrometry?

Pesticide pre-screening with minimal to no sample preparation utilizing one mass spectrometer to detect tens of common pesticides simultaneously.

### Co-authors:

*Joona Mikkilä, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland*  
*Paxton Juuti, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland*  
*Jussi Kontro, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
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 Tuesday 30 August 2022 from 14:00 to 15:30 hours

Jyri Mikkilä, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland  
 Verner Hemmilä, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland  
 Juha Kangasluoma, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland, Department of Physics and Institute for Atmospheric and Earth System Research, University of Helsinki, Helsinki, Finland  
 Aleksei Shcherbinin, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland  
 HJ Jost, Karsa Ltd., A. I. Virtasen aukio 1, 00560 Helsinki, Finland  
 Matti Rissanen, Aerosol Physics Laboratory, Physics Unit, Faculty of Engineering and Natural Sciences, Tampere University, Tampere, Finland

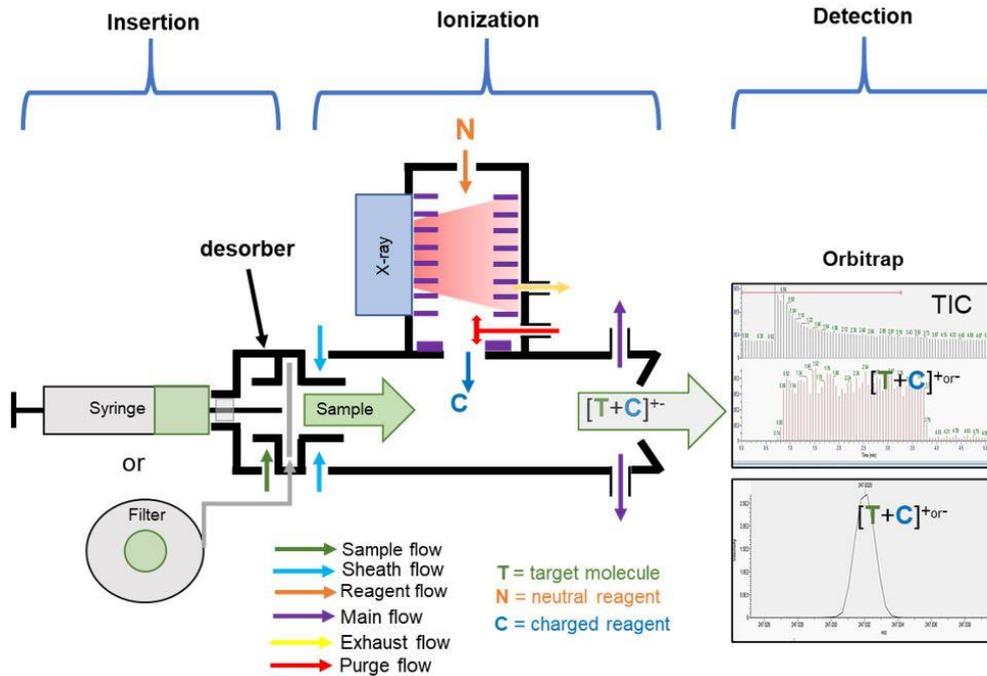


Figure 1. Calibrator and MION inlet coupled to Orbitrap MS

Poster number: IM-PA-149

## QUALITATIVE AND QUANTITATIVE DISTINCTION OF ISOMERS BY MEANS OF CHIRPED FEMTOSECOND LASER IONIZATION

Abstract ID: 702

Presenting author: Sebastian Broetz, Philipps-Universität Marburg

### Introduction

Femtosecond laser ionization mass spectrometry (fs-LIMS) is demonstrated to be a powerful analytical method providing access to the qualitative and quantitative distinction of structural isomers. The key point of the approach presented is a systematic variation of the spectral phase of the fs-laser pulses, which characteristically affects the fragmentation pattern observed in the mass spectra. Ultimately the method presented is capable of in-situ quantification of mixtures of isomers without chromatographic separation. Examples to be presented include a ternary mixture of fluorotoluenes, as well as mixtures of cineols and mixtures of linalool, geraniol and isopulegol. The accuracy of the approach is currently on the order of a few percent in molar composition.

### Methods

Laser ionization experiments have been conducted by shining fs-laser light into the ion source of a home-built linear time-of flight mass spectrometer. Control of the spectral phase is carried out in a folded 4f-pulse shaper including a liquid crystal display in the Fourier plane. fs-LIMS data have been recorded as a function of the spectral phase, with particular focus on a systematic variation of linear and quadratic chirp parameters. From the mass spectra exhibiting isomer-specific fragmentation pattern ion yield ratios of relevant signals have been extracted.

### Preliminary data (results)

Fig. 1 presents the ion yield ratio of the  $Y(m/z=109)$  (i.e. H loss) to  $Y(M^+)$  (i.e. molecular ion) signals of pure fluorotoluene isomers as a function of the linear chirp parameter  $\alpha$ . Clearly the three isomers can be unambiguously distinguished. Here the observable decreases in the order para-, ortho to meta isomer. For the subsequent quantification of a mixture composition it is essential that there are also spectral phase parameters for which this order is completely different, e.g. reversed. Ultimately the ion yield ratios of a real unknown mixture are measured for different chirp parameters and represented in terms of the contribution of pure isomer signals with the molar fractions as the adjustable parameter. These molar fractions are the result of the analysis. The approach makes the chromatographic separation of isomeric components obsolete. As an example fig. 2 presents the ternary representation of a mixture of ortho-, meta- and para-fluorotoluene with the real composition in green and the uncertainty of the fs-LIMS reconstruction as a red circle. First results of this approach have been reported in ref. [1]. More recent data will be presented, e.g. for a mixture of 1,4 Cineol and 1,8 Cineole, mixtures of terpenes (Linalool, Geraniol and Isopulegol) and mixtures of several alcohols.

[1] V. Schäfer, K.-M. Weitzel, *Analytical Chemistry*, 92, 5492–5499, (2020)

### Please explain why your abstract is innovative for mass spectrometry?

Chirped femtosecond laser ionization mass spectrometry allows quantification of mixtures of structural isomers without the need of prior chromatographic separation.

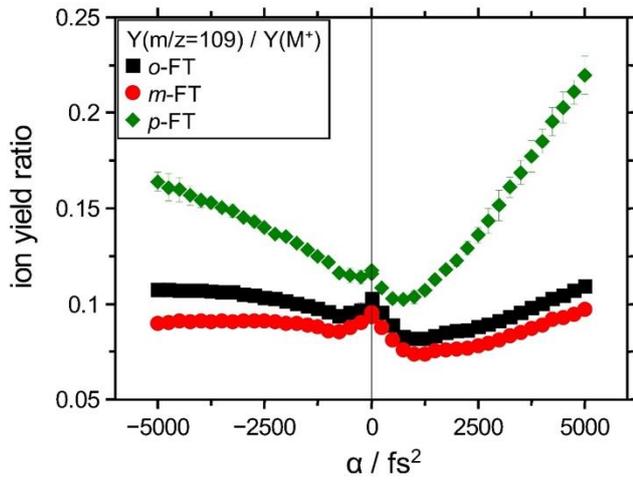
### Co-authors:

Karl-Michael Weitzel, Philipps-Universität Marburg

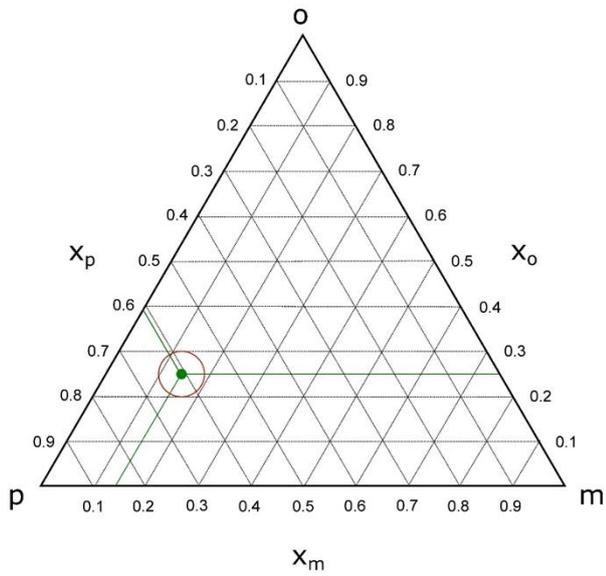
Viola Schäfer, Philipps-Universität Marburg

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Ion yield ratios for fluorotoluene isomers.



Ternary diagram of a mixture of fluorotoluene isomers.

Poster number: **IM-PA-150**

## REACTANT ION POPULATION IN HIGH KINETIC ENERGY ION MOBILITY SPECTROMETRY (HIKE-IMS) AT VARYING CORONA IONIZATION SOURCE VOLTAGE AND OPERATING PRESSURE

Abstract ID: 877

**Presenting author: Florian Schlottmann, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology, 30167 Hannover, Germany**

### Introduction

Regarding the operating pressure, High Kinetic Energy Ion Mobility Spectrometers (HiKE-IMS), being operated at pressures of 10 – 40 mbar, are settled between traditional ion mobility spectrometry and mass spectrometry. Reduced operating pressures allow for high reduced electric field strengths of up to 120 Td and lead to benefits like reduced cross-sensitivities, improvements in linearity as well as a broader range of detectable substances compared to traditional IMS. As the sensitivity in HiKE-IMS improves quadratically with increasing pressures at constant E/N, the operating pressure is now further increased to 60 mbar. Typically, HiKE-IMS use a corona ionization source for the formation of reactant ions that further ionize analytes. Here, the influence of increased pressure and corona voltage on the reactant ion population is investigated.

### Methods

Here, we investigate how pressure and operating voltage of the corona ionization source affects the reactant ion population in HiKE-IMS, as the reactant ions are crucial for product ion generation and sensitivity. In order to have comparable data, the ion mobility spectra are recorded at constant reduced electric field strength. The data are analyzed with respect to the total reactant ion current and the abundances of different reactant ion species. Especially the abundance of  $O_2^+$  is important for the ionization of non-polar substances like n-Hexane.

### Preliminary data (results)

Measurements show effects known from literature when increasing the pressure: Increasing corona ionization source ignition voltage [DOI: <https://doi.org/10.1109/TDEI.2003.1194116>], increasing total ion current [DOI: [10.1007/s13361-018-1970-6](https://doi.org/10.1007/s13361-018-1970-6)] and changes in reactant ion population [DOI: <https://doi.org/10.1021/jasms.0c00098>]. Furthermore, the amount of  $NO_x^+$  increases with increasing voltage at the corona ionization source [DOI [10.1016/j.jhazmat.2009.11.087](https://doi.org/10.1016/j.jhazmat.2009.11.087)].

However, increasing the voltage at the corona ionization source also leads to higher ion currents and the risk of an electric breakdown. When applying higher voltages to the corona ionization source at pressures above 30 mbar, a pronounced maximum total reactant ion current is observed, at even higher voltages the abundance of  $NO_x^+$  increases significantly. Thus,  $NO_x^+$  intensity can be used to identify the operating point of a corona ionization source and to avoid electric breakdown. Operation at low reactant ion current will lead to an unwanted reduced sensitivity, as sensitivity also depends on the amount of available reactant ions. Close to the maximum reactant ion current, the relative amount of  $NO_x^+$  is still significantly low and ionization is primarily driven by proton transfer reactions via  $H_3O^+$  or charge transfer reactions via  $O_2^+$ .

In summary, operation of HiKE-IMS at 60 mbar with high E/N > 100 Td is possible, necessary reactant ions such as  $O_2^+$  are available. Thus, non-polar substances like n-hexane can be detected in HiKE-IMS at concentrations below 10 ppbv, which would be impossible with traditional IMS operated at ambient pressure.

### Please explain why your abstract is innovative for mass spectrometry?

Reactant ion population of a corona discharge ionization source at different operating pressures and voltages in HiKE-IMS, applicable to mass spectrometry with corona discharge ionization.

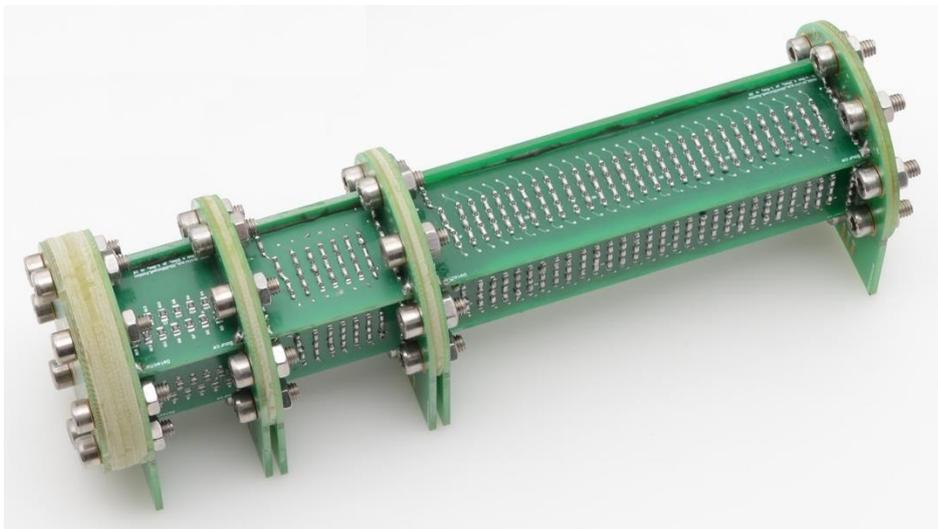
### Co-authors:

*Christoph Schaefer, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology, 30167 Hannover, Germany*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Stefan Zimmermann, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology,  
30167 Hannover, Germany*



Printed circuit board HiKE-IMS for operation at 60 mbar

Poster number: **IM-PA-151**

## ENHANCING PROTEOME COVERAGE USING NEGATIVE ION MODE PROTEOMICS

Abstract ID: **903**

**Presenting author: Pelayo Alvarez Penanes, Department of Biochemistry and Molecular Biology, University of Southern Denmark, DK5230 Odense M, Denmark**

### Introduction

Mass spectrometric analysis of proteins using negative ion mode has been overshadowed by the widespread use of positive ion mode proteomics. Application of negative ion mode is complicated by a number of challenges that need attention, such as corona discharge, spray stability and low intensity; hence, optimisation of both chromatographic and mass spectrometric conditions is of paramount importance. Moreover, interpretation of peptide fragmentation spectra has proven difficult with ion collision activation methods. We propose to investigate other parameters that can aid to reliable protein identification in negative ion mode, such as MS1-only data combined with prediction of liquid chromatography retention times and peptide charge-state-distribution.

Analysis in negative ion mode will increase protein coverage and gain further insights in the acidic part of the proteome.

### Methods

For the optimisation, several organic alkaline compounds (piperidine) at different concentrations and isopropanol (0.5-5%) as organic modifier were evaluated on a *E. Coli* tryptic digest and iRT peptide mixture, using 1-hour gradients. Once spray stability and comparable signal intensity was achieved, the best conditions were evaluated on two separate HeLa digests (trypsin and Glu-C) using short gradients (5-15 minutes) both in positive and negative polarity. All analyses were performed on an Orbitrap Lumos mass spectrometers using nano-electrospray ionization. For protein identification, a deep learning model based on exact mass and retention time has been applied.

### Preliminary data (results)

Mobile buffers at pH=9 with isopropanol at 3% concentration provided the best results in terms of spray stability and signal intensity, without compromising chromatographic performance. Higher isopropanol concentrations were detrimental for the elution of hydrophobic peptides (eluting too early in the chromatogram). Signal intensity was on the same order of magnitude as in positive ion mode (median intensity 2-3 times lower), and peak width was similar both under positive and negative polarity (with 85 % of the detected features below 4 seconds) for both trypsin and Glu-C digests. This trend has been observed for all gradients (5, 10 and 15 minutes) irrespective of the enzyme used for the digestion. Finally, the evaluation of charge-state-distribution revealed that it was reproducible and different for all 11 iRT peptides added to the mixture, implying that it can be used as an additional parameter for peptide identification.

Once the best conditions have been critically assessed and established, the final goal is the identification of the largest number of proteins possible. At the moment, the deep learning model provides an average retention time accuracy of 0.1 minutes in both polarities, further proving the validity of the conditions. Regarding mass accuracy, a 0.4 ppm mass accuracy could be achieved for data in positive ion mode and 1.4 ppm in negative. Current achievement reaches few hundreds of protein identifications in the negative mode, and further work and refinements of the methodology will pursue the goal of detecting 1,000 proteins in a single 5-minute gradient under negative polarity.

### Please explain why your abstract is innovative for mass spectrometry?

One of the first thorough studies in negative ion mode proteomics not requiring instrument modifications, with AI software application for protein identification using MS1 data acquired in this polarity.

### Co-authors:

Vladimir Gorshkov, Department of Biochemistry and Molecular Biology, University of Southern Denmark, DK5230 Odense M, Denmark

Mark V. Ivanov, V. L. Talrose Institute for Energy Problems of Chemical Physics, N. N. Semenov Federal Research

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours



*Center for Chemical Physics, Russian Academy of Sciences, Moscow 119334, Russia*

*Mihail V. Gorshkov, V. L. Talrose Institute for Energy Problems of Chemical Physics, N. N. Semenov Federal Research*

*Center for Chemical Physics, Russian Academy of Sciences, Moscow 119334, Russia*

*Frank Kjeldsen, Department of Biochemistry and Molecular Biology, University of Southern Denmark, DK5230 Odense M, Denmark*

Poster number: **IM-PA-152**

## **COLDSPRAY IONIZATION-MS: THE SEARCH FOR A MISSING LINK IN AN ARTIFICIAL PHOTOSYNTHESIS MODEL**

Abstract ID: **955**

**Presenting author: Noemí Cabello, Institute of Chemical Research of Catalonia (ICIQ), the Barcelona Institute of Science and Technology, Tarragona, Spain.**

### **Introduction**

Photosynthesis is essential for most of the life on Earth. Green plants use photosynthesis to transform carbon dioxide and water in oxygen and carbohydrates by capturing sunlight. The oxidation of water to dioxygen in the oxygen-evolving complex, the core of Photosystem II, is a milestone. Understanding this process is crucial for artificial photosynthesis development, in particular the O-O bonding formation and the species derived of it. We have studied by Coldspray ionization (CSI)-TOF-MS a ruthenium-based model to gain insight about this mechanism. This soft ionization source works at atmospheric pressure and is able to ionize compounds at low temperature. It allowed us to obtain mass spectra of the reaction of interest using the same temperature and solvent as in the synthetic version.

### **Methods**

Coldspray ionization source, designed by K. Yamaguchi and coworkers, is able to analyze ionic compounds at low temperature taking advantage of the increasing value of the dielectric constant when the temperature decreases. It is compatible with a large variety of solvents, from pure water to dichloromethane. In our case, the ruthenium-based reaction was studied by MS at the same temperature (20°C) and solvent (pure water) as these employed in the synthesis of the compounds. We coupled the Coldspray ionization source (Bruker Daltonics™) to a TOF mass spectrometer and injected directly the reaction mixture into the nebulizer, working in positive mode.

### **Preliminary data (results)**

The objective of this work is to afford some light in the oxidation of water during the photosynthesis. The step involving O-O bond formation can be explained, from a formal point of view, by two mechanisms: the coupling between two metal-oxyl radicals (M-O•) and the nucleophilic attack of water to metal-oxo species (M=O). However, evidences for the formation of metal-peroxo species under catalytic conditions after the O-O bond formation have not been reported to date. In our hands, this elusive peroxo intermediate **B** (Figure 1) has been isolated and characterized by different analytical techniques, including X-ray diffraction and mass spectrometry. It has been also generated *in situ* under catalytic conditions and detected as its [M-PF<sub>6</sub>]<sup>+</sup> directly from the reaction mixture by Coldspray ionization-TOF-MS in conditions that are relevant for catalytic water oxidation, starting from a previous reported ruthenium complex. In addition, we studied the kinetics and mechanism of the reaction by direct injection in H<sub>2</sub><sup>18</sup>O. Accurate mass data were obtained by performing calibration with clusters of proline and NaCl. Measured isotopic patterns are in good agreement with calculated ones.

### **References:**

1. C. Casadevall, et al.: Isolation of a Ru(IV) side-on peroxo intermediate in the water oxidation reaction, *Nat. Chemistry*, **13**, 800 (2021).
2. S. Sakamoto, et al.: Characterization of Self-Assembling Nano-Sized Structures by Means of Coldspray Ionization Mass Spectrometry, *Tetrahedron*, **56**, 955 (2000).
3. C. Casadevall, et al.: Spectroscopic, electrochemical and computational characterisation of Ru species involved in catalytic water oxidation. *Chem. Eur. J.* **22**, 10111 (2016).

### **Please explain why your abstract is innovative for mass spectrometry?**

CSI-TOF-MS detected intact, labile intermediates in unique conditions in the MS field: atmospheric pressure ionization, direct injection of reaction mixtures, low temperature and pure water as the injection solvent.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### **Co-authors:**

*Carla Casadevall, Institute of Chemical Research of Catalonia (ICIQ), the Barcelona Institute of Science and Technology, Tarragona, Spain.*

*Vanessa Martínez, Institute of Chemical Research of Catalonia (ICIQ), the Barcelona Institute of Science and Technology, Tarragona, Spain.*

*Carly Chédotal, Institute of Chemical Research of Catalonia (ICIQ), the Barcelona Institute of Science and Technology, Tarragona, Spain.*

*Julio Lloret-Fillol, Institute of Chemical Research of Catalonia (ICIQ), the Barcelona Institute of Science and Technology, Tarragona, Spain., Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.*

Poster number: **IM-PA-153**

## **IMPROVED SENSITIVITY FOR LOW ABUNDANT LIPIDS USING A VACUUM INSULATED HEATED ESI SOURCE**

Abstract ID: **990**

**Presenting author: Matthias Szesny, Bruker Daltonics GmbH & Co. KG**

### **Introduction**

Abundance of lipids in typical samples such as plasma extracts can vary drastically. Therefore, analytical equipment with a high sensitivity and dynamic range is required in lipidomics research. Here we present improvements in sensitivity provided by a newly introduced vacuum insulated heated ESI source (VIP-HESI) compared to a standard ESI source combined with the utilization of an extended lipid annotation capabilities using a rule-based annotation approach to minimize the number of false positive lipid IDs.

### **Methods**

Samples were analyzed on a timsTOF Pro system using negative polarity in PASEF mode. The sample set was acquired using the VIP-HESI and for comparison standard ESI source. To assess the limit of detection and dynamic range, dilution series of the commercially available SPLASH mix lipid standard (Avanti Lipids) was acquired both in the presence and absence of a reference serum (SRM1950, Sigma). Lipid annotations were determined using a rule-based lipid annotation, which applies a series of rules to observed lipid adducts and fragments to characterize lipid class and fatty acid composition.

### **Preliminary data (results)**

In comparison to the standard ESI source the VIP-HESI source shows the majority of analyzed compounds have a better LOD and an improved linear dynamic range, especially in negative mode. The LOD's were visually determined by S/N higher than 3. For some lipids, a gain in sensitivity up to a factor of 100 could be determined. The average gain in LOD for all analyzed lipids in a matrix free surrounding was around 23. All lipids showed an improved LOD by at least a factor of 3.

In matrix the average gain in sensitivity was around a factor of 5. All lipids showed improved LOD's with one exception for the PI 15:0-18:1(d7), where a matrix interference reduced the LOD slightly. For all the analyzed compounds, no higher degree of decomposition due to the heated ionization process could be observed.

The improved ionization process additionally results in a higher number of annotated lipids in the serum standard SRM 1950. To assess this, different SRM 1950 plasma extract equivalents on column were analyzed. Here an average gain of 20 % higher lipid annotations was revealed.

The improved LODs result also in a higher dynamic range. Data is presented here for the Lyso PE 18:1(d7) as a lipid example with a limit of quantification (LOQ) of 4900 ppt using standard ESI and 490 ppt using VIP-HESI. This LOQ is one order of magnitude lower whilst maintaining the linearity of quantitation.

### **Please explain why your abstract is innovative for mass spectrometry?**

The results show that the VIP-HESI source in combination with using PASEF data acquisition and rule-based annotation can lead to a deeper coverage of the lipidome.

### **Co-authors:**

*Sven Meyer, Bruker Daltonics GmbH & Co. KG*

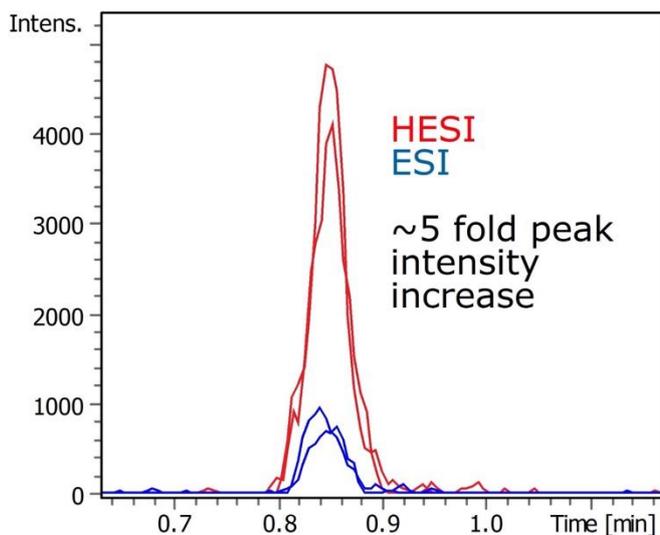
*Ansgar Korf, Bruker Daltonics GmbH & Co. KG*

*Nikolas Kessler, Bruker Daltonics GmbH & Co. KG*

*Aiko Barsch, Bruker Daltonics GmbH & Co. KG*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
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An unprecedented lipid shows a five times higher sensitivity

	Without matrix			With matrix		
	HESI neg [ppt]	ESI neg [ppt]	LOD ESI/HESI	HESI neg [ppt]	ESI neg [ppt]	LOD ESI/HESI
Lyso PC 18:1(d7)	238	793.3	<b>3.3</b>	238	2380	<b>10</b>
Lyso PE 18:1(d7)	163.3	1633.3	<b>10.0</b>	490	1633.3	<b>3.3</b>
PA 15:0-18:1(d7)	690	2300	<b>3.3</b>	6900	23000	<b>3.3</b>
PC 15:0-18:1(d7)	502	5020	<b>10</b>	1506	1506	<b>1</b>
PE 15:0-18:1(d7)	176.7	1766.7	<b>10</b>	1766.7	5300	<b>3.0</b>
PG 15:0-18:1(d7)	267	8900	<b>33.3</b>	890	8900	<b>10</b>
PI 15:0-18:1(d7)	85	8500	<b>100</b>	28333*	8500*	<b>0.3*</b>
PS 15:0-18:1(d7)	130	3900	<b>30</b>	1300	13000	<b>10</b>
SM d18:1-18:1(d9)	296	2960	<b>10</b>	986.7	2960	<b>3.0</b>
<b>Average gain</b>			<b>23.3</b>			<b>4.89</b>

LOD comparison between the VIP-HESI vs. Bruker Apollo II source

## **Session: Miniaturization, lab-on-a-chip, in situ applications**

Poster number: **IM-PA-154**

### **QUANTIFICATION OF ENDOCANNABINOIDS IN HUMAN CEREBROSPINAL FLUID USING A NOVEL MICRO-FLOW LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY METHOD**

Abstract ID: **810**

**Presenting author: Bingshu He, Leiden University**

#### **Introduction**

Endocannabinoids are a group of lipids that are involved in the regulation of numerous physiological processes. Their dysregulation in the central nervous system triggers various brain disorders such as Alzheimer's disease, Parkinson's disease, and migraine. However, the pharmaceutical and clinical studies on endocannabinoids in human cerebrospinal fluid (CSF) were sometimes hindered by low concentration levels of certain compounds such as anandamide (AEA) and 2-arachidonoyl glycerol (2-AG). Although a number of LC-MS methods have been developed for plasma, serum, and tissue, the quantification method for endocannabinoids in CSF is still in urgent requirement.

#### **Methods**

In this study, we developed a sensitive and robust method for the simultaneous quantification of endocannabinoids and related N-acyl ethanolamines in human CSF using micro-LC-MS/MS. The flow rate was downscaled to 4  $\mu\text{L}/\text{min}$  with a 0.3 mm inner diameter column, other settings, and parameters were also optimized correspondingly to adapt the microflow. Waters nanoAcquity LC coupled with Shimadzu triple quadrupole 8060 was used in this study. A minor modification to the Shimadzu Mikros Micro-ESI spray needle was carried out to improve its service life and the robustness of this method.

#### **Preliminary data (results)**

By using an injection volume of 3  $\mu\text{L}$ , our method reached limits of detection in the range from 0.6 to 1293.4 pM and limits of quantification in the range from 2.0 to 4311.3 pM while intra- and interday precisions were below 13.7%. 250  $\mu\text{L}$  CSF was required for sample preparation and 3  $\mu\text{L}$  was injected. After validation, the developed workflow was successfully used for the determination of eCBs in 288 human CSF samples.

The weak robustness of micro LC-MS methods has always been a major obstacle that keeps it away from the widespread application. Our method has shown its high sensitivity and robustness for high throughput analysis. It is promising to contribute to future brain disorder studies with human CSF samples.

#### **Please explain why your abstract is innovative for mass spectrometry?**

A minor modification of a novel spray needle was carried out to generate stable spray and improve the robustness of our method.

Poster number: **IM-PA-155**

## **MODIFIED TARGET SURFACE TO ENHANCE PERFORMANCE DURING MALDI ANALYSIS**

Abstract ID: **871**

**Presenting author: Andreas Baumeister, Shimadzu Europa GmbH**

### **Introduction**

Miniaturization, parallelization, and automation are crucial for modern approaches in drug discovery and biotechnology to maximize throughput, efficiency, and reducing expenses.

Aquarray developed a Droplet Microarray (DMA) technology for chemical synthesis, characterization and biological screening. The surface of a target slide / plate is chemically modified resulting in a high contrast in wettability between omniphilic spots and surrounding omniphobic areas. Thus, aqueous and organic liquids can be applied manually by a pipette or automated using a liquid dispenser to generate high-density arrays of nanodroplets.

Shimadzu MALDI mass spectrometers enable fast and sensitive characterization of chemical and biological samples. Here, we report a high-throughput workflow using the Aquarray DMA technology for fast sample preparation and high-sensitivity MALDI MS characterization down to the attomole range per spot.

### **Methods**

Shimadzu stainless steel targets were modified by Aquarray. Spot size (900  $\mu\text{m}$ ), distance between spots (225  $\mu\text{m}$ ) and shape of spots can be customized. This approach increased the number of samples on the target from 48 to 705.

Samples were prepared by non-contact liquid dispenser, I-DOT Mini AQ Edition (sample solution (GluFib peptide) and matrix solution (CHCA), 50 nL each) or pipetted manually (100 nL each). 80-90% of solvent was saved compared to standard sample preparation on non-modified MALDI targets (0.5  $\mu\text{L}$  each).

Two different Shimadzu bench-top instruments were used: MALDI-8020 (time-of-flight) and MALDImini™-1 (digital ion trap).

### **Preliminary data (results)**

The limit of detection for a peptide was evaluated on the MALDI-8020 bench-top TOF instrument. Glu1-Fibrinopeptide B (GluFib) could still be detected in a concentration of 10 amol on target. This is far below the specified sensitivity of 250 amol. The gain in sensitivity is about factor 25.

To compare these results, the sensitivity on the MALDImini-1 bench-top MALDI- digital ion trap (DIT) instrument was also evaluated. The limit of detection for GluFib was found to be around 50 amol. Compared to the specified sensitivity of 1 fmol, this corresponds to a gain factor of 20.

This evaluation showed that Aquarray's surface modification can enhance MALDI analysis in various dimensions:

Sensitivity: An increase by a factor of 20-25 could be observed.

Solvent consumption: The volume of sample and matrix solution could be reduced by a factor of 5-10 each.

Throughput: The number of samples on the target could be increased by a factor of 14. The use of a non-contact liquid dispenser allowed the automated sample preparation for high-throughput applications.

Both, lower solvent volume and higher sensitivity, help to save sample and facilitate analysis of rare samples. On two different MALDI instruments with two different mass analysis technologies, the approximate gain of sensitivity was around the same factor between 20-25. It is assumed that on other MALDI instruments a similar gain in sensitivity can be observed.

**Please explain why your abstract is innovative for mass spectrometry?**

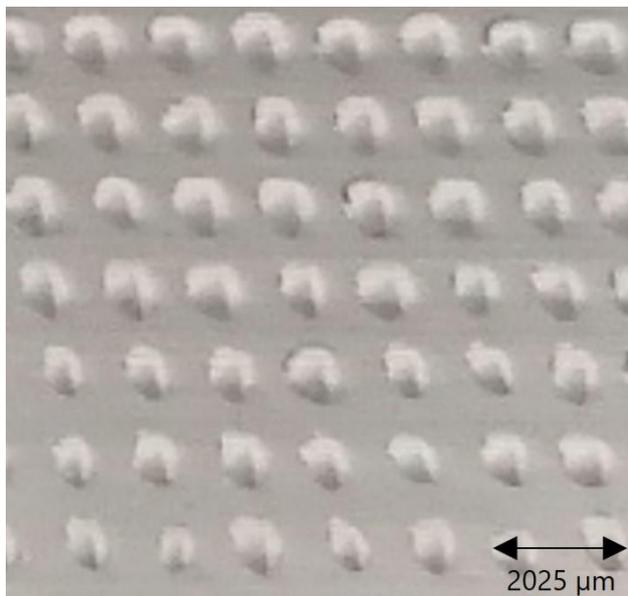
**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

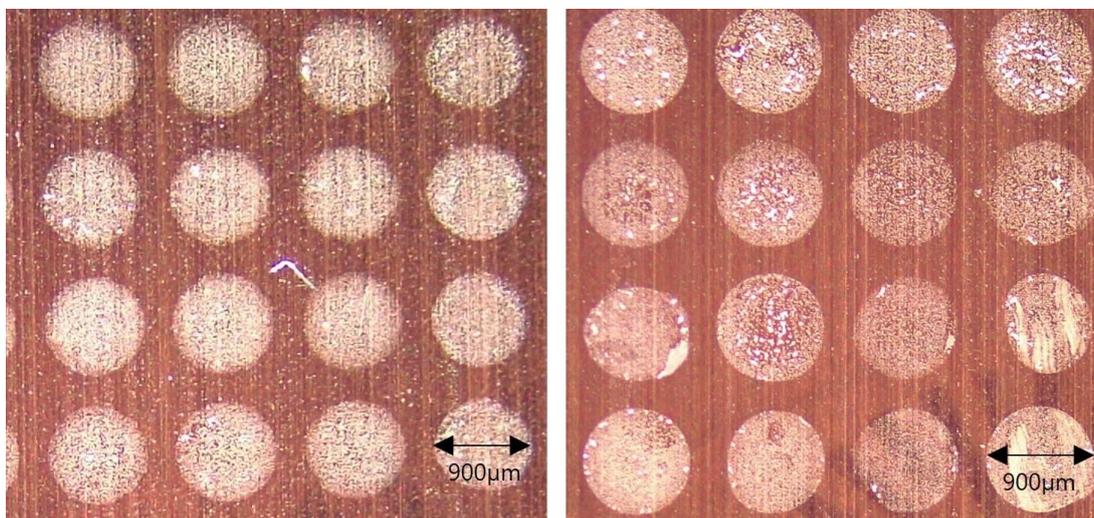
Droplet microarray surface modification of MALDI targets enhance sensitivity and throughput of the analysis and reduce sample and solvent consumption.

**Co-authors:**

*Maximilian Benz, Aquarray GmbH*  
*Holger Saal, Aquarray GmbH*



Water droplets show the omniphilic/omniphobic pattern after surface modification.



Samples prepared by non-contact liquid dispenser (left) or manually (right).

Poster number: **IM-PA-156**

## **INCREASING SENSITIVITY IN PROTEOMICS REQUIRED ALSO DECREASING SAMPLE ADSORPTION ON CONTAINER SURFACES**

Abstract ID: **969**

**Presenting author: Wafa Hechiche, SMBP LPC ESPCI Paris CNRS UMR 8249, PSL University, Sanofi Aventis R&D**

### **Introduction**

To address many medical and biological challenges, single-cell proteomic analysis of the proteome has become essential as it provides information on dynamic proteome change or cellular heterogeneity. However, the classical workflow used in proteomics is not adapted for a single cell or even for minimal quantities of biological material. It is therefore essential miniaturizing it to adapt it to a minimal amount of material. From cell isolation to LC-MS/MS analysis, each step of the sample preparation process requires careful development to increase the sensitivity of the analysis and reduce sample losses before the arrival of the sample in front of the mass spectrometer detector.

### **Methods**

In this sense, studying the container surfaces in contact with the samples is often neglected yet very important. One of the phenomena observed with proteins that represents a real challenge in single-cell proteomics is the non-specific adsorption of molecules of interest on surfaces. Thus, the adsorption of proteins and peptides limits the recovery yield and is deleterious to identify a maximum of peptides. This effect is even more visible with smaller quantities of samples.

### **Preliminary data (results)**

In the present work, we studied the surface nature to optimize sample preparation yield. We used two biocompatible materials: polypropylene (PP) and polydimethylsiloxane (PDMS). Working with a standard multiwell plate format, we decreased the wells' size to 10 $\mu$ l instead of the 58 $\mu$ L, the minimum commercially available size. By reducing the adsorption surface area, recovery and identification are expected to be improved. However, PDMS has been described to induce higher adsorption losses for specific molecules. This phenomenon can be minimized with surface treatments of these materials to limit protein and peptide adsorption on the surface.

Our results confirmed that reduction and treatment of contact surfaces are crucial parameters to improve protein recovery, identification robustness and peptide detection sensitivity when working in LC-MS/MS with low amounts of protein extracts or low numbers of human cells.

### **Please explain why your abstract is innovative for mass spectrometry?**

Reduction and treatment of contact surfaces are crucial parameters to improve protein recovery, identification robustness and peptide detection sensitivity when working in LC-MS/MS with low numbers of human cells

### **Co-authors:**

*Armelle Buzy, Sanofi Aventis R&D*

*Jean-Claude Guillemot, Sanofi Aventis R&D*

*Jacques Fattaccioli, Microfluidics Lab. ENS CNRS UMR 8640, PSL University*

*Joelle Vinh, SMBP LPC ESPCI Paris CNRS UMR 8249, PSL University*

Poster number: **IM-PA-157**

## **MINIATURIZATION OF 3D TISSUE SAMPLING WITH A NANOSECOND INFRARED LASER (NIRL) FOR DIFFERENTIAL QUANTITATIVE PROTEOMICS**

Abstract ID: **1001**

**Presenting author: Antonia Gocke, Section of Mass Spectrometric Proteomics, University Medical Center Hamburg-Eppendorf**

### **Introduction**

In recent years the usage of ultrashort pulsed infrared (IR) laser, pulsing at 2940 nm, the wavelength of the OH stretching band of water, for sample homogenization has yielded improvements in the efficiency and time needed for the sample preparation. Due to its efficiency and precision, IR lasers have shown great potential for high-resolution tissue sampling in proteomics or even single-cell proteomics. With the reduction of the tissue volume sampled, the protein yield is also limited, leading to difficulties when conducting a differential proteome analysis.

In this study we show steps in miniaturization of the sample volume and maximizing the protein yield by further improvements regarding the sample collection. The quality of the data was verified by performing a differential analysis of the proteome.

### **Methods**

Different sample volumes of bovine liver were ablated with a nanosecond infrared laser (NIRL). The aerosol was condensed on a glass slide and resuspended in buffer solution. After tryptic digestion, proteome analysis via liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed. The proteins were identified via a database search and statistically analysed with Perseus.

With the lowest sample volumes for reproducible proteomics results, different murine organs were sampled and analyzed as described above. Finally, the proteins that showed significantly different abundance in the respective organs were compared with the Human Protein Atlas to confirm organ specificity.

### **Preliminary data (results)**

In our first experiments we were able to reduce our sample volume from 700 nL down to 23 nL, corresponding to 3000 and 100 laser pulses, respectively still obtaining relatively high number of proteins. The reproducibility of the samples was confirmed by a principal component analysis (PCA) and Pearson correlation coefficients. The determination of sample volumes is based on optical coherence tomography (OCT) measurements before and after ablation.

In the second experiment, four different mouse organs (heart, colon, kidney and liver) were ablated and analyzed to confirm the applicability of the reduced sample volume. It could be shown that not only organ specific patterns were found with a high reproducibility, but also different tissue structures had no significant impact on the data quality.

For further improvements in sample preparation towards single-cell proteomics, new protocols and surfaces will be studied to eliminate unnecessary transfer steps minimizing the material loss.

### **Please explain why your abstract is innovative for mass spectrometry?**

Quantitative proteome analysis of different mouse organs could be performed on only 23 nL volumes, sampled as voxels of 500 x 500 x 100  $\mu\text{m}^3$  directly from the tissue.

### **Co-authors:**

*Manuela Moritz, Section of Mass Spectrometric Proteomics, University Medical Center Hamburg-Eppendorf*

*Killian Müller, Section of Mass Spectrometric Proteomics, University Medical Center Hamburg-Eppendorf*

*Hartmut Schlüter, Section of Mass Spectrometric Proteomics, University Medical Center Hamburg-Eppendorf*

*Jan Hahn, Section of Mass Spectrometric Proteomics, University Medical Center Hamburg-Eppendorf*

## Theme: Life sciences and health

### Session: Metabolomics

Poster number: LS-PA-002

## NMR BASED CSF METABOLOMICS IN TUBERCULOUS MENINGITIS: CORRELATION WITH CLINICAL AND MRI FINDINGS

Abstract ID: 19

Presenting author: Rashmi Parihar, CBMR SGPGIMS, LUCKNOW, INDIA

### Introduction

Tuberculosis is a major public health problem in developing countries and an emerging problem even in the western world. In 2015, there were 10.4 million new cases of tuberculosis, and 1.8 million deaths were due to tuberculosis (WHO, 2016). We report the potential of NMR-based metabolomics in CSF for discrimination of the definite and probable cases of TBM from control subjects and evaluate the correlation of metabolomics with clinical and radiological findings and outcomes.

### Methods

This is a retrospective study carried out at Sanjay Gandhi Postgraduate Institute of Medical Sciences and Center of Biomedical Research, Lucknow, India. The Institute Ethics Committee approved the project. The patients or their relatives consented to the study. **Inclusion criteria: Essential criteria: Supportive criteria: Clinical evaluation: Investigations: Cranial MRI: Treatment: Outcome: CSF collection and preparation: Acquisition of  $^1\text{H}$  NMR and data processing: Acquisition and processing of two-dimensional (2D) NMR : Metabolite Assignment: Statistical analysis:**

### Preliminary data (results)

We report the potential role of  $^1\text{H}$  Nuclear Magnetic Resonance (NMR) based metabolomics in tuberculous meningitis (TBM). We also correlate the significant metabolites with clinical-radiological parameters. Forty-three patients with TBM were included, and their severity of meningitis was graded as stages I to III, and patients with positive *M. tuberculosis* or its nucleic acid was considered as definite TBM.  $^1\text{H}$  NMR-based metabolomic study was performed on CSF samples, and the significant metabolites compared to healthy controls were identified. Outcome at three months was defined as death, poor and good based on the modified Rankin Scale. These metabolites were compared between definite and probable groups of TBM and also correlated with MRI findings. About 11 metabolites were found to be significant for distinguishing TBM from the controls. In TBM, lactate, glutamate, alanine, arginine, 2-hydroxyisobutyrate, formate, and cis-aconitate were upregulated, and glucose, fructose, glutamine, and myoinositol were downregulated compared to the controls. For differentiating TBM from the controls, the AUC of the ROC curve generated using these significant metabolites was 0.99, with a 95% confidence interval from 0.96 to 1, demonstrating that these metabolites were able to classify cases with good sensitivity and specificity. Lactate Concentration in CSF correlated with hemoglobin, CSF glucose, and infarction. The outcome did not correlate with metabolomics parameters. NMR-based CSF metabolomics has a potential role in differentiating TBM from the controls.

### Please explain why your abstract is innovative for mass spectrometry?

$^1\text{H}$  spectrum (1D  $^1\text{H}$  CPMG) of CSF samples from control, definite TBM, and probable TBM, respectively. The assignment of crucial metabolites is noted in the spectra, and the assignment.

Poster number: **LS-PA-003**

## **COMPETITIVE BINDING SCREENING ASSAY BASED ON LC-TANDEM MS FOR NATURAL PRODUCT INHIBITOR OF TNF-ALPHA**

Abstract ID: **21**

**Presenting author: Yongsoo Choi, Korea Institute of Science and Technology, University of Science and Technology**

### **Introduction**

Natural products (NPs) have played a significant role in drug discovery for diverse diseases, and numerous attempts have been made to discover promising NP inhibitors of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), a major therapeutic target in autoimmune diseases. However, NP inhibitors of TNF- $\alpha$ , which have the potential to be developed as new drugs, have not been reported for over a decade. To facilitate the search for new promising inhibitors of TNF- $\alpha$ , we developed an efficient competitive binding screening assay based on analytical size exclusion chromatography coupled with liquid chromatography-tandem mass spectrometry. Then, we will present preliminary results after applying this screening method to the NP library for the discovery of TNF- $\alpha$  inhibitors in HeLa cell.

### **Methods**

To develop a competitive binding screening assay, the known TNF- $\alpha$  inhibitor SPD304 and the NP compound were incubated with TNF- $\alpha$  in Tris buffer at room temperature for 20 min. After incubation, the mixture solution was loaded into a Bio-gel SEC column followed by spin-down. The eluted solution containing the ligand and TNF- $\alpha$  complex was washed three times, followed by another centrifugation at 13,000 g for 10 min. Then, the ligands were dissociated using 400  $\mu$ L of methanol. The ultrafiltrate containing the ligand was dried, reconstituted, and 2  $\mu$ L of the sample was injected into the LC-tandem MS system.

### **Preliminary data (results)**

The scheme illustrating the overall screening method described in the Figure. The known TNF- $\alpha$  inhibitor SPD304 was selected as a positive control or competitive ligand to validate the competitive binding screening assay using analytical SEC LC-tandem MS. First, sample of SPD304 incubated without TNF- $\alpha$  was analyzed as shown in the Figure, indicating that the small molecule SPD304 was efficiently removed after SEC and 10 kDa ultrafiltration. Next, when SPD304 was incubated with fresh TNF- $\alpha$ , a strong signal of SPD304 was detected at a retention time of 5.0 min after the process of analytical SEC. However, no SPD304 signal was detected when SPD304 was incubated with denatured TNF- $\alpha$ . This result confirmed that signal detection of SPD304 occurred only when it successfully bound to intact TNF- $\alpha$ . Using the validated method, a competitive binding screening assay will be performed with the natural product compound library. In this study, compounds with an inhibition level of SPD304 signal higher than 70% will be set as the moderate inhibitors.

### **Please explain why your abstract is innovative for mass spectrometry?**

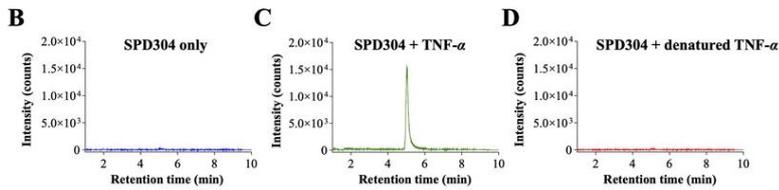
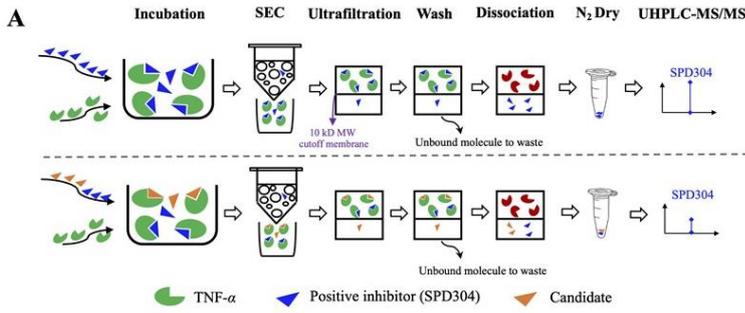
A competitive binding screening assay coupled with liquid chromatography (LC) tandem mass spectrometry (MS) was first applied for the screening of natural product inhibitors of TNF alpha.

### **Co-authors:**

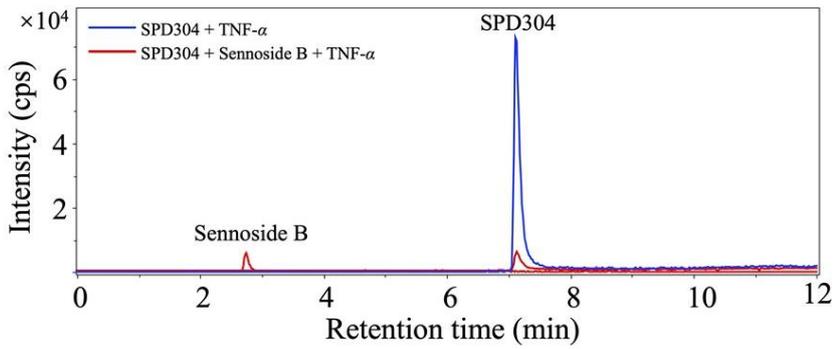
*Jeong Joo Pyo, Korea Institute of Science and Technology*  
*Keunwan Park, Korea Institute of Science and Technology*  
*Prasannavenkatesh Durai, Korea Institute of Science and Technology*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Method development and validation of competitive binding screening assay



Confirmation of competitive binding of a hit against TNF-alpha

Poster number: **LS-PA-004**

## **ANTI-INFLAMMATORY EFFECTS OF HOP BITTER ACIDS IN DENDRITIC CELLS REVEALED BY MRMS METABOLOMIC STUDIES**

Abstract ID: 23

**Presenting author: Matthias Witt, Bruker Daltonics GmbH & Co. KG**

### **Introduction**

Inflammation is a complex, evolutionarily conserved process that involves immune and non-immune cells in the host protection from harmful stimuli. Metabolic changes occur in macrophages and dendritic cells (DCs) leading to pro or anti-inflammatory phenotypes. The growing field of immunometabolism aims to target specific metabolic pathways to modulate inflammation, and natural compounds are more and more used for preventive action. To understand the metabolic shift toward anti-inflammatory phenotypes, metabolomics has emerged as prominent approach. In this study we employed flow injection analysis magnetic resonance mass spectrometry (FIA-MRMS) to highlight the metabolic changes in dendritic cells stimulated with lipopolysaccharide (LPS) and co-treatment with Hop Bitter acids. Results will underline a net metabolic reprogramming after treatment with Hop bitter acids.

### **Methods**

DCs from murine bone marrow (BMDCs) were generated from six- to eight-week-old mice. On day 10 of culture 25  $\mu$ M quercetin or 25  $\mu$ g/ml Hop B or Hop C fractions, were administered to the culture medium. Later DCs were stimulated with 1  $\mu$ g/ml of Salmonella typhimurium LPS. And supernatants were collected and stored for further analysis. Metabolites were extracted from cell pellets with ice cold methanol, the extracts were resuspended in MeOH and analyzed on an MRMS solarix XR 7T mass spectrometer using Electrospray ionization in positive and negative mode.

### **Preliminary data (results)**

Hop B and C fractions are mainly composed from alpha and beta bitter acids. BMDCs stimulated with LPS previously exposed to 25 $\mu$ g/ml of Hop B and C fractions respectively, showed a reduced production of several inflammatory cytokines including IL-6, IL-1 $\alpha$ , IL-1 $\beta$  and TNF in a cytokine assay. The C fraction was significantly more effective of B fraction. To elucidate the metabolic change in BMDCs we employed FIA-MRMS which enabled the rapid profiling of numerous key cellular metabolites involved in the shift from oxidative phosphorylation (OXPHOS) to glycolysis, in particular nucleosides and nucleotides, pentose phosphate pathway (PPP) and tricarboxylic acids cycle intermediates, fatty acids, glycerophospholipids and amino acids. Hop-C was effective to reduce the levels of many inflammatory hallmark metabolites such as succinate and citrulline, PPP intermediates, free fatty acids, revealing a shift toward an anti-inflammatory phenotype.

### **Please explain why your abstract is innovative for mass spectrometry?**

MRMS metabolomic studies provide metabolic changes in BMDCs stimulated by LPS and co-treated with Hop bitter acids.

### **Co-authors:**

*Emanuela Salviati, University of Salerno*

*Giulio Verna, University of Salerno*

*Marcello Chieppa, National Institute of Gastroenterology "S. de Bellis"*

*Eduardo Sommella, University of Salerno*

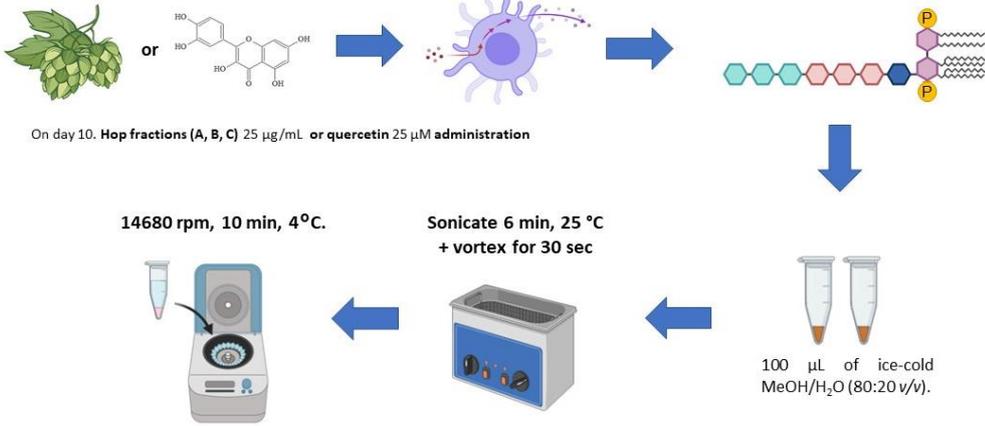
*Pietro Campiglia, University of Salerno*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

DCs from murine bone marrow (BMDCs) from six- to eight-week-old mice.

24 h later LPS stimulation (1  $\mu$ L/mL



Sample preparation protocol for metabolite extraction

Poster number: **LS-PA-005**

## **ACCURATE MASS SPECTRAL AND RETENTION INDEX LIBRARY FOR METABOLOMICS BASED ON QUADRUPOLE TIME-OF-FLIGHT GAS CHROMATOGRAPHY/MASS SPECTROMETRY**

Abstract ID: 37

**Presenting author: Remko Van-Loon, Agilent Technologies, Inc.**

### **Introduction**

Reference mass spectral libraries play an important role in compound identification of small molecules and metabolites. Databases for metabolic profiling commonly use derivatization techniques and retention time matching for GC/MS based studies. The use of high-resolution and accurate mass quadrupole-time-of-flight mass spectrometers makes it possible to use accurate mass spectral data to improve the confidence in compound annotation. Chemical ionization as a soft ionization method allows for improved detection of molecular ions and subsequent elemental formula calculations. Here we present a new accurate mass spectral and retention index library with both electron ionization (EI) and chemical ionization (CI) mass spectra.

### **Methods**

Metabolite reference standards were selected according to their frequency of occurrence from over 450 published GC-MS based studies at the Metabolomics Workbench website. A high-resolution accurate mass quadrupole-time-of-flight mass spectrometer connected to gas chromatography was utilized to acquire mass spectral data. Compounds were derivatized before measurement including a methoximation and trimethylsilylation process. Fatty acid methyl ester retention indices (FAMES) were utilized as retention index markers.

### **Preliminary data (results)**

Accurate mass spectra and retention indices of 600 unique metabolites, totaling 800 individual entries are covered in this library. The compound classes include lipids, amino acids, fatty acids, amines, alcohols, sugars, amino-sugars, sugar alcohols, sugar acids, organic phosphates, hydroxyl acids, aromatics, purines, and sterols as methoximated and trimethylsilylated mass spectra under 70 eV electron ionization. 25% of those spectra also have chemical ionization spectra recorded.

To test the metabolite coverage of the library five mouse samples (brain, kidney, liver, plasma, and serum) were acquired by GC-QTOF and processed with the Agilent MassHunter software and compared to results from the publicly available MS-Dial software.

### **Please explain why your abstract is innovative for mass spectrometry?**

An accurate mass spectral and retention index library for electron ionization and chemical ionization for metabolic profiling of biological matrices.

### **Co-authors:**

*Honglian Ye, University of California, Davis*  
*Wasim Sandhu, University of California, Davis*  
*Shunyang Wang, University of California, Davis*  
*Luis Valdiviez, University of California, Davis*  
*Tobias Kind, University of California, Davis*  
*Oliver Fiehn, University of California, Davis*

Poster number: **LS-PA-006**

## **SUPERCHARGING IN LC-MS/MS HORMONE ANALYSES: ENHANCING IONIZATION, NOT SENSITIVITY**

Abstract ID: **50**

**Presenting author: Vera de Kleijne, Endocrine Laboratory, Department of Clinical Chemistry, Amsterdam UMC, University of Amsterdam**

### **Introduction**

Hormone analysis plays an important role in the diagnosis and follow-up of endocrine disorders. Although hormones are frequently measured using immunoassays past decades, more and more hormones are nowadays measured using LC-MS/MS, due to the improved specificity and the ability to measure hormones simultaneously. However, it remains challenging to quantify hormones at very low concentrations using LC-MS/MS. One of the critical steps during hormone analysis that affects the sensitivity of the assay is the ionization process. Enhancing ionization efficiencies by the addition of supercharging reagents might be one way to improve sensitivity and to reduce the lower limit of quantitation.

### **Methods**

Therefore, we investigated whether the addition of the supercharging reagents *m*-nitrobenzyl alcohol (*m*-NBA), sulfolane, propylene carbonate, and *o*-nitroanisole increased ionization efficiency and improved assay sensitivity of insulin, oxytocin, sex steroids, and corticosteroids in stock solutions. Additionally, the influence of the supercharging reagents was tested in serum samples after sample pretreatment, to determine whether ionization would be enhanced similarly in routine analyses, and, subsequently, lead to higher sensitivity.

### **Preliminary data (results)**

The screening experiments showed that the impact of the supercharging reagents varied for each hormone; although the addition of *m*-NBA increased the signal of all hormones, ranging from 1.1- to 21-fold, the other reagents only enhanced ionization efficiencies for some hormones. Adding supercharging reagents during the hormone analysis in serum samples resulted in increased ionization as well. While the addition of these supercharging reagents did result in a signal increase in both stock solutions and serum samples, it did not improve the signal-to-noise ratio, as a simultaneous increase in noise was observed. In conclusion, even though supercharging reagents can enhance ionization efficiencies of hormones significantly, the findings from our study show that the addition of these reagents does not result in an improved sensitivity for hormone measurements with LC-MS/MS.

### **Please explain why your abstract is innovative for mass spectrometry?**

The addition of supercharging reagents does not result in improved sensitivity for hormone measurements with LC-MS/MS.

### **Co-authors:**

*Annemieke Heijboer, Endocrine Laboratory, Department of Clinical Chemistry, Amsterdam UMC, University of Amsterdam, Endocrine Laboratory, Department of Clinical Chemistry, Amsterdam UMC, Vrije Universiteit Amsterdam*  
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*Mariëtte Ackermans, Endocrine Laboratory, Department of Clinical Chemistry, Amsterdam UMC, University of Amsterdam*

Poster number: **LS-PA-007**

## **METABOLIC SIGNATURE PANEL SURROGATES THE HUMAN DRUG EXPOSURES - A METFORMIN MODEL**

Abstract ID: **69****Presenting author: Anas Abdel Rahman, King Faisal Specialist Hospital and Research Center, Alfaisal University**

### **Introduction**

Pharmaceuticals are exogenous molecules that drastically perturb endogenous metabolism, instantly reflecting the phenotype. In patients, a drug interaction is expected to return the metabolic profile to a healthy state in case of a positive therapeutic response. A unique metabolomics pattern is expected for drug interactions with multiple targets in healthy subjects. Untargeted metabolomics covered the maximum number of detected molecules. It categorized them based on their importance for identification using the metabolome-wide association study (MWAS) framework which was integrated into this study. MWAS evaluates the significant chemical with the disease outcome or biological response. Metformin, the first drug to be approved for human use without a clear mechanism of action, was our first model to be studied, where specific metabolic and lipidic patterns were reported.

### **Methods**

This project mainly relies on multiple time point samples collected from healthy volunteers after exposure to a single dose of medication. In the metformin model, plasma samples were collected from 26 healthy subjects at five-time points. These samples were analyzed using untargeted metabolomics and lipidomics analytical pipelines (**Figure 1**). The MWAS framework was used to find the endogenous metabolomics and lipidomics profile directly associated with each drug based on the known and unknown therapeutic target(s). The drug's specific patterns were validated with other drugs' patterns for more comprehensive drug interactions.

### **Preliminary data (results)**

A total of 111 metabolites involved in various biochemical processes were perturbed, with branched-chain amino acid (BCAA) being the most significantly altered pathway. Additionally, the Pearson similarity test revealed that 63 metabolites showed a change in their levels dependent on metformin level. Out of these 63, the level of 36 metabolites was significantly altered by metformin. Significantly altered metformin-dependent metabolites, including hydroxymethyl uracil, propionic acid, glycerophospholipids, and eicosanoids, pointed to fundamental biochemical processes such as lipid network signaling, energy homeostasis, DNA lesion repair mechanisms, and gut microbiota functions that could be linked to the multiple beneficial roles of metformin. The distinctive metabolic pattern linked to metformin exposure can be used as a metabolic signature to predict and assess the pharmacological effects of other drugs and chemical compounds.

### **Please explain why your abstract is innovative for mass spectrometry?**

This research program will be introduced as a computational pipeline interfaced with MS data. This platform will help reduce the drug effects in metabolomics for more reliable clinical studies

### **Co-authors:**

*Lina Dahabiyeh, The University of Jordan*

*Muhammad Mujammami, King Saud University*

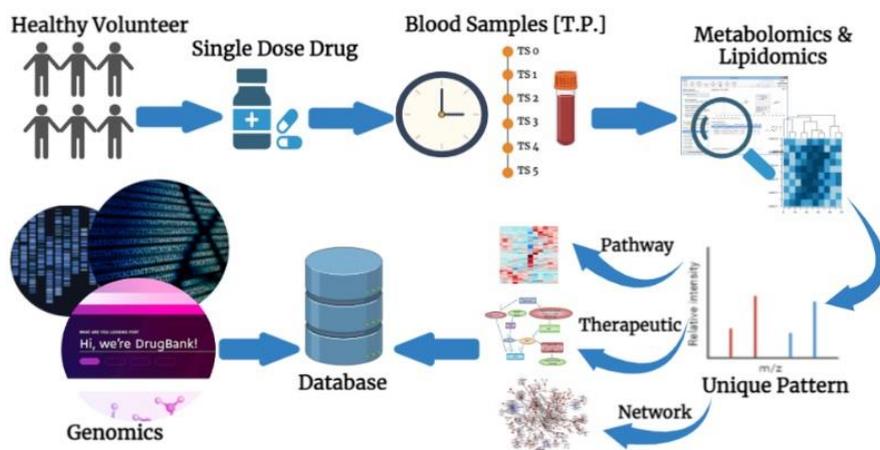
*Tawfiq Arafat, Jordan Center for Pharmaceutical Research*

*Hicham Benabdelkamel, King Saud University*

*Assim Alfadda, King Saud University*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



*Workflow of developing a database-based on single-dose human drug exposome*

Poster number: **LS-PA-008**

## **THE USE OF DRIED BLOOD SPOT SAMPLE FOR LC-MS/MS MONITORING OF PATIENTS WITH TYROSINEMIA TYPE I.**

Abstract ID: 171

**Presenting author: Josef Bártl, Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic**

### **Introduction**

Hereditary Tyrosinemia Type I (Tyr I) is an inborn error of tyrosine catabolism caused by fumarylacetoacetate hydrolase enzyme deficiency resulting in hepatic failure with comorbidities involving the renal and neurologic systems. There is a nitisinone (NTBC) drug treatment, that successfully reduces the production of toxic metabolites such as succinylacetone (SUAC) but results in hypertyrosinemia which must be mitigated by dietary restriction of tyrosine (Tyr).

### **Methods**

In order to assess the treatment effect, we developed LC-MS/MS method algorithm monitoring relevant concentration of Phenylalanine, Tyr, SUAC and NTBC based on dried blood spot (DBS) sampling. We change the common paradigm using plasma sample for NTBC determination by employing DBS sample instead.

### **Preliminary data (results)**

Compared with NTBC plasma concentration levels, DBS method exhibited a negative bias – 52% due to the lower concentration of this analyte in erythrocytes. This fact should be taken account during the matrix NTBC method transition. By employing 250 control samples the reference range for SUAC concentration in DBS was established 0.1 - 1.7  $\mu\text{mol/L}$ .

*Supported by MH CZ – DRO VFN64165.*

### **Please explain why your abstract is innovative for mass spectrometry?**

Use of DBS samples may facilitate and simplify the monitoring of patients with Tyr I due to the ease of collection and transport and may enhance efficacy in diagnosis procedure.

### **Co-authors:**

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*Karolína Kukačková, Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic*

*Renata Pinkasová, Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic*

*Daniela Píčová, Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic*

*Tomáš Honzík, Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic*

*Jiří Zeman, Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic*

*Karolína Pešková, Department of Pediatrics and Inherited Metabolic Disorders, General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic*

Poster number: **LS-PA-009**

## **INVESTIGATIONS INTO THE IN VITRO METABOLISM OF hGH AND IGF-I EMPLOYING STABLE-ISOTOPE LABELLED DRUGS AND MONITORING DIAGNOSTIC IMMUNUM IONS BY HIGH RESOLUTION/HIGH ACCURACY MASS SPECTROMETRY**

Abstract ID: **182**

**Presenting author: Sophia Krombholz, Institute of Biochemistry/ Center for Preventive Doping Research, German Sports University Cologne**

### **Introduction**

Studying the metabolism of prohibited substances is an essential element in anti-doping research in order to facilitate and improve detectability. Whilst pharmacokinetic studies on healthy volunteers are valuable, they are often difficult, not least due so safety reasons and ethical constraints, especially concerning peptidic substances which have to be administered parenterally. Hence, there is a growing need for suitable in vitro models and sophisticated analytical strategies to investigate the metabolism of protein- and peptide-derived drugs. These include human growth hormone (hGH) and its main mediator insulin-like growth factor-I (IGF-I), both prohibited in professional sports for their anabolic and lipolytic effects, while challenging in their detection, as they occur naturally in the human body.

### **Methods**

Within this study, the in vitro metabolism of hGH and IGF-I was investigated using a stable isotope-labelled reporter ions screening strategy (IRIS). After incubation in various biological fluids and tissue lysates (human skin S9 mix, liver s9 mix, serum and urine), a combination of liquid chromatography-high resolution mass spectrometry (LC-HRMS) and characteristic immonium ions generated by internal dissociation of the stable-isotope labelled peptidic metabolites enabled the detection and afterwards the sequencing of specific fragments. Additionally, the stability of several metabolites in human serum was assessed, as a first approach to examine their applicability as target analytes in sports drug testing.

### **Preliminary data (results)**

The formation of a variety of different metabolites for hGH and IGF-I in different biological fluids could be demonstrated and the corresponding structures determined within this study. A total number of 13 peptide fragments was successfully identified for hGH, with extensive metabolism observed in skin S9 mix and urine. Compared to the intact peptide, relatively small and predominantly singly or doubly charged fragments were found, comprising between 6 and 16 amino acids. Generally, our findings demonstrated a relatively high stability of IGF-I in serum, while a pronounced formation of metabolites was observed in skin and liver S9 mix. 9 metabolites of IGF-I were identified, with sequences ranging between 8 and 66 amino acids. Albeit most of the smaller fragments of hGH and IGF-I seem to be subject to further degradation in human serum, the considerable metabolism of both peptides in skin S9 mix provides a promising basis to potentially use these fragments to uncover the subcutaneous administration of the recombinant peptide hormones in future anti-doping applications. Overall, the applicability of the presented IRIS approach to larger and structurally complex peptides was shown, substantiating how this tool can be used to discover metabolites of further peptide-based drugs.

### **Please explain why your abstract is innovative for mass spectrometry?**

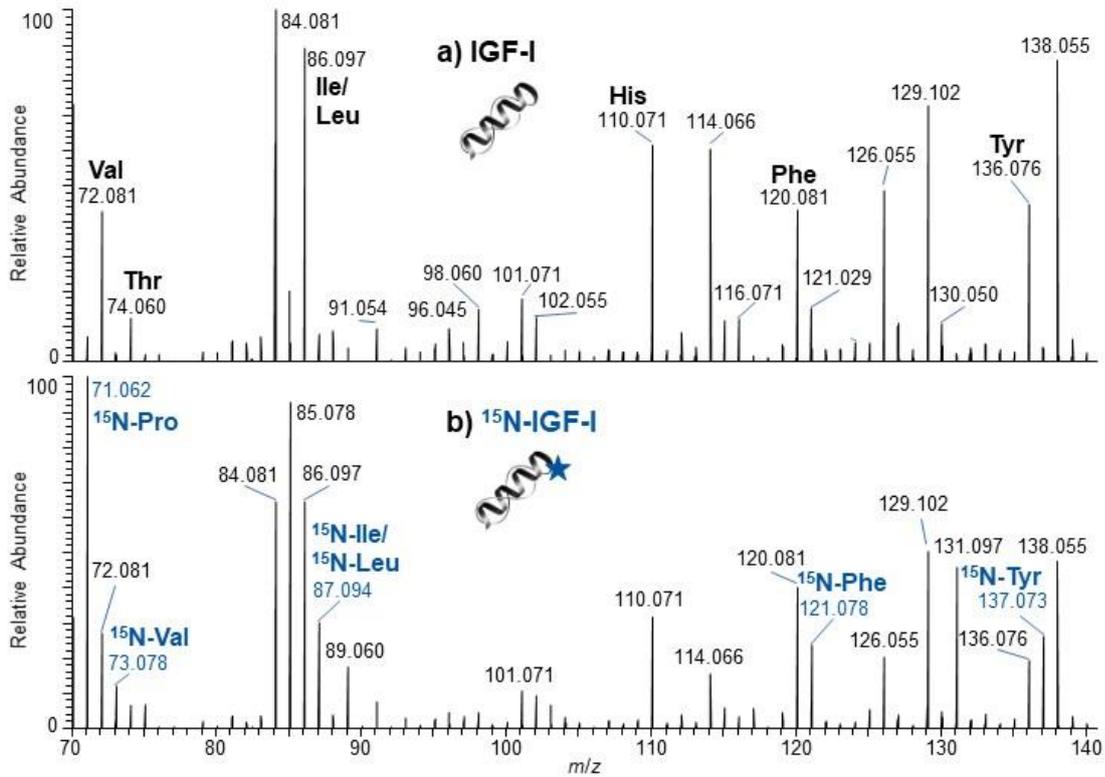
The study demonstrates how the combination of LC-HRMS and IRIS, using uniformly stable-isotope labelled peptides, can be used to explore the metabolism of structurally challenging peptide-based drugs.

### **Co-authors:**

*Andreas Thomas, Institute of Biochemistry/ Center for Preventive Doping Research, German Sports University Cologne  
Mario Thevis, Institute of Biochemistry/ Center for Preventive Doping Research, German Sports University Cologne,  
European Monitoring Center for Emerging Doping Agents (EuMoCEDA) Cologne/Bonn*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



AIF mass spectra depicting non-labelled and <sup>15</sup>N-labelled immonium ions.

Poster number: **LS-PA-010**

## **DEVELOPMENT, VALIDATION AND APPLICATION OF AN LC-MS/MS METHOD TO INVESTIGATE THE ROLE OF GLUTAMINE: GLUTAMATE RATIO AS A POTENTIAL PROGNOSTIC BIOMARKER IN A HEART FAILURE COHORT**

Abstract ID: **337**

**Presenting author: Helen Jordan, University of Leicester, Shimadzu**

### **Introduction**

26 million people live with a diagnosis of heart failure globally, this is set to increase with ageing populations. Patients with heart failure present with non-specific symptoms including chest pain and breathlessness. Diagnosis cannot be made on symptoms alone and is obscured by comorbidities. Current guidelines suggest measurement of serum BNP, with levels greater than >400ng/litre triggering further investigation. However levels of BNP are affected by not only heart failure but also obesity, renal function and pulmonary diseases. There is a need for new diagnostic biomarkers to improve accuracy and efficiency in diagnosing heart failure.

This presentation outlines the development and validation of an LC-MS/MS method in order to explore the potential of glutamine: glutamate ratio as a diagnostic biomarker of heart failure

### **Methods**

OPLS-DA and PCA analysis and Kruksal-Wallis tests identified significant m/z of interest from previous untargeted studies. The use of pathway searches, and metabolite databases found potential identities for those m/z. This formed the basis of targeted work.

Sample preparation was achieved by protein precipitation using 80% acetonitrile. Separation by liquid chromatography used an Aquity BEH 1.7 $\mu$ m, HILIC column, 100  $\times$  2.1mm, with a run time of 2 minutes. Detection was performed on the Shimadzu 8050 triple quadrupole

The method will be applied to 600 human plasma samples as part of a clinical study.

### **Preliminary data (results)**

7 metabolites were found to be significantly different ( $p < 0.05$ ) between heart failure, COPD and healthy groups of the clinical cohort. Of these 7 metabolites, the levels of L-glutamic acid (also known as glutamate) were found to be significantly different ( $p < 0.000$ ). L-Glutamic acid levels and their ratio with glutamine are reported in the literature as a potential prognostic biomarker for heart failure. Its diagnostic abilities have not yet been reported.

The LC-MS/MS method is currently being fully validated following FDA guidelines. Calibration curves were linear for all metabolites from 1 $\mu$ M to 5000 $\mu$ M with  $R^2$  values of 0.98 or better. Accuracy within-run was between 92-107% across the full range of concentrations. The between-run accuracy was between 88-112%. The within-run precision was between 4-11%.

### **Please explain why your abstract is innovative for mass spectrometry?**

Development of LC-MS/MS method for quantitation of glutamine and glutamate with short analysis time per sample (2 minutes) compared to commercially available full amino acid panels (>30 minutes).

Poster number: **LS-PA-011**

## **BUILDING MULTIDIMENSIONAL IN-HOUSE METABOLOMICS LIBRARIES FOR UNTARGETED METABOLOMICS WITH OPEN-SOURCE TOOLS**

Abstract ID: **410**

**Presenting author: Katyeny Manuela Da Silva, University of Antwerp**

### **Introduction**

Liquid chromatography (LC) - high resolution mass spectrometry (HRMS) is widely used to detect a broad range of metabolites in untargeted metabolomics. It allows the obtention of retention time (RT) and mass/charge ( $m/z$ ) information with an extended dynamic range and high mass accuracy in MS and MS/MS mode. Ion mobility (IM), hyphenated to LC-HRMS, is gaining significant interest to help increase confidence in annotation by using collision cross section (CCS) information. Despite advances in the field, annotation remains one of the bottlenecks in metabolomics due to the limited availability of high-quality MS/MS spectra (and CCS values) in public databases. In this study, a systematic workflow was developed to create a multidimensional metabolite library with open-source tools and inclusion of quality control (QC).

### **Methods**

Mass spectral data were acquired using three collision energies in an Agilent 6530 QTOF and 6560 drift tube (DT) IM-QTOF. The latter was also used to acquire  $^{DT}CCS_{N_2}$  values of different adduct ions. Three LC columns were used to obtain RT information in positive (+) and negative (-) electrospray ionization (ESI) modes (HILICON iHILIC-Fusion ESI +, HILICON iHILIC-Fusion(P) ESI - and ACQUITY UPLC BEH C18 ESI +/-). After compound lists generation, MS/MS spectra for different adducts were created using an R script created by Mass Bank EU. The data were converted to NIST msp format and evaluated using MS-LIMA.

### **Preliminary data (results)**

The workflow was used to create libraries for 608 polar metabolites and 147 lipids using different LC columns and ESI modes. Until now, 332 adduct ions were included. The RMassBank workflow creates text files with the instrumental conditions and chemical information. Different product ions were putatively annotated with the molecular formula. In order to evaluate whether the characteristic product ions of the compounds were retained after data processing, MS/MS spectra of different metabolite classes were elucidated with putative structures for visible fragment comparison with literature (e.g., triacylglycerols, amino acids, nucleobases, fatty acyls). The 95<sup>th</sup> percentile of the relative standard deviation of the RT of different compounds injected over 12 h was used as a QC criterion for inclusion (1% for HILICON iHILIC-Fusion ESI +). A total of 166 CCS values were already calculated (83 in ESI+ and 82 in ESI-). For each dataset, QA compounds showed an average CCS error <0.5% compared to experimental values in the CCS compendium. The already obtained results were compared to public spectral data within the GNPS environment. For metabolites with limited fragmentation, such as small amino acids, the cosine similarity score was usually high (> 0.8) when compared to other HRMS MS/MS data, however, when comparing metabolites such as prostaglandin E<sub>2</sub>, the score was < 0.05. This latter highlights the value of in-house shareable libraries to improve spectra matching and the addition of orthogonal information, such as RT and CCS values, to improve confidence in annotation.

### **Please explain why your abstract is innovative for mass spectrometry?**

Systematic user-friendly workflow based on open-source tools to create multidimensional high-quality libraries for LC-(IM)-HRMS untargeted metabolomics.

### **Co-authors:**

*Maria van de Lavoie , University of Antwerp*

*Rani Robeyns , University of Antwerp*

*Elias Iturraspe , University of Antwerp, Vrije Universiteit Brussel*

*Lisa Verheggen , University of Antwerp*

*Adrian Covaci , University of Antwerp*

*Alexander L.N. van Nuijs , University of Antwerp*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

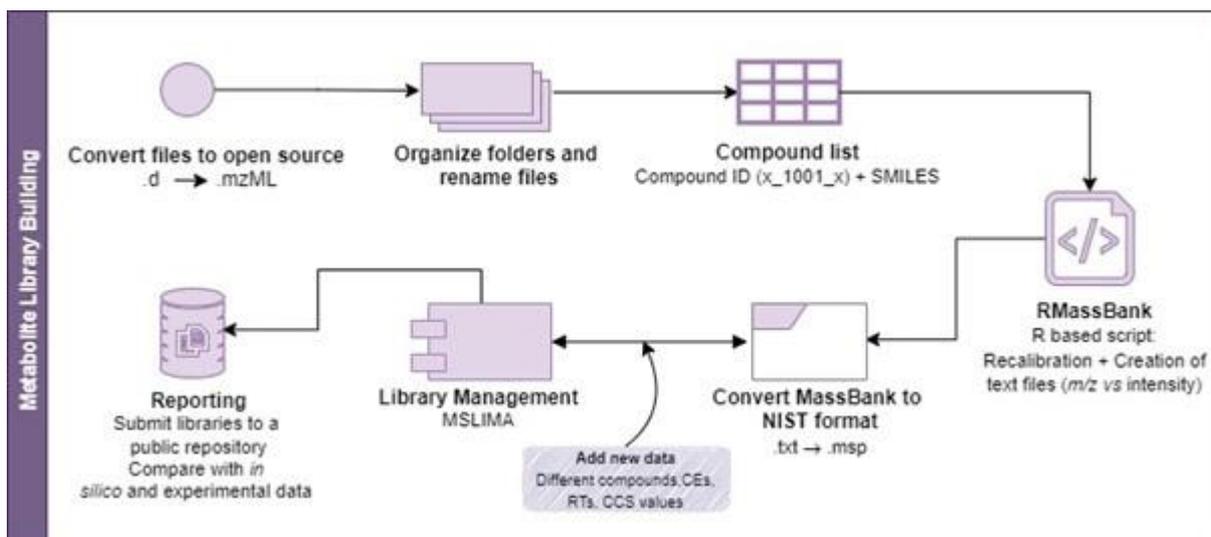


Figure 1. Library building workflow with open-source tools.

Poster number: **LS-PA-012**

## **APPLICATION OF THE HRMS TO METHOD DEVELOPMENT AND VALIDATION OF THE QUANTITATION OF TECOVIRIMAT IN HUMAN PLASMA.**

Abstract ID: **440**

**Presenting author: Alexander Chernonosov, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia**

### **Introduction**

Smallpox is a highly contagious disease; it is only slightly contagious inferior to measles and chickenpox. Until recently, there were no effective drugs to treat smallpox. However, in July 2018, the US Food and Drug Administration (FDA) registered the first smallpox drug, Tecovirimat (TPOXX). In Russia, an analogue of tecovirimat, a new chemical compound - NIOCH-14 was obtained, which has a similar activity against orthopoxviruses. This compound is a prodrug for tecovirimat and is converted into it when ingested. Due to the novelty of tecovirimat and NIOCH-14, a series of clinical, preclinical, toxicological etc. studies are required. For these purposes, developed and validated methods for the quantitative determination of tecovirimat in human plasma are required.

### **Methods**

This research aims to develop and validate the high-resolution mass spectrometry parallel reaction monitoring (PRM) method of tecovirimat quantification in human plasma and compare it with previously reported MRM methods. The PRM method allows more accurate determination of analytes using larger tolerance (up to 10 ppm) due to simultaneously accurate detection of not only the whole molecular ion but its fragments. At the same time, the aggregation of the peak areas of different fragments increases the sensitivity of the method due to the higher signal, in comparison to methods where only whole molecules are analyzed by the HRMS method.

### **Preliminary data (results)**

This research demonstrated the PRM method's advantages in LC-HRMS analyses, which allows us to reach LOQ 10 ng/ml. The PRM method was validated in precision, accuracy, selectivity, and linearity within the 10-5000 ng/ml concentration range following FDA guidelines. In addition, the samples containing tecovirimat showed good stability after three freeze-thaw cycles, within 48 hours at 4 C in autosampler and storage for 1.5 months at -20 C. Thus, the PRM method shows could be applied for tecovirimat and NIOCH-14 determination in preclinical or clinical pharmacokinetic studies.

The work was supported by a Russian government-funded project for ICBFM SB RAS (22-24-00697).

### **Please explain why your abstract is innovative for mass spectrometry?**

This HRMS method was developed to improve the tecovirimat' molecule detection accuracy without compromising the rest of the method parameters.

### **Co-authors:**

*Galina Zakabluk, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia*

*Vladimir Koval, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia, Novosibirsk State University, Pirogova Str. 2, Novosibirsk, 630090, Russia*

Poster number: **LS-PA-013**

## **DEVELOPMENT AND VALIDATION OF A MULTIPLE REACTION MONITORING METHOD TO DETERMINE TECOVIRIMAT AS THE PRIMARY NIOCH-14 METABOLITE IN HUMAN PLASMA.**

Abstract ID: **444**

**Presenting author: Galina Zakabluk, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia**

### **Introduction**

Even though in 1980 WHO declared the eradication of smallpox, there are still two places of storage of the variola virus in the world. There are also zoonotic orthopoxvirus infections that pose a danger to humans. In 2018, SIGA Technologies developed tecovirimat (TPOXX, ST-246), the first drug in the world to be approved by the US Food and Drug Administration to treat smallpox. Another one is NIOCH-14 synthesized in Russia as an analogue of tecovirimat. The NIOCH-14, being a prodrug of tecovirimat, shows similar activity against smallpox and metabolizes into tecovirimat in the body. Therefore its pharmacokinetic properties could be characterized by tecovirimat detection. Thus, a developed and validated method is required to quantify the concentration of the drug in human plasma.

### **Methods**

Experiments were carried out to develop and validate the method of mass spectrometric monitoring of multiple reactions (MRM) for the subsequent quantitative determination of tecovirimat in blood plasma. MRM is a highly specific and sensitive mass spectrometry method that is able to selectively determine the number of compounds in complex mixtures. For each compound, only a specific parent ion and corresponding specific daughter ions are isolated, but only one daughter ion is selected for analysis. Since all other ions are ignored, the method becomes more sensitive while maintaining exceptional accuracy.

### **Preliminary data (results)**

We validated the LC-MS/MS method according to the EMA (European Medicines Agency) guidelines for the validation of bioanalytical methods. In the analysis by the MRM method, the LOQ was reached at 10 ng/ml. The MRM method was validated for precision, accuracy, selectivity, and linearity over a 10–2500 ng/mL concentration, with results within tolerance limits. The analyte contained in the samples showed good stability after three freeze-thaw cycles, within 48 hours at 4 °C in autosampler and storage for 44 days at -20 °C. Thus, the method complies with the EMA requirements and can be used for the quantitative determination of tecovirimat and NIOCH-14 in human plasma. The method was applied in the preclinical study of NIOCH-14, where tecovirimat was analyzed as the primary metabolite of NIOCH-14.

The work was supported by a Russian government-funded project for ICBFM SB RAS (22- 24-00697).

### **Please explain why your abstract is innovative for mass spectrometry?**

This MRM method has been developed and validated for the quantitative determination of tecovirimat and NIOCH-14 in human plasma.

### **Co-authors:**

*Alexander Chernonosov, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia*

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Poster number: **LS-PA-014**

## INTEGRATION OF METABOLOMICS RESEARCH INTO TRANSLATIONAL CLINICAL CARE: CEREBROSPINAL FLUID BIOMARKERS FOR NEUROINFLAMMATION

Abstract ID: **477**

**Presenting author: Jinni Jingya Yan, Kids Neuroscience Centre, The Children's Hospital at Westmead, Faculty of Medicine and Health, University of Sydney, NSW, Australia, Clinical School, The Children's Hospital at Westmead, Faculty of Medicine and Health, University of Sydney, NSW, Australia, Department of Biochemistry, The Children's Hospital at Westmead, NSW, Australia**

### Introduction

Inflammation of the brain is increasingly recognised as important in encephalitis, but also neurodevelopmental, neuropsychiatric and neurodegenerative processes. The high mortality and morbidity rates of neuroinflammatory diseases drives significant interest in the investigation of biomarkers to define neuroinflammation and explore mechanisms involved in the regulation of central nervous system immune responses.

### Methods

An untargeted cerebrospinal fluid (CSF) metabolomics study investigated a cohort of fourteen patients with acute encephalitis and age-matched non-inflammatory neurological disease controls ( $n=14$ ) using high resolution mass spectrometry followed by subsequent multivariate and univariate statistical methods. The preliminary untargeted approach identified a useful potential panel for neuroinflammation. To translate the research data, we developed and clinically validated a translational targeted LC-QQQ/MS method for the simultaneous measurement of thirteen CSF metabolites. This method was applied to a cohort of encephalitis patients ( $n=11$ ), epilepsy group ( $n=24$ ) and non-inflammatory neurological disease controls ( $n=13$ ).

### Preliminary data (results)

The tryptophan-kynurenine pathway contributed nine key metabolites. There was a statistical increase of kynurenine, quinolinic acid, and anthranilic acid in patients with encephalitis, whereas tryptophan, 3-hydroxyanthranilic acid, and kynurenic acid were decreased. The nitric oxide pathway contributed four metabolites, with elevated asymmetric dimethylarginine and argininosuccinic acid, and decreased arginine and citrulline in patients with encephalitis. An increase in the CSF kynurenine/tryptophan ratio ( $p<0.001$ ), anthranilic acid/3-hydroxyanthranilic acid ratio ( $p<0.001$ ), asymmetric dimethylarginine/arginine ratio ( $p<0.001$ ), and neopterin ( $p<0.001$ ) strongly predicted neuroinflammation.

### Please explain why your abstract is innovative for mass spectrometry?

We present a time and cost efficient LC-MS/MS method for routine applicability to translational clinical care.

### Co-authors:

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Poster number: LS-PA-015

## GC-MS ANALYSIS AND SYNTHESIS OF STABLE ISOTOPE-DERIVATIVE OF A NEW 20-KETO-STEROID S42

Abstract ID: 478

Presenting author: Hui-Chung Wen, Department of Chemistry, University of Cologne, Greinstr. 4, D-50939 Cologne, Germany

### Introduction

Selective Androgen Receptor Modulators (SARMs) have been intensely studied due to their anabolic properties combined with less side effects based on their tissue selectivity.[1] In 2009, a new synthetic 20-keto-steroid S42 was introduced as a new SARM candidate.[2] Since S42 showed anabolic and anti-catabolic effects on cultured myotubes, it was proposed to be applied as a cure for muscle-related diseases.[3] For these reasons S42 has a certain potential for illicit sports doping application.[4] However, fundamental understanding of the GC-MS behavior of S42 and of relevant derivatives including the formation mechanisms of prominent fragment ions, is the indispensable basis for reliable qualitative and quantitative analysis of this pharmaceutical and of its metabolites in body fluid matrices.

### Methods

Steroid S42 was synthesized according to a procedure reported earlier by *Uyanik et al.*[5], using pregnenolone acetate as a starting material (Figure 1). Accurate ion mass determinations as well as gas chromatography-electron ionization MS experiments were carried out on an Thermo Scientific Exactive GC (ThermoFisher Scientific) instrument. Besides S42, we synthesized a quadruply deuterated isotopologue D4-S42 by conventional *keto-enol* chemistry and the trimethyl-silyl-ether derivative TMS-S42 according to standard protocols. *Trans*-8-methyl-1-acetyl-hydrindane, was also synthesized to serve as a simplified model compound to study the complex water loss reaction observed in EI MS computationally.[6-8]

### Preliminary data (results)

Besides the signal of the radical molecular ion  $[M]^{+•}$  at  $m/z$  296.2129 an important signal of a water-loss fragment ion  $[M-H_2O]^{+•}$  at  $m/z$  278.2025 was observed. The mechanism of the water-loss and the origin of the hydrogens expelled with the water molecule were investigated very closely. For that purpose, we analyzed D4-S42 (deuterium labelled at C-17 and C-21) with EI-MS. The unambiguous loss of H<sub>2</sub>O evidenced that the hydrogens at C-17 and C-21 are not involved. Further confirmation of this assumption delivered the EI-MS analysis of *trans*-8-methyl-1-acetyl-hydrindane and of *trans*-8-methyl(d<sub>3</sub>)-1-acetyl-hydrindane.[7] Concluding, we assume that in an initial step, a hydrogen in  $\gamma$ -position is transferred from either C-12 or C-18 to the carbonyl oxygen in a McLafferty-type rearrangement reaction. Subsequently, a  $\beta$ -fragmentation opens ring D and paves the way for the ultimate release of water. This mechanistic view is in accord to earlier reports by *Djerassi et al.*[7]

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[2] L. Min *et al.*, *Endocrinology* **2009**, 150, 5606-5616.

[3] Y. Muta *et al.*, *Biochem. Biophys. Rep.* **2019**, 17, 177-181.

[4] M. Thevis *et al.*, *Anal. Chem.* **2020**, 92, 506-523.

[5] C. Uyanik *et al.*, *J. Chem. Res.* **2006**, 417-419.

[6] P. T. Lansbury *et al.*, *JACS* **1975**, 97, 394-403.

[7] C. Djerassi *et al.*, *Chemische Berichte* **1968**, 101, 1018-1034.

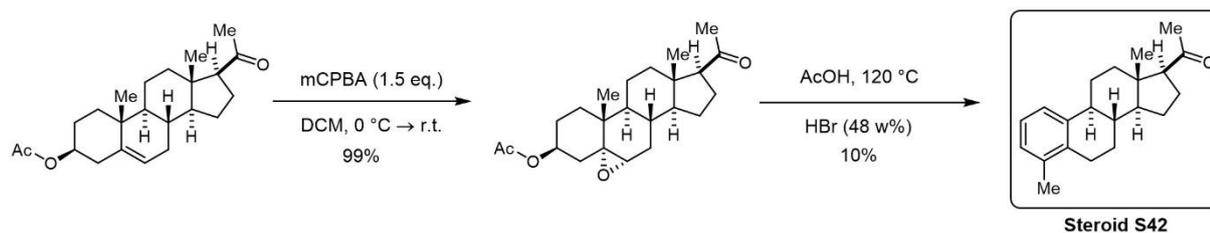
[8] Milena Vermeer, *Bachelor Thesis*, **2022**, University of Cologne.

**Please explain why your abstract is innovative for mass spectrometry?**

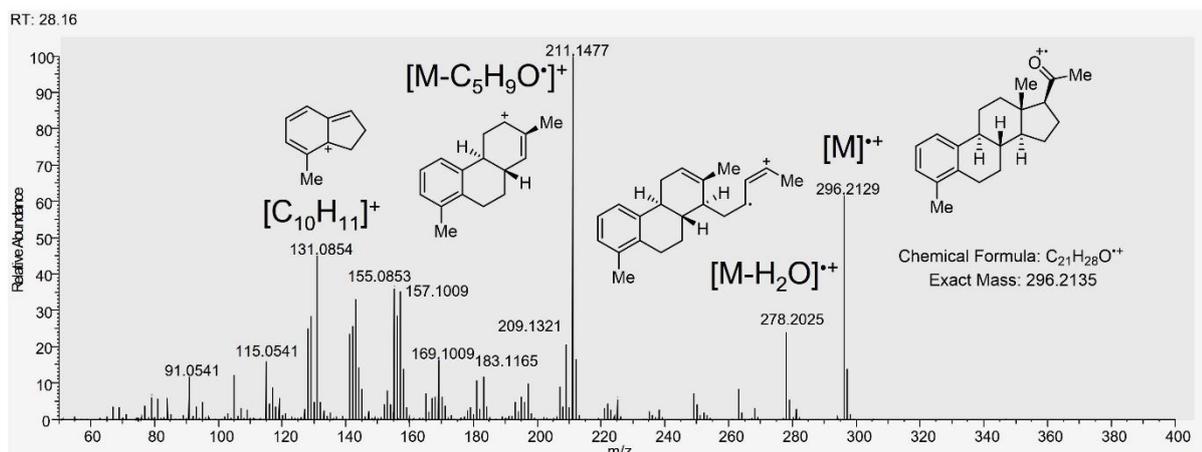
Synthesis of a new 20-keto-steroid S42 and of derivatives along with the profound analysis and interpretation of GC-EI-Mass spectra.

**Co-authors:**

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 Hans-Günther Schmalz, Department of Chemistry, University of Cologne; Greinstr. 4, D-50939 Cologne, Germany  
 Mario Thevis, Institute of Biochemistry, German Sport University Cologne; Sportpark Müngersdorf 6, 50933 Cologne, Germany  
 Mathias Schäfer, Department of Chemistry, University of Cologne; Greinstr. 4, D-50939 Cologne, Germany



**Figure 1.** Synthesis of S42 [5]; *m*CPBA: *meta*-Chloroperoxybenzoic acid.



**Figure 2.** GC-MS spectrum of S42.

Poster number: **LS-PA-016**

## ENHANCED 4D WORKFLOWS USING TIMS FOR ADVANCING SMALL MOLECULE RESEARCH

Abstract ID: 491

**Presenting author: Aiko Barsch, Bruker Daltonics GmbH & Co. KG**

### Introduction

Small molecule analyses in metabolomics, pharma and applied research often face similar challenges. Analysis of complex samples for detection, identification and (semi-) quantification of known and unknown analytes across a broad mass and dynamic range. Recent advances in high resolution LC-MS in combination with trapped ion mobility separation (LC-TIMS-MS) address the needs for rapid and confident identifications. TIMS capabilities to separate coeluting isobaric and isomeric analytes and collisional cross sections (CCS) can increase confidence in compound identification. The TIMS enabled PASEF provides extensive MSMS precursor coverage in single injections.

Here we present an optimized workflow for LC-PASEF data acquisition and processing enabling broad profiling and screening for knowns and unknown across a wide mass range in single shot injections.

### Methods

Serum and urine metabolites were separated using RP chromatography. A mixture of 32 drugs covering a mass range from m/z 69 (Imidazole) to m/z 917 (Tylosine A) (Sigma-Aldrich) was spiked in diluted urine. Data was acquired using a timsTOF Pro 2 in PASEF mode and processed using TASQ and MetaboScape 2023 software (Bruker). A TargetList containing CCS values for metabolites derived from Unified CCSCompendium[1] and predicted CCS values were used for annotation. Retention times from Bruker HMDB Personal Library 2.0 and MS/MS references from HMDB Personal Library 2.0, MetaboBase Personal Library 3.0 and NIST 2020, respectively

### Preliminary data (results)

We enhanced the parallel accumulation serial fragmentation (PASEF) data acquisition routines for small molecules by optimizing instrument acquisition parameters and routines. This resulted in a new default method for metabolomics, pharma and applied research questions requiring a broad profiling method for screening and quantitation in a single acquisition mode for target samples. We investigated urine and human plasma extracts with and without spiked in reference standards to establish a novel qual/quant routine workflow. The software solutions TASQ and MetaboScape run on the same server and utilize the same raw data acquired. This provides CCS-enabled screening / quantitation and profiling / ID workflows, respectively.

Toxicologically relevant test compounds were investigated in TASQ and linear dynamic ranges of quantitation > 3.0 orders of magnitude determined (e.g. Alprazolam). TIMS separation provided enhanced quantitation capabilities by mobility separating target compounds from co-eluting background noise.

Using the MetaboScape software solution MSMS spectra acquired with two different collision energies were annotated as endogenous and xenobiotic metabolites by matching to reference library spectra. The automatic, TargetList based, annotation of e.g. Naproxen, Indole-3-acetate, and Paraxanthine in human plasma extracts was substantiated by matching accurate (mass below 1ppm), retention time (below 0.01 min vs. HMDB 2.0 reference) and CCS (below 1% vs. CCS Compendium reference[1]). Oleic acid missed MSMS and CCS reference information in the utilized library and CCS reference resources. Based on the known structure, automatic in-silico fragmentation and CCS prediction in MetaboScape enabled tentative annotation of this compound in the human plasma sample.

[1]<https://doi.org/10.1039/C8SC04396E>

### Please explain why your abstract is innovative for mass spectrometry?

In summary we developed a CCS-enabled, single shot LC-MS/MS data acquisition routine providing broad screening, profiling, quantitation and ID capabilities for metabolomics, pharma and applied research.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### **Co-authors:**

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*Mathias Szesny, Bruker Daltonics GmbH & Co. KG*

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*Viola Jeck, Bruker Daltonics GmbH & Co. KG*

*Mohamed Elsadig, Bruker Daltonics GmbH & Co. KG*

*Michael Krause, Bruker Daltonics GmbH & Co. KG*

*Birgit Schneider, Bruker Daltonics GmbH & Co. KG*

*Carsten Baessmann, Bruker Daltonics GmbH & Co. KG*

Poster number: LS-PA-017

## TANDEM MASS SPECTRAL LIBRARY IN METABOLOMICS: FRAGMENTATION SPECTRA OF SODIATED SPECIES AS ADDITIONAL AND VALUABLE METABOLITE IDENTIFIER

Abstract ID: 517

**Presenting author: Annelaure Damont, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, F-91191 Gif sur Yvette, France**

### Introduction

Electrospray, the most popular ionization technique used in mass spectrometry-based metabolomics, has the particularity to competitively generate both intact protonated or deprotonated molecules and **adduct ions**, sodium being the most frequent monoatomic cation entering their composition. With the spread and generalization of untargeted tandem mass spectrometry experiments, product ion spectra of sodium-containing entities are information-rich and have to be carefully considered. Indeed, even though product ion spectra of sodiated chemical standards are gradually enriching spectral libraries, their value for **metabolite identification** is still largely underestimated. Here, we demonstrate the **unique fragmentation patterns** displayed by collision-induced dissociation of sodium-containing species in both positive and negative modes, and their high complementarity with product ion spectra of protonated and deprotonated molecules.

### Methods

Solutions of about twenty pure metabolite standards were prepared at 1 mg/mL and injected either by direct infusion (3 mL/min) or by flow-injection (100 mL/min) prior to ESI-MS/MS analysis. Product ion spectra were recorded both in positive and negative modes on an **Orbitrap Tribrid Fusion** or on a **LTQ Orbitrap Elite** (Thermo Scientific) at a resolution of 120,000 under both non-resonant (**HCD**) and resonant (**CID**) excitation conditions. Various normalized collision energies (NCE) were applied (from 0 to 80%). Hydrogen/deuterium exchanges (HDX) were also conducted. Density functional theory calculations using the Gaussian09 package are ongoing.

### Preliminary data (results)

Through the study of a selection of about 20 common metabolites, mainly amino and organic acids, collected ESI-MS/MS data clearly revealed that collision-induced dissociation of sodiated ionic species produces spectra **significantly different** from their protonated ( $[M+H]^+$ ) or deprotonated ( $[M-H]^-$ ) counterparts. They proved to be highly **informative product ion spectra** resulting from specific fragmentation mechanisms compared to protonated or deprotonated molecules. These specific covalent-bond cleavages involving **sodium retention** in product ions but also in neutral losses, have been investigated by comparing CID and HCD spectra of all considered ions. It has been evidenced that such fragmentation behaviors are obtained when the sodium cation is integrated into a charged structure as a **sodium salt**, formed at a deprotonated chemical function (e.g., carboxylate, phosphate, phenolate), the free charge being located at another functional group. Noteworthy, sodium-containing ions are not limited to the positive mode: fragmentation of deprotonated sodium salt (e.g.,  $[(M-H+Na)-H]^-$ ), ions that are rarely considered in metabolomics, also produces highly informative collisional spectra. Additionally, the importance of the charge location in the **underlying fragmentation processes** is discussed and quantum calculation are ongoing to validate our proposals. Overall, we demonstrate the benefits of considering MS/MS data generated from sodiated entities, complementarily to  $[M+H]^+$  and  $[M-H]^-$  fragmentation data, to **improve metabolite annotation confidence level**. Considering the number of metabolites featuring chemical groups capable of forming salts with  $Na^+$ , systematic integration of these data into annotation workflows should be considered.

### Please explain why your abstract is innovative for mass spectrometry?

Product ion spectra of metabolite-derived sodium species as a source of specific and valuable structural information. Higher confidence in metabolite annotation provided by fragmentation of sodium adduct ions.

### Co-authors:

Chenqin CAO, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, F-91191 Gif sur Yvette, France

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Anais LEGRAND, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, F-91191 Gif sur Yvette, France*

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*Yves GIMBERT, Sorbonne Université, Faculté des sciences et de l'ingénierie, Institut Parisien de Chimie Moléculaire (IPCM), Paris, France, Département de Chimie Moléculaire, UMR CNRS 5250, Université Grenoble Alpes, Grenoble, France*

*Alain PERRET, Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France*

*Amina WOODS, NIDA IRP, NIH Structural Biology Unit Integrative Neuroscience Branch, 333 Cassell Drive, Baltimore, Maryland 21224, United States*

*Christophe JUNOT, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, F-91191 Gif sur Yvette, France*

*Jean-Claude TABET, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, F-91191 Gif sur Yvette, France, Sorbonne Université, Faculté des sciences et de l'ingénierie, Institut Parisien de Chimie Moléculaire (IPCM), Paris, France*

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Poster number: **LS-PA-018**

## MULTIDIMENSIONAL SEPARATION IN METABOLOMICS BASED ON DIFFERENTIAL MOBILITY SPECTROMETRY WITH BINARY MODIFIERS AND HIGH RESOLUTION MASS SPECTROMETRY

Abstract ID: 521

**Presenting author: Lysi Ekmekciu, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva, Switzerland**

### Introduction

One LC dimension separation in LC-MS-based metabolomics is often not sufficient to get all peaks resolved due to the high physico-chemical variety of the metabolites. Improved peak capacity can be achieved using 2DxLC-MS but the transfer of the analytes from the first LC dimension to the second LC dimension can be challenging. In Differential Mobility Spectrometry the addition of different modifiers enables to tune the selectivity of the separation and can be used as an additional separation dimension. In the present work, we have compared the separation performance of LC(RP)xDMS-HRMS and LC(HILIC)xDMS-HRMS with different modifier conditions, and with respect to the peak capacity, orthogonality, and sensitivity.

### Methods

A mix of 53 analytes (Mix53) present in urine including endogenous compounds, drugs and metabolites along with different urine samples were investigated. Chromatographic separation was achieved using 2.1 x 150 mm columns either for reversed phase chromatography (RPLC) or hydrophilic interaction liquid chromatography (HILIC). The mass spectrometer is a quadrupole time-of-flight (TOF 6600+, Sciex) equipped with a DMS cell (SelexIon, Sciex). For the DMS experiments the separation voltage (SV) was set at 4000 V and the compensation voltage (CoV) was scanned with step of 1.5V, within 1 sec. The modifiers investigated were 2-propanol, ethanol, acetonitrile, cyclohexane and toluene.

### Preliminary data (results)

Based on previous studies from our group, it was shown that cyclohexane can be applied as a DMS non-clustering modifier and opens the use of binary mixtures without the change of the hardware setup. Mix of a cyclohexane with 2-propanol 97:3 (v/v) as DMS modifier showed good RPLCxDMS/MS separation for Mix53 and urine samples without significant loss of sensitivity as usually observed with pure 2-propanol. For the Mix53 with many analytes co-elution, 18 analytes could be clearly identified with 2-propanol modifier while with the binary mix 22 additional analytes (n=40) could be successfully detected using the two dimensions. Furthermore, different orthogonal DMS separation selectivity could be obtained with the different modifiers in particular comparing ethanol and toluene. This is due to different type of interactions between the charged molecules and the solvent to form the clusters. DMS requires scanning of CoV (1.5V step) and approximately 40 experiments can be performed with fast acquiring TOF allowing to investigate a CoV range of about 60V. The approach is suitable as well as for RPLC and HILIC LCxDMS-MS analysis of urine showed that significantly more analytes could be characterized with better confidence without compromising analysis time as with a single LC-MS approach. No correlation between LC retention time and compensation voltage was observed, which confirms the orthogonality of LC and DMS for low molecular weight compounds analysis. Comprehensive hyphenation DMS and RPLC or HILIC has shown increased peak capacity and improved data quality compatible with data independent acquisition (DIA) workflows.

### Please explain why your abstract is innovative for mass spectrometry?

Improved peak capacity and selectivity in metabolomics by using LCxDMS-MS/MS with binary modifiers using data independent acquisition.

### Co-authors:

*Gérard Hopfgartner, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva, Switzerland*

Poster number: LS-PA-019

## CCS-ENABLED TIMSTOF PRO WORKFLOW FOR IN VITRO HUMAN LIVER MICROSOME DRUG METABOLITES PROFILING AND CHARACTERIZATION

Abstract ID: 590

Presenting author: Xuejun Peng, Bruker Scientific LLC

### Introduction

Fast and accurate profiling and characterization of drug metabolites play a critical role in preclinical and clinical development stages to assist lead compound optimization, screening drug candidates, find active or potentially toxic metabolites. In this work, a non-targeted trapped ion mobility (TIMS) enabled timsTOF Pro PASEF workflow was conducted to investigate, profile, and characterize drug metabolites from in vitro human liver microsome incubation experiments. Drug metabolites were postulated by utilizing BioTransformer, a knowledge and machine learning based approach to predict small molecules metabolism. Metabolite structures were elucidated by MetFrag in-silico fragmentation and CCS-enabled annotation of biotransformation products.

### Methods

A time-series experiment was conducted by spiking human liver microsomes (Promega) and Fentanyl (Sigma) into a pre-incubated NADPH regeneration system at 37°C, aliquoting 100 µL of reaction solution at 0, 5, 15, 30, 45, 60, 90 and 120min. Reactions were stopped by adding cold acetonitrile. Samples were centrifuged at 12,000rpm at 4°C for 10min. The supernatant was transferred into sample vial and 5µL was injected (n=3) for two biological replicates. Analysis was performed by Elute UHPLC timsTOF Pro (Bruker) with PASEF data acquisition and ESI positive mode. Data analysis was conducted in DataAnalysis 5.3 and MetaboScape 2022 (Bruker).

### Preliminary data (results)

PASEF provides fast MS/MS acquisition speed at full sensitivity following ion mobility separation to deeply profile low abundant metabolite; Data analysis and feature findings of the drug and metabolites were conducted in MetaboScape with the T-ReX<sup>®</sup>4D algorithm applied for automatic feature extraction and alignment; The ion mobility ( $1/K_0$ ) and collisional cross sections (CCS) were calculated and listed in a feature table; BioTransformer tool was used to predict drug metabolites based on Cytochrome P450 Phase I biotransformation. All postulated metabolites from enzymatic reactions of hydroxylation (aromatic, aliphatic, amide at O- or P- location), N-deacetylation, alkyl oxidation and N-oxidation were investigated, and its chemical structures were characterized based on different metabolic locations. Metabolites were annotated based on mass accuracy and isotope pattern matching, further confirmed by MetFrag in-silico MS/MS fragmentation, as well as the enabled CCS prediction or CCS database.

Fentanyl (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub>) was used as example to outline the workflow of drug metabolites profiling and structure characterization. Fentanyl is mainly metabolized in liver by CYP3A4 into norfentanyl through oxidative N-dealkylation at the piperidine ring; it is also amide hydrolyzed to despropionyl-fentanyl or alkyhydroxylated to hydroxy-fentanyl which is further N-dealkylated to hydroxy-norfentanyl. Numerous isobaric metabolites formed which could be differentiated and characterized by retention time, isotopic pattern matching, MS/MS with MetFrag in-silico fragmentation, and TIMS enabled CCS verification.

In this proof-of-concept time-course drug metabolism study, TIMS enabled timsTOF Pro PASEF workflow and data analysis in MetaboScape 2022 allow CCS-enabled drug metabolite profiling and characterization.

### Please explain why your abstract is innovative for mass spectrometry?

CCS enabled characterization of isobaric drug metabolites by ion mobility timsTOF Pro PASEF

Co-authors:

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Lucy Woods, Bruker Scientific LLC*

*Matthias Szesny, Bruker Daltonics GmbH & Co KG*

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Poster number: LS-PA-020

## DISTINCTIVE METABOLOMICS PATTERN ASSOCIATED WITH HYPERTHYROIDISM TREATMENT

Abstract ID: 593

**Presenting author: Hicham Benabdelkamel, Proteomics Resource Unit, Obesity Research Center, College of Medicine, King Saud University, P.O. Box 2925 (98), Riyadh 11461, Saudi Arabia**

### Introduction

Hyperthyroidism is characterized by an increase in the production of thyroid hormones which affects several metabolic processes and energy expenditure. However, these changes' underlying mechanisms and metabolic implications are not well understood. Metabolomics is a comprehensive analytical approach that allows qualitative and quantitative analysis of alterations in metabolite levels within biological systems in response to specific stimuli and pathogenesis. In this study, the potential pathways and metabolic patterns associated with the hyperthyroidism treatment are explored using untargeted metabolomics. The study included 20 patients (age  $38.6 \pm 12.1$  years) patient at hyperthyroid state with FT4:  $35.4 + 9.9$  pmol/L and TSH  $0.014 + 0.014$  mIU/L, and post-treatment with anti-thyroid drugs (euthyroid state) with FT4:  $17.0 + 2.8$  pmol/L and TSH:  $0.6 + 0.5$  mIU/L.

### Methods

The plasma metabolites were extracted using 50% ACN in MeOH. The metabolic alteration associated with anti-thyroid therapy was investigated using liquid chromatography- high-resolution mass spectrometry. The untargeted metabolomics data was analyzed using both univariate and multivariate analyses using MetaboAnalyst v5. The significant metabolic pattern was identified using the lab standard pipeline, which included database searching in The Human Metabolome Database, LipidMap, LipidBlast, and METLIN. The identified metabolites were pathway and network analyzed for the significant connection with the cellular metabolism and disease system

### Preliminary data (results)

A total of 12522 mass ion features were detected in both positive and negative ionization modes. After several filtration processes such as alignment, peak picking, and missing value removal, 2499 features were retained for statistical analysis. The multivariate analysis using PCA and OPLS-DA model showed some separation and entire separation between groups (pre- and post-hyperthyroid), respectively. The robustness of the created models was evaluated by the fitness of model (R<sup>2</sup>Y) and predictive ability (Q<sup>2</sup>) values, which were 0.995 and 0.573, respectively (**Figure 1**). The univariate analysis Volcano Plot (FDR p-value  $\leq 0.05$ , FC 1.5) revealed 83 significantly dysregulated metabolites; 53 and 30, up- and down-regulated in post- compared to pre-hyperthyroid, respectively. 40 compounds were putatively identified and involved in lipid metabolism, such as Octanoylcarnitine, PC(20:1/0:0). The potential disease biomarkers were evaluated using Receiver Operating Characteristic (ROC) curve for sensitivity and specificity of detecting the condition. For instance, the ROC curve's Area Under the Curve (AUC) values for Heptadecanoyl Ethanolamide and Methylpentadecanal were 0.92 and 0.91, respectively. Ten features at the exploratory ROC curve using PLS-DA with crossvalidation (CV), have AUC value 0.82 (95% CI).

### Please explain why your abstract is innovative for mass spectrometry?

This distinctive metabolic pattern has the potential power for disease detection and thyroid treatment monitoring. Our metabolomics analysis indicated alterations in the abundance of several metabolites involved in the disease.

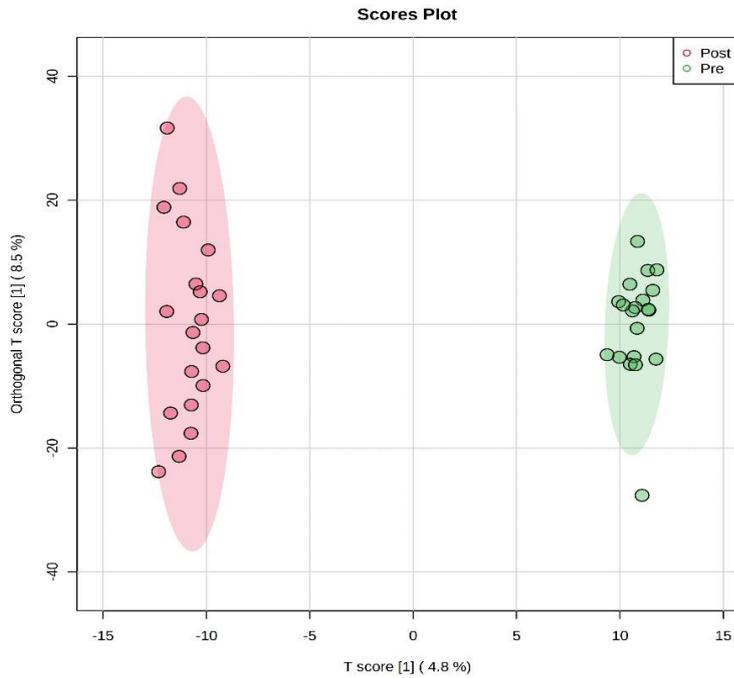
### Co-authors:

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Mohthash Musambil, Proteomics Resource Unit, Obesity Research Center, College of Medicine, King Saud University, P.O. Box 2925 (98), Riyadh 11461, Saudi Arabia

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Assim A Alfadda, Proteomics Resource Unit, Obesity Research Center, College of Medicine, King Saud University, P.O. Box 2925 (98), Riyadh 11461, Saudi Arabia, Department of Medicine, College of Medicine and King Saud Medical City, King Saud University, P.O. Box 2925 (98), Riyadh 11461, Saudi Arabia



oPLS-DA -separation between post (red) and pre(green) treatment groups

Poster number: **LS-PA-021**

## HIGH RESOLUTION ION MOBILITY TIMSTOF PRO FOR THE FAST SEPARATION AND CHARACTERIZATION OF ISOMERIC BILE ACIDS

Abstract ID: **594**Presenting author: **Xuejun Peng, Bruker Scientific LLC**

### Introduction

To develop and establish a fast analytical method for the separation of isomers has been challenges over the past four decades. Structural or spatial isomers can exhibit different chemical activity, potency, toxicity, and behavior in biological system. Chromatography (HPLC, SFC, GC, TLC) and capillary electrophoresis are commonly used techniques for isomer separation but can be costive and time consuming. After the thalidomide tragedy, the isomer purity is under strict regulations in drug discovery and development. In this work, a fast flow injection trapped ion mobility (TIMS) timsTOF Pro workflow was established for the fast separation of bile acid isomers, where gas phase ions are separated when subjected to a potential gradient due to differences in their shapes and sizes.

### Methods

Bile acid isomers of deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), isodeoxycholic acid (iso-DCA), lithocholic acid (LCA), isolithocholic acid (iso-LCA) were dissolved in methanol at 50 $\mu$ g/mL and 1.0 $\mu$ g/mL; data was acquired for 30 seconds by flow injection Elute UHPLC-timsTOF Pro 2 (Bruker) in ESI negative mode with TIMS and PASEF enabled; data was processed in DataAnalysis 5.3 and MetaboScape2022<sup>®</sup> (Bruker). An established 4D-metabolomics method (m/z 20-1300 Da; ion mobility 1/K<sub>0</sub> 0.45 – 1.45 V.s/cm<sup>2</sup>) was optimized to achieve the highest ion mobility resolution by adjusting TIMS duty cycle, ramp time, and ion mobility range etc..

### Preliminary data (results)

Bile acids are a large family of molecules consisting of a four-ring steroid structure with various side chains, which are synthesized from cholesterol in the liver and actively secreted along with cholesterol and phospholipids into the bile. Bile acids involve a wide range of biological functions including the adsorption of dietary lipids and fat-soluble vitamins, signaling molecules with diverse endocrine and paracrine functions to regulate bile acid, lipid, and glucose metabolism, modulate temperature and energy. Bile acids are cytotoxic when present in abnormally high concentration.

Deoxycholic acid (DCA, C<sub>24</sub>H<sub>40</sub>O<sub>4</sub>), chenodeoxycholic acid (CDCA, C<sub>24</sub>H<sub>40</sub>O<sub>4</sub>), isodeoxycholic acid, and (iso-DCA, C<sub>24</sub>H<sub>40</sub>O<sub>4</sub>) are bile acid isomers with same molecule formula and different hydroxy group location and orientation. By flow injecting the mixed standard solution, mass spectral ion of m/z 391.2854 [M-H]<sup>-</sup> was observed; its extracted ion mobilogram (EIM) displays three baseline separated peaks achieving ion mobility resolution at 166.7 (DCA), 176.7 (CDCA) and 199.2 (iso-DCA); and the calculated CCS values of 200.9 (DCA), 206.2 (CDCA), and 199.2 (iso-DCA) Å<sup>2</sup>, which are in good agreement with the reported CCS values in  $\Delta$ CCS error of 0.59% (DCA), 2.33% (CDCA) and 0.00% (iso-DCA). Similarly, lithocholic acid (LCA, C<sub>24</sub>H<sub>40</sub>O<sub>3</sub>) and isolithocholic acid (iso-LCA, C<sub>24</sub>H<sub>40</sub>O<sub>3</sub>) were also successfully separated achieving high ion mobility resolution of 206.6 (LCA) and 203.9 (iso-LCA). When analyzing the mixed standard solution, all five bile acid isomers achieved good separation from each other. Each of the EIM peaks was verified by spiking its pure standard and further confirmed by MS/MS data.

### Please explain why your abstract is innovative for mass spectrometry?

Report a high throughput analytical workflow for isomers separation using high resolution ion mobility timsTOF Pro system

### Co-authors:

*Lucy Woods, Bruker Scientific LLC*

*Matthias Szesny, Bruker Daltonics GmbH & Co KG*

*Beixi Wang, Bruker Scientific LLC*

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Poster number: LS-PA-022

## CCS-ENABLED TIMSTOF PRO WORKFLOW FOR IN VITRO HUMAN LIVER MICROSOME DRUG METABOLITES PROFILING AND CHARACTERIZATION

Abstract ID: 619

Presenting author: Xuejun Peng, Bruker Scientific LLC

### Introduction

Fast and accurate profiling and characterization of drug metabolites play a critical role in preclinical and clinical development stages to assist lead compound optimization, screening drug candidates, find active or potentially toxic metabolites. In this work, a non-targeted trapped ion mobility (TIMS) enabled timsTOF Pro PASEF workflow was conducted to investigate, profile, and characterize drug metabolites from in vitro human liver microsome incubation experiments. Drug metabolites were postulated by utilizing BioTransformer, a knowledge and machine learning based approach to predict small molecules metabolism. Metabolite structures were elucidated by MetFrag in-silico fragmentation and CCS-enabled annotation of biotransformation products.

### Methods

A time-series experiment was conducted by spiking human liver microsomes (Promega) and Fentanyl (Sigma) into a pre-incubated NADPH regeneration system at 37°C, aliquoting 100 µL of reaction solution at 0, 5, 15, 30, 45, 60, 90 and 120min. Reactions were stopped by adding cold acetonitrile. Samples were centrifuged at 12,000rpm at 40°C for 10min. The supernatant was transferred into sample vial and 5µL was injected (n=3) for two biological replicates. Analysis was performed by Elute UHPLC timsTOF Pro (Bruker) with PASEF data acquisition and ESI positive mode. Data analysis was conducted in DataAnalysis 5.3 and MetaboScape 2022 (Bruker).

### Preliminary data (results)

PASEF provides fast MS/MS acquisition speed at full sensitivity following ion mobility separation to deeply profile low abundant metabolite; Data analysis and feature findings of the drug and metabolites were conducted in MetaboScape with the T-ReX@4D algorithm applied for automatic feature extraction and alignment; The ion mobility ( $1/K_0$ ) and collisional cross sections (CCS) were calculated and listed in a feature table; BioTransformer tool was used to predict drug metabolites based on Cytochrome P450 Phase I biotransformation. All postulated metabolites from enzymatic reactions of hydroxylation (aromatic, aliphatic, amide at O- or P- location), N-deacetylation, alkyl oxidation and N-oxidation were investigated, and its chemical structures were characterized based on different metabolic locations. Metabolites were annotated based on mass accuracy and isotope pattern matching, further confirmed by MetFrag in-silico MS/MS fragmentation, as well as the enabled CCS prediction or CCS database.

Fentanyl (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub>) was used as example to outline the workflow of drug metabolites profiling and structure characterization. Fentanyl is mainly metabolized in liver by CYP3A4 into norfentanyl through oxidative N-dealkylation at the piperidine ring; it is also amide hydrolyzed to despropionyl-fentanyl or alkyhydroxylated to hydroxy-fentanyl which is further N-dealkylated to hydroxy-norfentanyl. Numerous isobaric metabolites formed which could be differentiated and characterized by retention time, isotopic pattern matching, MS/MS with MetFrag in-silico fragmentation, and TIMS enabled CCS verification.

In this proof-of-concept time-course drug metabolism study, TIMS enabled timsTOF Pro PASEF workflow and data analysis in MetaboScape 2022 allow CCS-enabled drug metabolite profiling and characterization.

### Please explain why your abstract is innovative for mass spectrometry?

CCS enabled characterization of isobaric drug metabolites by ion mobility timsTOF Pro PASEF

### Co-authors:

Lucy Woods, Bruker Scientific LLC

Matthias Szesny, Bruker Daltonics GmbH & Co KG

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

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Poster number: LS-PA-023

## PHYSIOLOGICAL AND POSTTRAINING EFFECTS OF TAKING ECDYSTEROIDS SUPPLEMENT. A MULTIVARIATE ANALYSIS OF THE HUMAN SERUM METABOLOME

Abstract ID: 662

**Presenting author:** Patrizia Leogrande, Antidoping laboratory FMSI, Institute of Pharmacy, Freie Universität Berlin

### Introduction

Metabolites, known to be responsible for a variety of biological functions (signaling molecules, biomarkers, and modulators of physiological processes), form the metabolome. Thanks to its highly dynamic nature, it is an instantaneous indicator of system perturbations induced by diet, training, etc. Since specific metabolic phenotypes may result from years of training, discrimination of physiological effects from those caused by the administration of substances prohibited by the World Anti-Doping Agency may be a valid analytical strategy for the interpretation of adverse analytical findings.

This work aims to characterize the athletes serum profile after the administration of ecdysteroids, natural steroid hormones recently proved to enhance physical performance. The combination between mass spectrometry and chemometric tools may allow to differentiate physiological effects from post-training and intake-driven effects.

### Methods

Serum samples consisted in 4 groups: control (200 mg of *Peak Ecdysone* without training); placebo (200 mg of placebo/day with training); Ec1 (200 mg/day of *Peak Ecdysone* with training) and Ec2 (800 mg/day of *Peak Ecdysone* with training). Metabolic profiling was measured by using a SCIEX Triple Quad LC-MS/MS system coupled with the Biocrates Absolute-IDQ p180 kit (Innsbruck, Austria) that allows quantifying a wide panel of metabolites, including amino acids, biogenic amines, acylcarnitines, sphingomyelins, glycerophospholipids, and monosaccharides (>90% glucose). Principal component analysis and orthogonal least squares discriminant analysis were performed using SIMCA (Umetrics, Sartorius, Malmö, Sweden).

### Preliminary data (results)

Unsupervised data analysis, performed to follow natural data aggregation, showed similarities between placebo and the tested dose of ecdysteroids supplementation (Ec1 group), which was also confirmed by principal component analysis. Merging Ec1 and Ec2 into a single group coded as *treated*, a clear discrimination was observed with the control and placebo groups (Figure 1). Supervised analysis was performed by evaluating two conditions at a time. Discrimination between the placebo and control groups revealed the influence of phosphatidylcholines on the control samples, as well as when the placebo vs Ec1 and placebo vs Ec2 group pairs were examined. Indeed, phosphatidylcholines were found to be a down-regulated feature with a dose-dependent effect (Figure 2). The most relevant features highlighted by the S-plots were evaluated individually to evaluate the biological interpretation of their variation. For example, histamine was the most relevant variable in discriminating the conditions considered, showing a marked increase in the training conditions. This alteration may be due to the release during the exercise of endorphins that stimulate immune cells to release chemicals, such as histamine. This response represents a key element of exercise. The alpha-amino adipic acid showed training-driven down-regulation. However, taking the ecdysteroids supplement appears to reduce its suppression. Trans-4-hydroxyproline (hydroxylated proline derivative) was also found to be an up-regulated feature. This variation showed a slight suppression after repeated intake of ecdysteroids supplementation. Considering that hydroxyproline increases in response to hypoxia and oxidative stress, the change in trans-4-hydroxyproline could suggest an antioxidant function for the supplement.

### Please explain why your abstract is innovative for mass spectrometry?

Coupling mass spectrometry with chemometrics allows the refined interpretation of the metabolome in biomatrices, combining a wide scan window with a good enough combination of sensitivity, specificity, rapidity, and robustness.

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

**Co-authors:**

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*Andreas Luch, German Federal Institute for Risk Assessment (BfR)*  
*Patrick Diel, Institute for Cardiovascular Research and Sports Medicine, German Sport University Cologne*  
*Maria Kristina Parr, Institute of Pharmacy, Freie Universität Berlin*

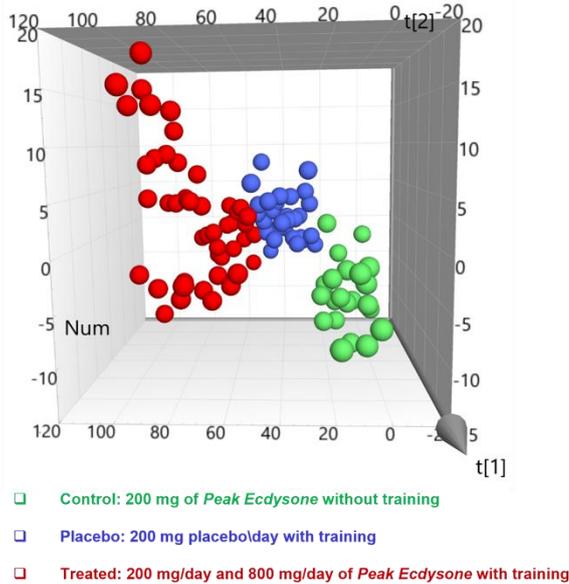


Figure 1. PCA score plot shows clear discrimination among the conditions examined

PCA score plot shows clear discrimination among the conditions examined

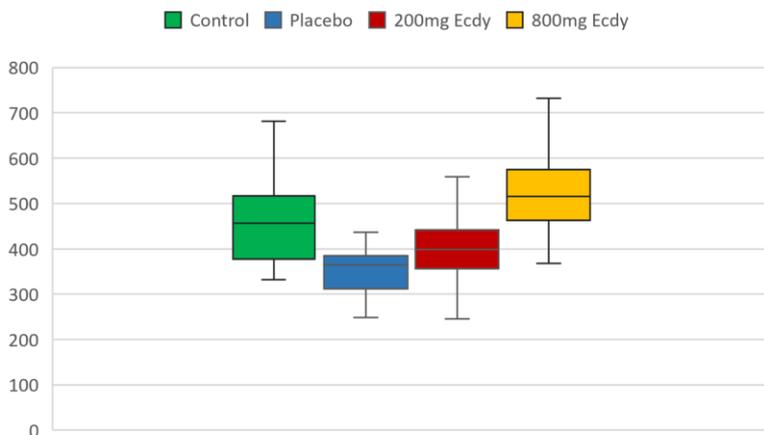


Figure 2. Box plot of phosphatidylcholine variation shows dose-dependent effect

Box plot of phosphatidylcholine variation shows dose-dependent effect

Poster number: **LS-PA-024**

## UTILIZATION OF DUAL COLUMN LC-HRMS FOR NON-TARGETED METABOLOMICS TO INVESTIGATE ALTERED ROOT EXUDATE EXPRESSION IN THE PRESENCE OF PLANT NEIGHBORS

Abstract ID: 672

**Presenting author: Alexandra Bennett, University of Natural Resources and Life Sciences, Department of Chemistry, Institute of Analytical Chemistry**

### Introduction

Growing cover crops is an effective way to manage soil and reduce weed propagation in agricultural settings. Resource competition is cited as a common way in which cover crops prevent weeds from developing early in the growing season (Osipitan *et al.* 2018). However, the production of allelopathic compounds may give certain cover crop species a heightened advantage as these compounds are defined by their ability to impact the growth, reproduction, and development of other species. Non-targeted liquid chromatography (LC) high resolution mass spectrometry (HRMS) can be utilized to assess low molecular weight compounds released by the roots of previously identified (Sturm *et al.* 2018) allelopathic cover crops in response to the presence of weedy neighbors.

### Methods

Black oat (*Avena strigosa*) and buckwheat (*Fagopyrum esculentum*) cover crops were propagated in a split root setup with either (i) no neighbor, (ii) a homospecific neighbor, or (iii) heterospecific weedy neighbor redroot pigweed (*Amaranthus retroflexus*). Root exudates were extracted with a water fraction, plants were given 24 hours to regenerate metabolites, and again exudates were extracted with a methanol fraction. Extractions were concentrated and subsequently analyzed via a non-targeted dual column LC-HRMS method utilizing a quadrupole time of flight mass spectrometer (QTOFMS). Non-blank signals were annotated and mapped to a network and differential expression between conditions was calculated.

### Preliminary data (results)

A chemical annotation pipeline was developed based off a mixture of 44 chemical standards known to be exudated by roots of agricultural plants. MS FINDER annotated the formula for standards. Fragmentation patterns and chemical formulas were imported into CANOPUS where chemical compound classes were computed. Results support the accuracy of the computational annotation pipeline.

After blank signal, noise, and in-source fragmentation removal and adduct aggregation, approximately 1500 to 2000 features remained in the aligned raw data for positive and negative mode for each condition. Differential analysis between growth setups was done by performing unpaired t tests on features that passed data filtration. Additionally, a fold change of 5 and 10 between different conditions was assessed.

Compounds from an in-house database generated from aforementioned chemical standards were identified in root exudate samples with a high degree (level 1) of confidence. More compounds were given probable identification with a lower confidence (level 2) based off database or in silico fragmentation. The formula was annotated with MS FINDER (Confidence level 4). CANOPUS annotated chemical class, bringing their confidence level up to 3. Confidence levels were based off of Schymanski *et al.* 2014.

Finalized data is being computed; preliminary data shown is for non-split root of buckwheat in negative mode. Compounds which clustered into a network larger than 5 nodes were reported in Figure 1. Clusters shown are from the same experimental condition but are too unrelated to form connecting edges. Finalized networks highlight differentially expressed and confidence level 1 compounds.

### Please explain why your abstract is innovative for mass spectrometry?

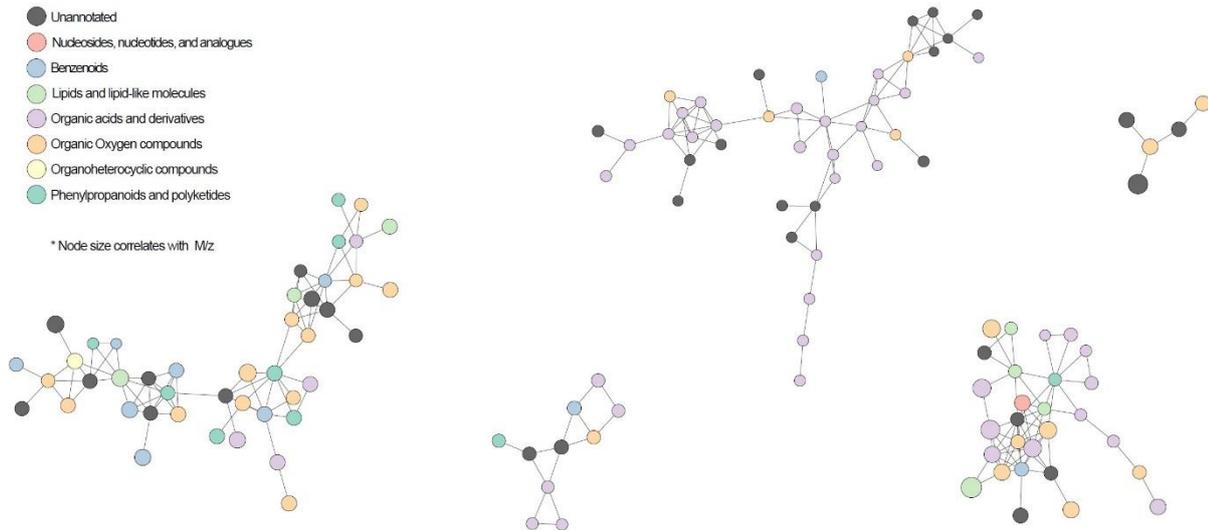
Application of a dual column setup and HRMS for establishment of a molecular network of root exudates

**Co-authors:**

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Çağla Görkem Eroğlu, Agroscope, Herbology in Field Crops and Viticulture, Plant-Production Systems*  
*Teresa Steininger-Mairinger, University of Natural Resources and Life Sciences, Department of Chemistry, Institute of Analytical Chemistry*  
*Markus Puschenreiter, University of Natural Resources and Life Sciences, Department of Forest- and Soil Sciences, Institute of Soil Research*  
*Aurélie Gfeller, Agroscope, Herbology in Field Crops and Viticulture, Plant-Production Systems*  
*Judith Wirth, Agroscope, Herbology in Field Crops and Viticulture, Plant-Production Systems*  
*Stephan Hann, University of Natural Resources and Life Sciences, Department of Chemistry, Institute of Analytical Chemistry*



Preliminary network from buckwheat negative mode data.

Poster number: LS-PA-025

## FINGERPRINTING OF TEA VARIETIES USING A NOVEL UNTARGETED METABOLOMICS WORKFLOW

Abstract ID: 738

Presenting author: Daniel Hermanson, Thermo Fisher Scientific

### Introduction

Tea is the second most consumed beverage worldwide after water. The geographical origin in combination with manufacturing and packaging processes cause metabolic changes in tea. Here we describe an untargeted metabolomics approach utilizing liquid chromatography-mass spectrometry for the detection and annotation of phytochemicals to classify and characterize tea with a focus on flavonoids. The annotation of flavonoids in tea presents significant analytical challenges given the presence of numerous isomeric species and shared fragmentation patterns. By developing and leveraging a spectral library of flavonoids we demonstrate the ability to simultaneously compare the metabolic profiles of different tea types and fully annotate both known and unknown flavonoids using a novel data acquisition and processing workflow

### Methods

Twenty-four different types of tea including green, black, and herbal varieties were brewed in triplicate. Pools of each variety of tea were also created for MS<sup>n</sup> data acquisition using a Deep Scan AcquireX acquisition workflow and Real-time library search (RTLS) method based on a spectral library generated from authentic flavonoid standards. Data was acquired using a Thermo Scientific™ Vanquish™ UHPLC system with Thermo Scientific™ Hypersil GOLD™ column and Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ mass spectrometer. Data analysis was performed using Thermo Scientific™ Compound Discoverer™ software for compound identification and differential analysis.

### Preliminary data (results)

The untargeted analysis of the tea varieties resulted in the detection of over 15000 non-background compounds across the 24 different tea types analyzed. By employing the AcquireX Deep Scan workflow to intelligently collect fragmentation data on the pooled tea samples, 94% of these compounds had fragmentation spectra for use in compound annotation. Further insights were achieved through the use of RTLS to automatically collect MS<sup>n</sup> data based on the MS<sup>2</sup> spectral match to a known flavonoid spectral library. Combining these acquisition strategies with the use of neutral loss scoring to identify compounds containing signature sugar losses and compound class scoring to identify compounds demonstrating signature fragment ions allowed for the putative classification of over 250 compounds as flavonoids. While performing a principal component analysis on all of the detected compounds yielded some differentiation of black and green tea from herbal tea varieties, filtering the results to the putative flavonoids provided clear differentiation of each class of tea variety as well as the specific types. To annotate the structures of the putative flavonoids assignment of molecular formula, online database searching and ranking with mzLogic™ scores, spectral matching on the acquired MS<sup>n</sup> data to both a flavonoid spectral library containing retention time information and the mzCloud™ spectral library, molecular networking, *in silico* fragmentation and scoring of candidates using FISH scoring, and searching against the built in flavonoid mass list were performed. These strategies allowed for the confident identification of the constituent flavonoids in tea that discriminate different varieties and types.

### Please explain why your abstract is innovative for mass spectrometry?

A workflow for differentiating tea varieties using a novel data acquisition and processing strategy using differential analysis and compound annotation with MS<sup>n</sup>

### Co-authors:

Rahul Deshpande, Thermo Fisher Scientific

Bashar Amer, Thermo Fisher Scientific

Brandon Bills, Thermo Fisher Scientific

Ralf Tautenhahn, Thermo Fisher Scientific

Poster number: **LS-PA-026**

## **NEW PEAK DETECTION (PYCO) AND ISOTOPE GROUPING (PRISM) ALGORITHMS FOR AN IMPROVED COMPOUND DETECTION WORKFLOW**

Abstract ID: **747**

**Presenting author: Pedro Navarro, Thermo Fisher Scientific**

### **Introduction**

A reliable peak detection algorithm is key for any compound detection workflow. Here we introduce the new detection workflow in Compound Discoverer 3.3 software, based on a novel parameter-free peak detection algorithm, Pyco, and a redesigned isotope grouping algorithm, Prism. We show that this new approach works more consistently on various chromatography methods, as peak integration does not rely on restrictive peak model assumptions, such as Gaussian shape or symmetry.

Using peak quality metrics, we corroborate that both identification and quantification results have been significantly improved. We were able to reduce false positive compounds by avoiding over-splitting of peaks. The new detection workflow is not only more precise, but also more efficient, reducing the overall computational time for processing.

### **Methods**

The Pyco algorithm can be split into preprocessing, preliminary integration, and consolidation steps. In the first stage linear interpolation is used to fill trace gaps occurring due to loss of signal or coalescence. After gap filling, we apply the AirPLS algorithm for baseline removal and the trace is then smoothed using either Total Variation Denoising followed by a Gaussian smoothing or a Savitzki-Golay Filter, depending on the trace resolution.

We generate peak candidate features using derivative information for preliminary peak search and then perform consolidation, using a statistical approach to merge separate peaks into a unified one.

### **Preliminary data (results)**

We have benchmarked the new detection workflow by using several datasets. The dataset '1100 Compounds' from Li et al<sup>1</sup> has been employed for accounting the number of true and false positives and assessing the quantification precision and accuracy. The number of true positives quantified has more than doubled when comparing with the old workflow, while false positives have been drastically reduced. The reduction in false positives also improves quantification quality, (CV values from 40% to 15%). Relative quantification is also improved, spotting the expected compound ratios, and showing an improved precision even with more quantified compounds. In terms of selectivity and sensitivity, the new workflow shows a better performance than others compared in Li et al<sup>1</sup>.

The new workflow is also faster and consumes less RAM resources, allowing for more efficient processing and the processing for large scale studies. For example, processing compound detection in a study containing ~2400 raw files took 26 hours by using a 32GB RAM, Xeon E5 @2.40GHz workstation with an SSD disk.

The workflow is further enhanced through a customizable peak rating system, which yields a peak quality rating based on technical replicate area precision and peak quality parameters such as peak jaggedness and modality, as defined in Chetnik et al<sup>2</sup>. This customized peak rating allows for the filtering of peaks in the processing workflow based on a customizable user-defined minimum rating, rather than an arbitrary intensity threshold value.

1. Li et al. *Analytica chimica acta* (2018)
2. Chetnik et al. *Metabolomics* (2020)

### **Please explain why your abstract is innovative for mass spectrometry?**

A new, parameter-less, faster, and more sensitive compound detection workflow with improved peak integration for accurate relative quantitation.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### **Co-authors:**

*Yovany Cordero Hernandez, Thermo Fisher Scientific*

*Konstantin Ayzikov, Thermo Fisher Scientific*

*Martin Strohalm, Thermo Fischer Scientific*

*James Dillon, Thermo Fisher Scientific*

*Alexander Tiegel, Thermo Fisher Scientif*

*Ute Comberg, Thermo Fisher Scientific*

*Ralf Tautenhahn, Thermo Fisher Scientific*

*Alexander Makarov, Thermo Fisher Scientific*

*Christoph Henrich, Thermo Fisher Scientific*

Poster number: **LS-PA-027**

## **TECHNOLOGIES FOR CHARACTERIZATION, DETECTION AND ISOLATION OF EXTRACELLULAR VESICLES AS BIOMARKERS**

Abstract ID: 771

**Presenting author: Ehsan Manouchehri Doulabi, Department of Immunology, Genetics & Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden**

### **Introduction**

Extracellular Vesicles (EVs) are membrane-bound phospholipid nanovesicles with a broad size range between 30 to 5000 nm in diameter, secreted by all cells. EVs mediate intracellular communication and regulate biological processes. Circulating EVs are shown to have high stability in the body fluids, and also they are containing the originated cell's molecules, which make them promising and potential biomarkers at very low concentrations. Since EVs are heterogeneous in the body fluids, information about organ-specific biomarkers especially proteins on the EV's surface is valuable information to use EVs as diagnostic and therapeutic tools. Therefore, there is a need for highly sensitive and specific techniques for the characterization and identification of EVs in bulk or on a single level.

### **Methods**

To address the shortcoming in methodology to characterize EV's surface proteins, we have established a protocol for high throughput profiling EV's surface proteins by using non-membrane permeable biotin labeling and high-resolution mass spectrometry (HRMS) in combination with flow cytometry-based proximity ligation assay (Exo-PLA) and solid-phase proximity ligation assay (SP-PLA). The protocol was used to investigate the profiles of surface proteins on EVs isolated from seminal fluids (Prostasome) and those purified from the human prostate cancer cell line PC3.

### **Preliminary data (results)**

In total, 1730 proteins were identified in both prostasomes and PC3 cell lines' EVs of which 273 proteins have previously not been reported as EVs proteins. Furthermore, 1014 surface proteins were identified of which 457 proteins were detected in all 3 technical sample replicates, and at least 730 proteins in 2 replicates. Seventy-four prostate-specific Unique proteins were identified to be enriched on the surface of EVs, and the presence of a subset of the most expressed ones were verified using Exo-PLA and SP-PLA on the intact EVs, including SEMG1, PTGDS, AKAP4, CRISP1, and GAPDS. The advantage of using an unbiased MS approach in combination with affinity-based technologies allows characterization and sensitive detection of EVs as potential biomarkers, but this approach also allows isolation of EVs subpopulations in body fluids for further downstream analysis.

### **Please explain why your abstract is innovative for mass spectrometry?**

Our methodology presented here for profiling surface proteins will be a powerful technique for use of EVs in biological as well as medical research.

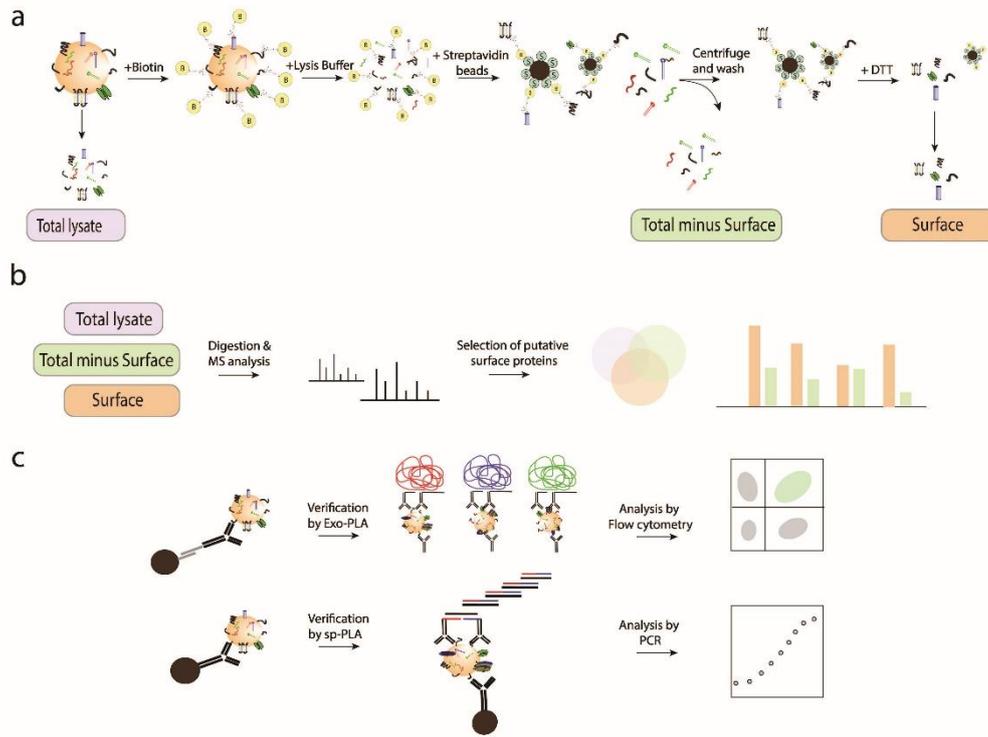
### **Co-authors:**

*Claudia Fredolini, Department of Immunology, Genetics & Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*

*Radosa Gallini, Department of Immunology, Genetics & Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*

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Figure 1



Poster number: LS-PA-028

## MICROBIAL DEGRADATION OF HYALURONAN: STABLE ISOTOPE LABELLING STRATEGY

Abstract ID: 817

Presenting author: Matej Simek, Contipro a.s.

### Introduction

Hyaluronan has been used as a dietary supplement for many years for its anti-inflammatory properties, ability to increase skin moisture and relieve of knee and chronic pain. It is suggested that high-Mw hyaluronan does not transit through the intestinal wall, however, it may be cleaved into shorter fragments by bacterial enzymes of complex population of gut microbiota. These fragments and other bacterial metabolites can be absorbed by the host intestinal epithelium and have a beneficial impact on the host. The aim of this study is to identify these metabolites by stable isotope–assisted metabolomic analysis.

### Methods

<sup>13</sup>C-hyaluronan (1.6 MDa) was prepared by growing *Streptococcus zooepidemicus* on <sup>13</sup>C-D-glucose. <sup>13</sup>C-labelling efficiency was inspected by LC-MS analysis of unsaturated hyaluronan disaccharide ( $\Delta$ AN2) produced after enzymatic digestion with hyaluronidase from *Streptococcus pneumoniae*. For metabolomics study, cecum from oMM<sup>12</sup>-colonized mice was removed and transferred into the anaerobic chamber. Afterwards, hyaluronan (500  $\mu$ g ml<sup>-1</sup>) was added and after 15, 30, 60 and 120 min samples were withdrawn and extracted with chilled methanol. HILIC-MS analysis was performed on BEH Amide column under basic conditions. Data were collected with Orbitrap Exploris 240 using AcquireX data acquisition mode and processed in Compound Discoverer.

### Preliminary data (results)

The LC-MS analysis of <sup>13</sup>C- $\Delta$ AN2 revealed high efficiency of <sup>13</sup>C labelling, with 83 % of monomers labelled at all carbon positions, 10 % labelled only at monosaccharide rings with none <sup>13</sup>C at the acetyl moiety of N-acetylglucosamine and 7 % of monomers were labelled in different manner. The purity of <sup>13</sup>C-labelling was sufficient for stable isotope–assisted metabolomic analysis of bacterial degradation of hyaluronic acid. However, non-uniform incorporation of <sup>13</sup>C atoms into hyaluronan structure indicates multiple pathways of metabolization of <sup>13</sup>C-glucose into hyaluronan. Thus, proposed LC-MS analysis of enzymatically degraded hyaluronan may serve for fluxomic analysis and subsequently for the control of biotechnological production of hyaluronic acid.

The study of microbial degradation of hyaluronan revealed that the <sup>13</sup>C-hyaluronan was completely metabolized by cultivated cecal content of oMM<sup>12</sup> mice within 120 min. With the decrease of <sup>13</sup>C-hyaluronan concentration, the production of  $\Delta$ AN2 was observed. The <sup>13</sup>C-label was further identified in several low-*M<sub>w</sub>* metabolites including short chain fatty acids. High level of incorporation (>25 %) was identified in C<sub>6</sub>H<sub>10</sub>O<sub>6</sub> which corresponds to unsaturated glucuronic acid. Similarly, high incorporation <sup>13</sup>C-label was observed in N-acetylglucosamine-6-phosphate (>30 %) and uridine diphosphate N-acetylglucosamine (>12 %). The high level of incorporation and number of eight <sup>13</sup>C atom within N-acetylglucosamine derivatives implies that these molecules are formed by direct conversion from  $\Delta$ AN2. The metabolization of hyaluronan by oMM<sup>12</sup> gut microbiota indicate that the orally administered hyaluronan may be efficiently metabolized into several metabolites, which may be available to the host and possibly biologically active.

### Please explain why your abstract is innovative for mass spectrometry?

Stable isotope–assisted metabolomics can be successfully employed for metabolite identification of even high molecular weight compounds such as hyaluronic acid and for investigation of its biotechnological production.

### Co-authors:

Martin Schwarzer, Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Tereza Foglova, Contipro a.s.

Dagmar Srutkova, Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Martina Hermannova, Contipro a.s.

Vladimir Velebny, Contipro a.s.

Poster number: **LS-PA-029**

## **A NOVEL LC-MS/MS METHOD FOR THE DETERMINATION OF SELECTED VITAMIN D METABOLITES AND ITS CLINICAL APPLICATION**

Abstract ID: **827**

**Presenting author: Ludmila Máčová, Institute of Endocrinology**

### **Introduction**

Due to the recognized pleiotropic positive effects involving immune system modulation and neuroprotection, vitamin D has become one of the most abundant analytes in clinical laboratories in the just-receding COVID-19 pandemics. As the number of analyzes performed increases, so do the analytical requirements for laboratory determination of vitamin D. Considering the vitamin D's lipophilic nature, tendency to bind to a protein transporter, and extremely low concentrations, its measurement is accompanied by several analytical challenges. A large number of structurally similar precursors and metabolites often lead to cross-reactions and overestimation of results when conventional routine immunoreaction-based methods are used. For the high specificity and sensitivity, chromatographic methods with mass spectrometry detection have become preferred for the evaluation of vitamin D status.

### **Methods**

The method uses the principle of liquid chromatography separation in tandem with mass spectrometry. Chromatographic separation was preceded by sample precipitation and liquid-liquid extraction with hexane. The HPLC system consisted of the ExionLC AD system and the separation was performed on a Phenomenex Kinetex PFP column with a binary flow gradient water/methanol with 0.1% formic acid. The MS/MS detection was accomplished using Sciex QTRAP 6500+ mass spectrometer operated in positive electrospray ionization and the multiple reaction monitoring mode (MRM). Three deuterated analogues were used as internal standards.

### **Preliminary data (results)**

A highly sensitive LC-MS/MS method has been developed enabling the simultaneous quantitation of 25-hydroxyvitamin D<sub>3</sub>, 25-hydroxyvitamin D<sub>2</sub>, 24,25-dihydroxyvitamin D<sub>3</sub>, and epimers 3-epi-25-hydroxyvitamin D<sub>3</sub> and 3-epi-25-hydroxyvitamin D<sub>2</sub> in human plasma. The developed method was successfully validated in accordance with FDA guidelines. VD-DDC Mass Spect Gold® as zero matrix and spiked serum samples were used to determine validation parameters. In addition, two different levels of certificate reference material ClinChek® Serum Control from RECIPE was used to ensure internal quality assurance of 25-hydroxyvitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>2</sub>. High linearity was achieved in the concentration range of 0.5-100 ng/mL without the need for derivatization. R<sup>2</sup> > 0.997 was achieved for each parameter. The novel method was applied in a study where vitamin D levels were measured in relation to COVID-19. The results showed a significant relationship between vitamin D levels and COVID-19 risks, and data will also be presented.

*The study was supported by MH CZ - DRO (Institute of Endocrinology - EÚ, 00023761).*

### **Please explain why your abstract is innovative for mass spectrometry?**

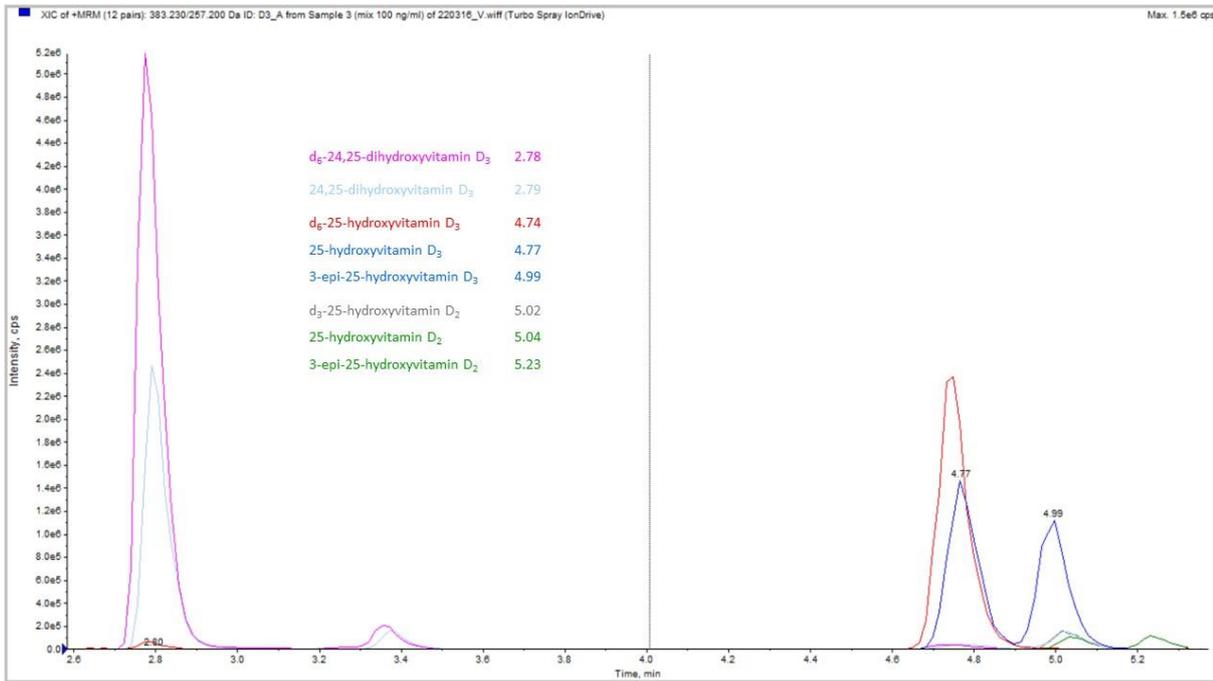
A rapid MS method has been developed for simultaneous determination of vitamin D metabolites in human plasma. The method tends to be a useful tool for monitoring vitamin D saturation.

### **Co-authors:**

*Marie Bičíková, Institute of Endocrinology  
Martin Hill, Institute of Endocrinology*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Chromatogram showing separation of vitamin D metabolites in standard solution

Poster number: **LS-PA-030**

## **RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (REIMS) FOR OXIDATIVE STRESS BIOMARKER DISCOVERY IN PSEUDOMONAS AERUGINOSA**

Abstract ID: **838**

**Presenting author: Rob Bradley, Department of Life Sciences, Imperial College London**

### **Introduction**

Rapid evaporative ionisation mass spectrometry (REIMS) allows for the high-throughput analysis of biological samples whilst requiring limited sample preparation. REIMS has shown promise for the rapid classification of microorganisms, however, less is known about the applicability of REIMS for biomarker discovery and mechanistic studies.

*P. aeruginosa* is a gram-negative bacteria that causes life-threatening infections in immunocompromised patients. The innate immune system is known to generate antimicrobial oxidants to defend against invading pathogens. However, the mode of killing and importance of such oxidants in the immune response is unclear.

In this study, REIMS was tested as a technique to identify biomarkers of oxidative stress in *P. aeruginosa* exposed to range of oxidants, resulting in potential insight into the stress protection systems employed by the bacteria.

### **Methods**

Deletion mutants were generated in genes known to play a role in the oxidative stress response of *P. aeruginosa*. These mutants were grown, alongside WT, in LB media to mid-exponential phase in the absence and presence of a range of oxidants. The cultures were washed using ammonium acetate, pelleted and analysed using laser-assisted REIMS (LA-REIMS).

### **Preliminary data (results)**

In total, 6 different oxidants were tested. For each oxidant, REIMS was able to reliably distinguish between untreated and treated samples over 4 individual days of experimentation. The classification accuracy determined using the Random Forest classification algorithm was close to 100% at the oxidant concentrations used in the study.

A number of features were identified as potential biomarkers of oxidative stress. Some of these markers were oxidant-specific whereas others appear to be general to oxidative stress. Preliminary identification of the metabolites suggests a range of molecules are impacted by the stress. These include quorum sensing signalling molecules that control the production of virulence factors in *P. aeruginosa* and phospholipid molecules that constitute the cell membrane of the bacteria.

For a number of the deletion mutants analysed in the study, a distinct metabolic profile was recorded. The presence or lack of certain molecules in these mutants can shed light on the role the relevant gene plays in the oxidative stress protection mechanisms employed by *P. aeruginosa*. The finding that REIMS is sufficiently sensitive to provide mechanistic insight pairs favourably with the high-throughput nature of the technique. For example, a large number of mutants could be rapidly screened to identify molecules of interest to a wide range of mechanistic questions.

### **Please explain why your abstract is innovative for mass spectrometry?**

Novel application of REIMS to biomarker discovery and mechanistic insight in the model organism *P. aeruginosa*.

### **Co-authors:**

*Daniel Simon, Department of Metabolism, Digestion and Reproduction, Imperial College London*  
*Zoltan Takats, Department of Metabolism, Digestion and Reproduction, Imperial College London*  
*Huw Williams, Department of Life Sciences, Imperial College London*

Poster number: LS-PA-031

## DIFFERENTIATION OF AYAHUASCA SAMPLES ACCORDING TO ORIGIN AND RELIGIOUS GROUPS USING MULTIVARIATE STATISTICAL ANALYSIS OF LC-MS DATA

Abstract ID: 842

**Presenting author:** Taynara Matos, Laboratory of Bioanalytics and Integrated Omics (LaBIOmics), Institute of Chemistry, University of Campinas

### Introduction

Ayahuasca is a brew used by indigenous people from the Amazonia region for medicinal, spiritual, and cultural purposes. Preparation of the brew usually is the same: boiling layers of washed leaves of *P. viridis* with crushed *B. caapi* liana and water<sup>2</sup>. The main compounds found in the brew belong to the alkaloid metabolite class<sup>1</sup>.  $\beta$ -carbolines from the liana inhibit the monoamine oxidase enzyme whereas *N,N*-dimethyltryptamine acts as agonist on the serotonergic receptors<sup>3</sup>. Ayahuasca has demonstrated antidepressive, anxiolytic, and antiaddictive effects<sup>4,5</sup>. When considering the potential of ayahuasca as a new phytomedicine to treat depression, it is important to establish which parameters can significantly modify the composition of the brew.

### Methods

126 ayahuasca samples were collected from ceremonies held in different countries (Estonia, Finland, Greece, USA, Brazil, and Italy) and from different religious groups (Santo Daime, shamanic, neoshamanic, and União do Vegetal - UDV). Initially, samples were separated in UDV, Estonia (EST), and the others in the European (EUR) samples group. Analysis was made using an UHPLC-ESI(-)-MS method. Data preprocessing was made by *Progenesis Q1 2.0* software and the data pretreatment and statistical analysis were performed on *MetaboAnalyst 5.0*.

### Preliminary data (results)

PCA score plots demonstrated that most of the QC samples are centralized and grouped together, thus indicating a satisfactory and reproducible analysis method. PCA score plots also present a tendency towards clustering between EST and EUR samples, whereas the Brazilian (UDV) samples tend to separate from all European samples. In addition, there is a greater dispersion of EUR samples, whereas the UDV samples are clustered. Samples from abroad of Brazil (*Exterior* - EXT) were grouped and compared to UDV samples. PLS-DA was used to discriminate samples and find potential biomarker candidates for samples differentiation. The sum of squares captured by the model ( $R^2$ ), the cross-validated  $R^2$  (also known as  $Q^2$ ), and the prediction accuracy were evaluated of 10-fold cross validation resulting in values above 0.7. The AUC of ROC obtained value ( $> 0.9$ ) reinforces the difference between the sample groups analyzed. PLS-DA showed that EXT x UDV and Santo Daime x UDV share the same VIP scores.

### Please explain why your abstract is innovative for mass spectrometry?

An innovative use of UHPLC-ESI-MS associated with chemometrics analysis was developed to unraveling the ayahuasca's minor compounds and generating new information to understand the biochemistry of depression

### Co-authors:

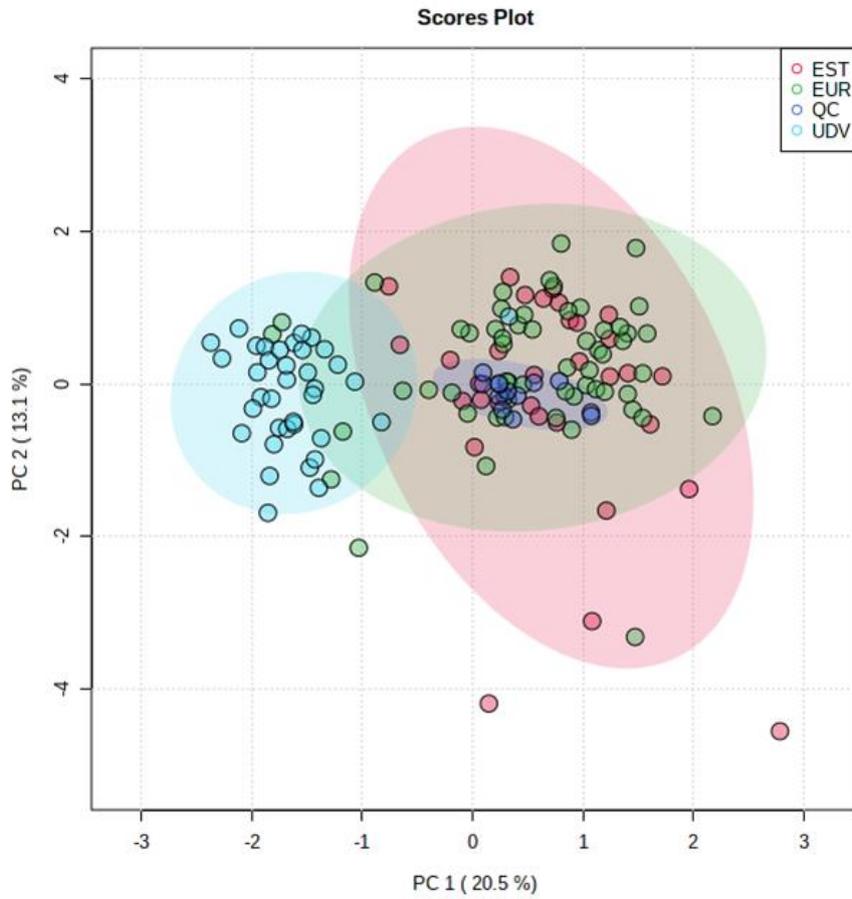
Alex Silva, Health Sciences Postgraduate Program, São Francisco University

Andreia Porcari, Health Sciences Postgraduate Program, São Francisco University

Alessandra Sussulini, Laboratory of Bioanalytics and Integrated Omics (LaBIOmics), Institute of Chemistry, University of Campinas

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



PCA of ayahuasca samples varying the origin.

Poster number: **LS-PA-032**

## **METABOLIC SIGNATURE OF ETHANOL-INDUCED HEPATOTOXICITY IN HEPARG CELLS BY LC-MS-BASED UNTARGETED METABOLOMICS**

Abstract ID: **943**

**Presenting author: Elias Iturraspe, Toxicological Center, University of Antwerp, Belgium, Department of In Vitro Toxicology and Dermato-cosmetology, Vrije Universiteit Brussel, Belgium**

### **Introduction**

Alcoholic liver disease is highly prevalent but poorly identified and characterized, leading to knowledge gaps, which impairs early diagnosis. Excessive alcohol consumption is known to alter lipid metabolism, followed by progressive intracellular lipid accumulation, resulting in alcoholic fatty liver disease.

### **Methods**

In this study, HepaRG cells were exposed to ethanol at IC<sub>10</sub> and 1/10 IC<sub>10</sub> for 24 and 48 h. Metabolic alterations were investigated intra- and extracellularly with liquid chromatography–high-resolution mass spectrometry. Ion mobility was added as an extra separation dimension for untargeted lipidomics to improve annotation confidence.

### **Preliminary data (results)**

Distinctive patterns between exposed and control cells were consistently observed, with intracellular upregulation of di- and triglycerides, downregulation of phosphatidylcholines and phosphatidylethanolamines, sphingomyelins, and S-adenosylmethionine, among others. Several intracellular metabolic patterns could be related to changes in the extracellular environment, such as increased intracellular hydrolysis of sphingomyelins, leading to increased phosphorylcholine secretion. Carnitines showed alterations depending on the size of their carbon chain, which highlights the interplay between  $\beta$ -oxidation in mitochondria and peroxisomes. Potential new biomarkers of ethanol-induced hepatotoxicity have been observed, such as ceramides with a sphingadienine backbone, octanoylcarnitine, creatine, acetylcholine, and ethoxylated phosphorylcholine. The combination of the metabolic fingerprint and footprint enabled a comprehensive investigation of the pathophysiology behind ethanol-induced hepatotoxicity.

### **Please explain why your abstract is innovative for mass spectrometry?**

Metabolomics and lipidomics platforms were developed in-house using innovative techniques such as HILIC and drift tube ion mobility hyphenated to MS. To improve fragmentation coverage, iterative exclusion DDA was applied.

### **Co-authors:**

*Katyeny Manuela da Silva, Toxicological Center, University of Antwerp, Belgium*

*Rani Robeyns, Toxicological Center, University of Antwerp, Belgium*

*Maria van de Lavoie, Toxicological Center, University of Antwerp, Belgium*

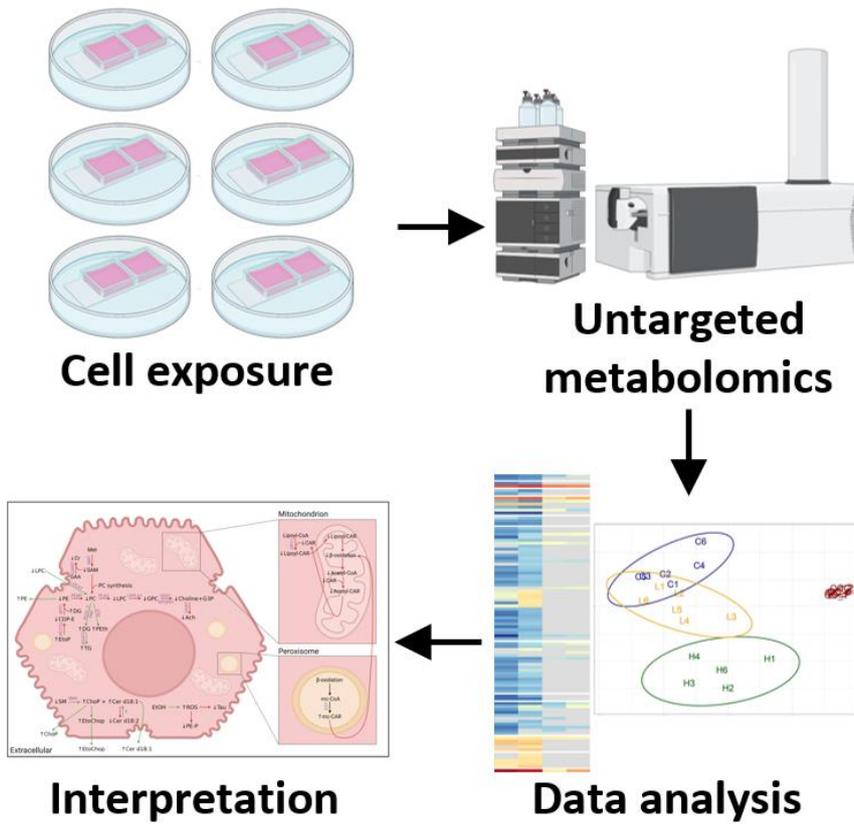
*Tamara Vanhaecke, Department of In Vitro Toxicology and Dermato-cosmetology, Vrije Universiteit Brussel, Belgium*

*Alexander L.N. van Nuijs, Toxicological Center, University of Antwerp, Belgium*

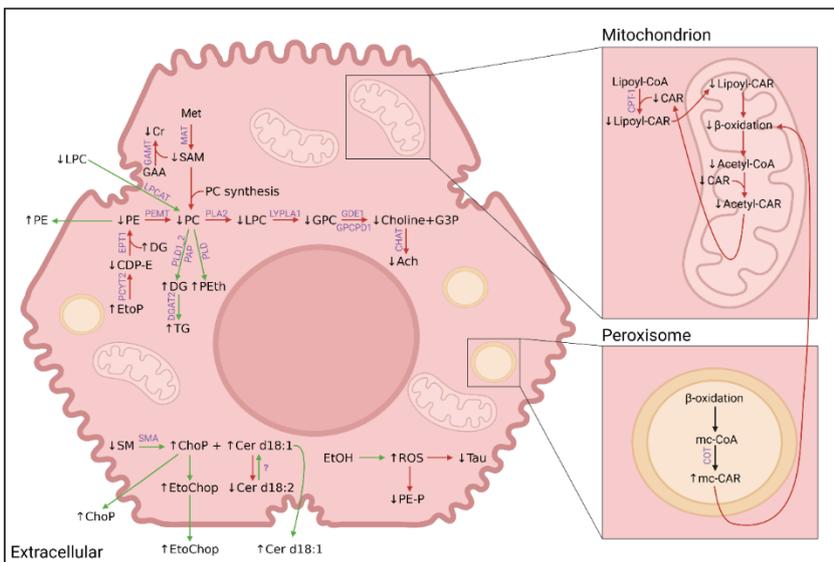
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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Graphical abstract.



Metabolic changes in HepaRG cells after ethanol exposure.

Poster number: **LS-PA-033**

## **EVALUATION THE DRUG-INDUCED LIVER INJURY POTENTIAL OF SAXAGLIPTIN THROUGH THE IDENTIFICATION OF REACTIVE METABOLITES**

Abstract ID: **946**

**Presenting author: Ki Young Kim, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea**

### **Introduction**

Saxagliptin has been used as an antidiabetic drug by promoting insulin secretion and lowering glucagon concentration. In 2018, the possibility of drug-induced liver injury (DILI) in DPP-4 inhibitors, which was approved by FDA, was announced through Liver-tox. DILI cases of DPP-4 inhibitors including saxagliptin have been published, but no studies have been reported on detailed mechanisms and metabolic pathways for saxagliptin. Vildagliptins are known to bind cysteine to pyrrolidine rings containing cyanide groups, and saxagliptin also has azabicyclohexane.

### **Methods**

Our in vitro studies have confirmed that when L-cysteine was used as a trapping agent, cysteine was bound to saxagliptin, and these results suggest that saxagliptin can form irreversible bonds with endogenous proteins. We are currently conducting an in vivo metabolism study of saxagliptin in rat. After oral administration of saxagliptin (10, 50, and 100 mg/kg) in rats, plasma, bile, and liver samples were collected for LC-MS/MS analysis.

### **Preliminary data (results)**

The sample pretreatment method for the bile, plasma, and liver was optimized. In bile sample, a total of 21 saxagliptin metabolites, including 4 novel metabolites (saxagliptin cysteine conjugate, hydroxysaxagliptin cysteine conjugate, saxagliptin glutathione conjugate, and hydroxysaxagliptin glutathione conjugate), were found. These results suggest that saxagliptin can form irreversible bonds with cysteine residue of endogenous proteins. We are currently identifying the saxagliptin metabolites in plasma and liver tissues to comprehensively elucidate the in vivo metabolism of saxagliptin in rats.

### **Please explain why your abstract is innovative for mass spectrometry?**

Using LC-MS/MS, the possibility of Drug-induced Liver Injury (DILI) in Saxagliptin, a type 2 diabetes treatment, was confirmed.

### **Co-authors:**

*Yeo Jin Jeong, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea*  
*So young Park, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea*  
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*Mass spectrometry convergence research institute, Kyungpook National University, Daegu 41566, South Korea*

Poster number: **LS-PA-034**

## **IN VIVO METABOLISM OF DONEPEZIL IN RATS USING NON-TARGETED METABOLOMICS**

Abstract ID: **947**

**Presenting author: Eun-Ji Park, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea**

### **Introduction**

Donepezil is a reversible acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. Donepezil has low side effects and has been used commercially in many countries. Nevertheless, cases of drug-related hepatotoxicity have been reported. There is a lack of data available on the metabolic profile of donepezil and the mechanism of liver damage it causes. In our previous study, the in vitro metabolism of donepezil was investigated using non-targeted metabolomics and as a result, new metabolites and a potential reactive metabolite were identified. They also demonstrated non-targeted metabolomics technique combined with molecular networking is an effective tool to detect unknown drug metabolites. In this study, the in vivo metabolism of donepezil in rats was elucidated using LC-MS/MS based on a non-targeted metabolomics approach.

### **Methods**

After oral administration of donepezil (10 and 30 mg/kg) in rats, urine, feces, and liver samples were collected for LC-MS/MS analysis. The data were processed to molecular networking and multivariate data analysis to identify metabolites.

### **Preliminary data (results)**

In rat urine, a total of 26 donepezil metabolites, including 6 novel metabolites (*O*-desmethyl-*N*-desbenzyl donepezil, dihydroxydonepezil, hydroxydonepezil glucuronide, and so on), were found. Phase I (*O*-demethylation, didemethylation, *N*-debenzylation, *N*-oxidation, and hydroxylation) and phase II (glucuronidation and sulfate conjugation) reactions were the main biotransformation pathways of donepezil in rats. We are currently also identifying the donepezil metabolites in the rat feces and liver to comprehensively elucidate the in vivo metabolism of donepezil in rats.

### **Please explain why your abstract is innovative for mass spectrometry?**

This study proves that a non-targeted metabolomics approach using LC-MS/MS is a reliable tool to identify unknown drug metabolites and proposes the in vivo metabolic pathway of donepezil.

### **Co-authors:**

*Ki-Young Kim, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea*  
*So-Young Park, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea*  
*Kyung-Sik Song, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea*  
*Kwang-Hyeon Liu, BK21 FOUR Community-Based Intelligent Novel Drug Discovery Education Unit, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, South Korea*  
*Mass spectrometry convergence research institute, Kyungpook National University, Daegu 41566, Korea*

Poster number: **LS-PA-035**

## **ASSOCIATION OF TRYPTOPHAN PATHWAY-RELATED METABOLITES: DEPLETION OF DIETARY ARYL HYDROCARBON RECEPTOR LIGANDS INDUCED ATOPIC DERMATITIS IN MICE**

Abstract ID: **949**

**Presenting author: You Mee Ahn, Korea Institute of Oriental Medicine**

### **Introduction**

The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor sensing xenobiotic chemicals of exogenous and endogenous origin, including phytochemicals, microbial bio-products, and tryptophan photoproducts, and is highly expressed in the epidermis. AhR has gained special attention because it plays a crucial role in photoaging, epidermal differentiation, and immunomodulation.

### **Methods**

We assigned C57BL/6 mice to a conventional diet, an AhR ligand-free diet (AHR), or an AhR ligand-free diet plus 2,4-dinitrochlorobenzene -induced atopic dermatitis (AHRAD). To identify the relationship between improvement of AD symptoms and skin metabolites, we conducted an untargeted metabolomics approach using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) followed by multivariate statistical analysis.

### **Preliminary data (results)**

In this study, we investigated the change of tryptophan pathway-related metabolites in the skin and observed the skin barrier function and immune response. AHR and AHRAD mice showed high transepidermal water loss (TEWL), indicating a loss of skin barrier integrity. A partial least squares-discriminant analysis (PLS-DA) score plot revealed distinct metabolic patterns of the skin obtained from the control, AHR, and AHRAD mice. Furthermore, metabolites related to the tryptophan pathway include tryptophan, serotonin, kynurenine, kynurenic acid, anthranilic acid, 3-hydroxyanthranilic acid, 3-hydroxykynurenine were also evaluated. We found that tryptophan, serotonin and 3-hydroxykynurenine were significantly altered by the AHRAD mice compared AHR mice. These results suggest that AhR ligand deficiency may exacerbate atopic dermatitis symptoms. Nevertheless, further studies are required to elucidate the detailed roles of AhR in atopic dermatitis skin diseases.

### **Please explain why your abstract is innovative for mass spectrometry?**

We also verified that metabolomics studies using UPLC/Q-TOF MS provide comprehensive understanding of atopic dermatitis skin diseases.

Poster number: **LS-PA-036**

## **FOCUS ON QUALITY IN A FULLY INTEGRATED SOFTWARE SOLUTION FOR EXPLORING METABOLIC PROFILING DATASETS**

Abstract ID: **976**

**Presenting author: Nikolas Kessler, Bruker Life Sciences Mass Spectrometry Division**

### **Introduction**

Metabolic profiling is routinely employed in biomedical research demanding high quality data at various scales of clinical and population phenotyping. In recent years, numerous publications have focused on quality control in metabolomics, describing increased interest and cooperation within the field. MetaboScape is a quality-focused software solution for the processing of untargeted metabolic profiling data that integrates the field's best practices to support the user's comprehensive investigation and control of metabolomics data quality. We assessed quality control measures on a batch of more than 1000 urine sample analyses acquired on a UHPLC-HR-QTOF system. The study design featured regularly interleaved pooled quality control (QC) samples for assessment of technical variation and use in data filtering, within-batch correction, and quality assessment.

### **Methods**

Three distinct human urine samples and three pairwise mixtures were diluted 1:3 with water and centrifuged. The supernatant was aliquoted for chromatographic separation using a linear reversed-phase gradient with a 15 minute cycle time. LC-MS data (ESI positive) were acquired on an Impact II QTOF-MS (Bruker Daltonics). Every seventh injection corresponded to a QC sample which was an equal-parts mixture of each sample in the study.

The ion source region was cleaned and the detector was tuned prior to sample analysis which was conducted in two distinct intended batches.

Quality assessment and within-batch correction have been performed in MetaboScape.

### **Preliminary data (results)**

Feature filtering based on the reoccurrence across replicate QC sample measurements, the observed relative standard deviation (RSD) across QCs, and the observed ratio of total and technical variation (measured across study and QC samples, respectively) are supported and illustrated. Clear and interactive visualisations of data quality are provided which aid the user in conveniently selecting and filtering data based on these criteria. Markers for highlighting QC outliers are included, making disturbances during the batch immediately visible. Within-batch correction is also supported, leveraging the QC sample data to account for feature-specific run-order-dependent intensity drifts using a LOESS function and large bandwidth to prevent overfitting. Finally, the quality of annotation reporting is also considered, with five-tiered Annotation Quality (AQ) (m/z, RT, isotope pattern, MS/MS and CCS) scores presented in a concise but comprehensive manner. This was exemplified here using a target list derived from the HMDB 2.0 database. Taken together, these capabilities enable users to confidently control and review the quality of their metabolic profiling data.

### **Please explain why your abstract is innovative for mass spectrometry?**

LC-HRMS workflow for enabling large scale metabolic profiling research with streamlined quality assessment and within-batch correction in an integrated software solution

### **Co-authors:**

*Aiko Barsch, Bruker Life Sciences Mass Spectrometry Division*

*Konstantin Schwarze, Bruker Life Sciences Mass Spectrometry Division*

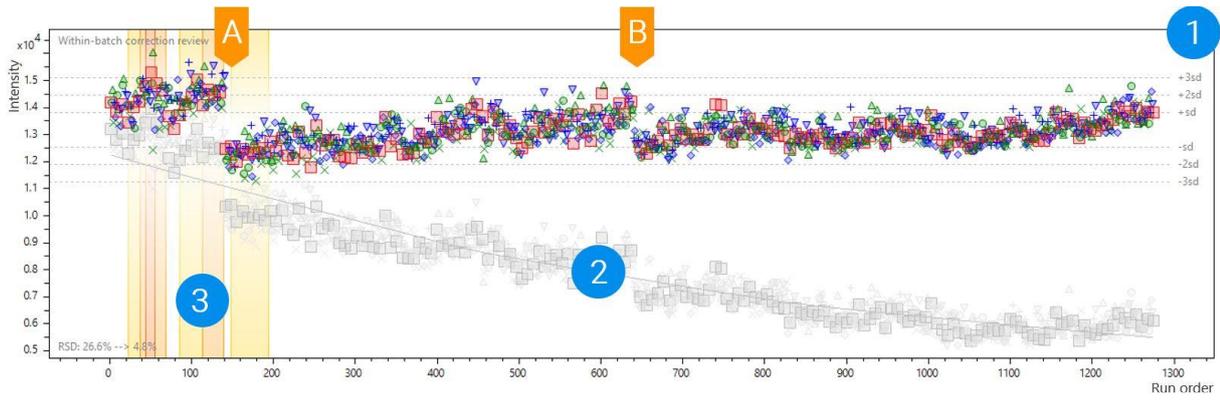
*Sofie Weinkouff, Bruker Life Sciences Mass Spectrometry Division*

*Heiko Neuweger, Bruker Life Sciences Mass Spectrometry Division*

*Matthew Lewis, Bruker Life Sciences Mass Spectrometry Division*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Within-batch correction of a strongly decomposing compound.

Poster number: **LS-PA-037**

## **ENHANCED UNTARGETED METABOLOMICS WORKFLOW USING LC-QTOF AND METABOSCAPE FOR ANALYSIS OF GUT MICROBIAL METABOLISM**

Abstract ID: **984**

**Presenting author: Erica Forsberg, Bruker Daltonics, San Diego State University**

### **Introduction**

Untargeted metabolomics data analysis strategies must handle large data files and produce biologically and statistically relevant data. There is an unmet need for an efficient single bioinformatic solution for handling these data sets. Metaboscape, Bruker's integrated untargeted metabolomics software solution, was used to analyze a model gut microbe system exposed to the synthetic hormones ethinyl estradiol and levonorgestrel found in common oral contraceptives. A comparison was made with the open source strategies XCMS and GNPS. Results indicate significant down regulation of amino acids in *Bacteroides fragilis*, up regulation in *Lactobacillus rhamnosus*, and a mediating effect in a co-culture. This may indicate that supplementation of a probiotic may ameliorate the effects of synthetic hormones on gut microbial metabolism.

### **Methods**

*Bacteroides fragilis* and *Lactobacillus rhamnosus* were grown anaerobically and exposed to ethinyl estradiol and/or levonorgestrel versus control. Hormone/bacterial conditions were grown until late-log phase. Cell pellets were washed in minimal media, then quenched with acetonitrile:methanol:water. Extracts were dried and reconstituted in acetonitrile:water. Untargeted metabolomics data was acquired using RP chromatography coupled with a Bruker Impact II QTOF using autoMSMS in positive mode. XCMS was used for feature detection and statistical analysis of metabolites between hormone/bacterial conditions. GNPS was used for MSMS fragmentation pattern matching. A comparison was made with Metaboscape as a complete untargeted data analysis workflow.

### **Preliminary data (results)**

Comparisons were performed between metabolite features from high resolution MS data from extracts of *Bacteroides fragilis*, *Lactobacillus rhamnosus*, and a mixed co-culture with the following hormone conditions: 0.7  $\mu\text{M}$  ethinyl estradiol, 3  $\mu\text{M}$  levonorgestrel, 50  $\mu\text{M}$  levonorgestrel, and 0.7  $\mu\text{M}$  ethinyl estradiol with 3  $\mu\text{M}$  levonorgestrel versus the "no hormone" controls.

A total of 1808 features were found to be dysregulated in *Bacteroides fragilis* hormone conditions, 1468 were dysregulated in *Lactobacillus rhamnosus*, and 825 were dysregulated in the mixed co-culture using XCMS Online. Although XCMS is suitable for feature detection and statistical analysis, it lacks the ability to perform MSMS fragmentation pattern matching. GNPS utilizes a cosine similarity algorithm to perform fragmentation spectral matching. Thus, this workflow was used to validate dysregulated metabolites. To expedite feature detection and annotation, Bruker Metaboscape software provided one complete workflow without the need for data conversion or transfer of data between platforms. Use of PCA loading plots and volcano plots allowed for fastidious identification of dysregulated features, coupled with rapid metabolite annotation to validate feature identity using the MetaboBASE 3.0 Spectral Library.

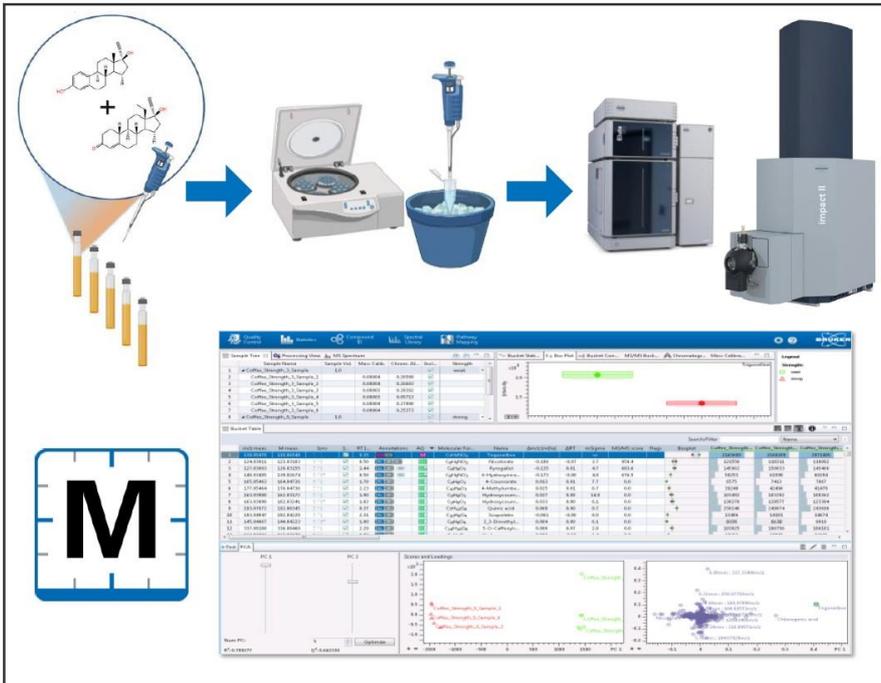
Several amino acids were significantly downregulated in *Bacteroides fragilis* in many hormone conditions of ethinyl estradiol and levonorgestrel. In *Lactobacillus rhamnosus* cultures, amino acids were also found to be significantly upregulated in many hormone conditions. In the co-culture, there was a mixture of up and down regulation of these amino acids with substantially less fold change indicating a mediating effect. Trends were consistent between open source XCMS/GNPS and Metaboscape workflows.

### **Please explain why your abstract is innovative for mass spectrometry?**

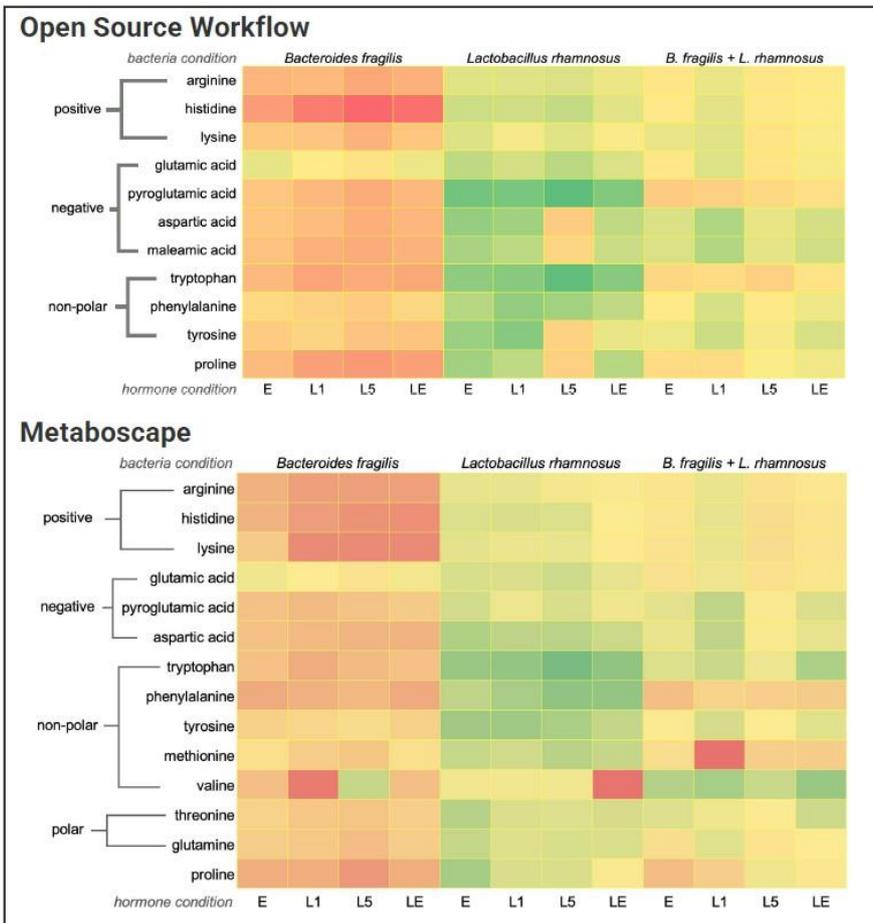
Untargeted metabolomics data analysis with Metaboscape provides fast and efficient metabolite annotation and statistical analysis of gut microbial metabolomes exposed to synthetic hormones.

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Untargeted metabolomics workflow for gut microbial metabolism



Heatmap of amino acids in different bacterial/hormone conditions

Poster number: **LS-PA-038**

## STUDY AND STATISTICAL ANALYSIS OF SMALL MOLECULES IN SNAKE VENOM

Abstract ID: **991**

Presenting author: **Luis L. Alonso, Division of BioAnalytical Chemistry**

### Introduction

Venomous snakebite is one of the most complex Neglected Tropical Diseases (NTDs) amongst others due to the high variability of the toxins and other compounds within snake venoms. Most research efforts focus on the toxins within this biological matrix, but the knowledge regarding the metabolites inside venoms is limited. The insights that could be gained by investigating these molecules remains unknown to a large extent, and thus the potential of studying them is large. Due to the complexity of this matrix, multivariate statistical analysis becomes a paramount technique when analyzing it.

### Methods

RP-HPLC separation was carried out using a Shimadzu HPLC system and a Waters Xbridge Peptide BEH300 C18 analytical column (100x4.6 mm), 3.5  $\mu$ m particle size and a 300Å pore size with a 0.5mL/min flow. MS small molecule detection was performed using a MaxIs QTOF mass spectrometer via an ESI source operating in positive ion mode with: temperature 220°C, capillary voltage 4.5 kV, gas flow 8.0 L/min, Nebulizer pressure 1.8 Bar. Data analysis was done by algorithms written in Julia language.

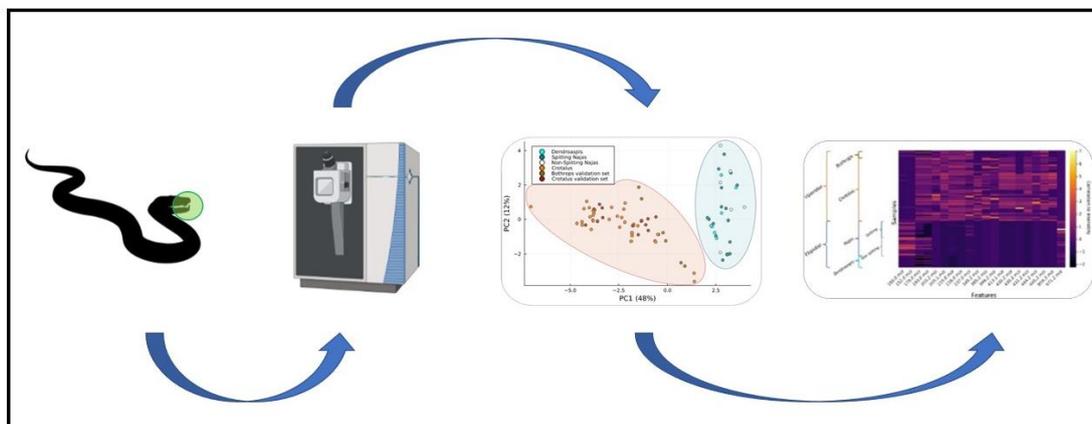
### Preliminary data (results)

Among the different tested models, the best one was set to be the one that contained 20 variables and used a Support Vector Machine (SVMs) to perform the classification. An underlying pattern within the data able to differentiate between the two families was found, and all samples coming from test sets were correctly identified.

Said 20 features were traced back to the metabolites from which they came by MS/MS and/or by standard addition - depending on their commercial availability-, and a biological explanation for the linkage between the levels of said molecules and the taxonomic unit they come from was formulated. Thus, metabolic studies of snake venom offer another perspective on snake venom and open a new path of research to gain more insight about this NTD.

### Please explain why your abstract is innovative for mass spectrometry?

A workflow for semi-supervised analysis of untargeted MS metabolomics data was developed

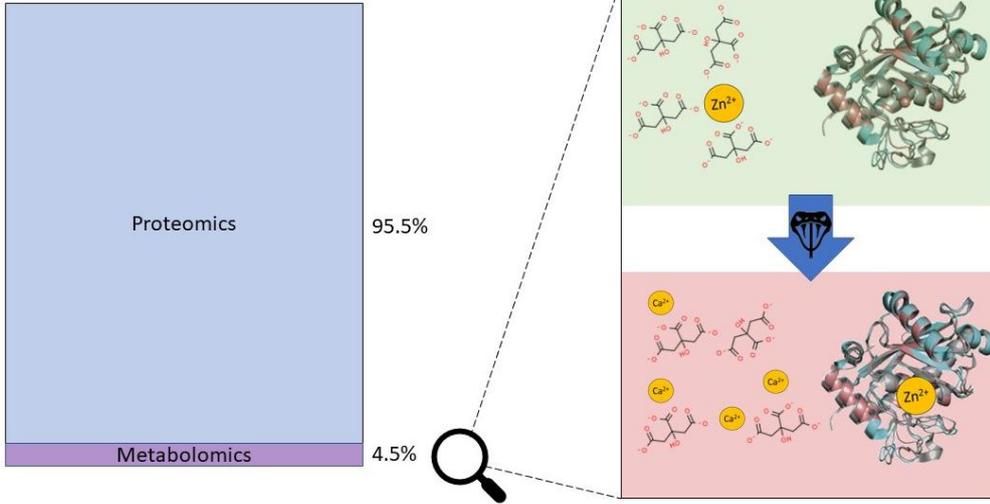


**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

From snake venom to its most relevant metabolites

Snake venom publications (2018-22)



Metabolomic research can aid in the hollistic understanding of venoms

Poster number: **LS-PA-039**

## A MULTI-ACQUISITION-MODE STRATEGY FOR IN-DEPTH METABOLOMICS ANALYSIS

Abstract ID: **1012**

**Presenting author: Jason Causon, SCIEX**

### Introduction

When performing untargeted metabolite identification from biological matrices, several strategies can be applied. On HRMS systems, untargeted analysis is performed using information dependent acquisition/data dependent acquisition (IDA/DDA). While this approach enables the identification of both expected and unknown metabolites, it suffers from gaps in MS/MS coverage or poor-quality MS/MS. In previous work, we showed that activation of the Zeno trap increased the MS/MS signal and peak area for polar metabolite fragments by up to 14x, leading to improved quantification and identification.

Here, we use a wider array of biological sample types to investigate using the Zeno trap with SWATH DIA. The benefit of this Zeno SWATH DIA approach is the untargeted nature of collecting MS/MS data on every precursor.

### Methods

Urine samples were obtained (N=5) from Zucker diabetic fatty (ZDF) rats, male and female, and Sprague Dawley (SD) rats, male and female. NIST 1950 human plasma, *E. Coli* credentialed cell and yeast extracts were also used. Urine and plasma were subjected to protein precipitation. *E. Coli* and yeast extracts were reconstituted in acetonitrile: water. Chromatography was performed on a Phenomenex Luna Omega Polar C18 (150 x 2.1 mm) or F5 (150 x 2.1 mm) columns coupled to the ZenoTOF 7600 system operated in DDA, MRM<sup>HR</sup>, SWATH and Zeno SWATH modes. Data were processed with SCIEX OS software or OneOmics.

### Preliminary data (results)

We explored the wider utility of using the Zeno trap with SWATH compared to IDA/DDA and MRM<sup>HR</sup>. We initially used the rat urine samples to make the comparison by looking at spectral quality using the different acquisition strategies and sample loading volumes while assessing raw signal and the number of identified metabolites. The first analysis compared a 0.2 $\mu$ L injection of 1:10 diluted rat urine using Zeno MRM<sup>HR</sup> and Zeno SWATH acquisition and a 2  $\mu$ L injection using SWATH acquisition. Cyclic AMP, which showed a 2-fold (Log<sub>2</sub>) change in the biological set, was chosen and extracted for the *m/z* 136.0602 fragment ion (10 mDa mass extraction window, MEW). The XIC peak intensities were 4.8e4, 5.6e4 and 5.5e4 cps, respectively. The 0.2 $\mu$ L injection using Zeno MRM<sup>HR</sup> and Zeno SWATH with the enhanced duty cycle showed the same sensitivity as the 2 $\mu$ L injection without the Zeno trap, highlighting the 10-fold gain in sensitivity.

Another benefit of the increased sensitivity of Zeno MS/MS with SWATH acquisition is flexibility in the number and distribution of the variable windows due to fast acquisition rates. The variable window method employed here is 80 windows between *m/z* 80 and 650, with accumulation times of 5 ms. A larger number of smaller windows increases selectivity while high scan rates maintain the number of data points across the LC peak, which was ~10 points across 6-second peak widths.

Analysis of identified metabolites that meet the 5-tier scoring will be presented.

### Please explain why your abstract is innovative for mass spectrometry?

With the Zeno trap enabled, MS/MS for SWATH acquisition closely matches targeted Zeno MRM<sup>HR</sup> sensitivity.

### Co-authors:

*Jose Castro-Perez, SCIEX*

Poster number: LS-PA-040

## COMPREHENSIVE METABOLITE CHARACTERIZATION USING ORTHOGONAL MS/MS DATA

Abstract ID: 1017

Presenting author: Eva Duchoslav, SCIEX

### Introduction

Qualitative capabilities of high-resolution mass spectrometry (HRMS), such as automated LC-MS/MS workflows using collision induced dissociation (CID), have been crucial for pharmaceutical drug development to investigate the metabolism of candidate modalities at the early stages of development. Recent HRMS technology advancements, including improvements in the duty cycle, enabled the application of electron activated dissociation (EAD) on LC timescales and the integration of this complementary MS/MS fragmentation mechanism into LC-MS/MS workflows, providing a more confident characterization of the compounds of interest. This presentation reports on the utility of “orthogonal” MS/MS fragmentation of complementary TOF MS precursors for a more comprehensive characterization of metabolites.

### Methods

Verapamil, buspirone and nefazodone were incubated in rat hepatocytes at a 1  $\mu$ M, and quenched with acetonitrile at 0-, 30- and 120-minutes. Phenomenex Luna Omega Polar C18, 150 mm column, using a 5  $\mu$ L injection volume was used with 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) over 4.75 minutes, from 5%B to 95%B with a total runtime of 6.5 minutes. The samples were analyzed in data-dependent mode using Zeno CID IDA and Zeno EAD IDA on a ZenoTOF 7600 system. Data was processed with a prototype version of Molecule Profiler software.

### Preliminary data (results)

The information-dependent acquisition MS/MS coverage for TOF MS peaks of interest were analyzed in Molecule Profiler software where the parent annotation is used in the proposal and ranking of structures of candidate metabolites. For the metabolites that have structures sufficiently different from the parent drug and thus yield significantly different fragmentation fingerprints, the annotation of fragments with respect to a hypothetical structure is used without relying on the annotation of the MS/MS spectrum of parent drug. We utilize the MS/MS of  $[M+Na]^+$  and the protonated precursors. To date, from 15 studied metabolites that covered hydroxylations and glucuronide conjugations either on parent drug or a cleavage product, the automated structure proposal gave the correct answer at rank 1 for 14 metabolites. Reconciling the evidence for a site of modification from the Zeno CID and Zeno EAD MS/MS data reduced the ambiguity in the structure proposal and enabled confident elucidation of positional isomers. We found that for the glucuronide conjugates, the EAD data provided more specific MS/MS fragments than CID and was instrumental in correctly pinpointing the site of modification. With the improvement of TOF MS/MS sensitivity due to Zeno trap activation, we found that the low abundance, accurate MS/MS fragments of  $[M+Na]^+$  helped to confirm and complement the evidence supporting the structure characterization of drug metabolites.

### Please explain why your abstract is innovative for mass spectrometry?

Utilizing sensitive orthogonal MS/MS fragmentation of complementary TOF MS precursors enables confident metabolite structure characterization.

### Co-authors:

*Disha Thakkar, SCIEX*

*Yves Le Blanc, SCIEX*

*Jason Causon, SCIEX*

## Session: MS in Structural biology - Crosslinking MS

Poster number: LS-PA-041

### STUDYING NUCLEOTIDE AND NUCLEOTIDE - PROTEIN PHOTO-CROSSLINKING USING CRYOGENIC IR SPECTROSCOPY

Abstract ID: 32

**Presenting author: Dominika Strzelecka, Institut für Chemie und Biochemie, Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4–6, 14195 Berlin, Germany, Institut Européen de Chimie et Biologie, University of Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, IECB, F-33600 Pessac, France**

#### Introduction

Photochemical crosslinking is an analytical technique for the characterization of RNA-protein interactions in complexes. Intermolecular crosslinks can be generated by exposure of native RNA-protein complexes to short UV light (254 nm). However, UV-photo-crosslinking is relatively nonspecific with low yield and the reaction is therefore still poorly understood. Covalent crosslinks may form not only under laboratory conditions but also occur in a skin cells during exposure to sunlight. UV induces the formation of covalent linkages between nucleobases and produces pyrimidine dimers. In a cell, up to 100 such reactions might occur per second. Although those pathological changes are usually corrected by a complex machinery of repair enzymes, some cross-links remain and result in wrong base pairing during replication. This leads to mutagenesis and ultimately cancer.

#### Methods

The first step to understand the mechanism of photo-crosslinking in detail is to investigate the structure of the resulting products. The structure of a few crosslinking products was reported in the literature, however, due to their low abundance it is difficult to unambiguously assign a particular structure. In order to close this gap we here aim to unravel the structure of nucleotide-amino acid and dimeric nucleotide products in the gas phase using nanoelectrospray ionization - ion mobility mass spectrometry (nESI-IMS) and cold-ion IR spectroscopy.

#### Preliminary data (results)

Using UV light (254 nm) we obtained four different photoproducts: uridine 5'-monophosphate (UMP) with cysteine (Cys), thymidine 5'-monophosphate (TMP) with cysteine, aUMP-dimer and a TMP-dimer. For the analysis, precursor ions were fragmented in-source and IR-spectra were recorded. The experimental IR-spectra of the fragments were compared to the theoretical spectra obtained by DFT. In case of UMP we identified the uracyl cyclobutane dimer as the major product of photo-crosslinking.

#### Please explain why your abstract is innovative for mass spectrometry?

The study demonstrates that tandem MS in combination with gas-phase infrared spectroscopy of ions is perfectly suited to unravel the structure of complex, low-abundant molecules of biological relevance.

#### Co-authors:

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Poster number: **LS-PA-042**

## **A PROTEIN-NUCLEIC ACID CROSSLINKING NODE FOR PROTEOME DISCOVERER 2.5 SOFTWARE**

Abstract ID: **177**

**Presenting author: Aditi Sharma, Thermo Fisher Scientific (Bremen) GmbH**

### **Introduction**

In recent years workflows around crosslinking mass spectrometry (XL-MS) of protein-DNA and protein-RNA complexes have made great strides, but data processing and visualization still remains a challenge. The introduction of OpenMS crosslinking search engine NuXL (manuscript in preparation) has significantly improved data processing and crosslink detection between proteins and RNA/DNA moieties. Thermo Scientific™ Proteome Discoverer™ 2.5 software (PD) offers interactive visualization and node-based data processing capabilities. Making the novel NuXL tool available inside the PD graphical user interface (GUI) makes state-of-the art computational methods for XL discovery easy to configure, execute and visualize.

### **Methods**

We developed a novel plugin compatible with PD 2.5 that provides custom nodes for nucleic-acid cross-link analysis with result visualization. The plugin is written in C# and wraps configuration and execution of NuXL as well as parsing and processing of the produced cross-linking results.

### **Preliminary data (results)**

After installation of the NuXL Proteome Discoverer nodes, the user can conveniently configure the tool through the Proteome Discoverer GUI, executing the search and visualizing the results. Presets for several crosslinking workflows are readily available and can be selected by users to match their experimental setup. After the results are generated, the XL-FDR level filtered crosslink spectrum matches are made available for inspection in the results table. Manual inspection of crosslink spectrum matches is possible through our custom extension to Proteome Discoverer that allow visualizing cross-link spectra along with extensive fragment annotations provided by the NuXL search engine.

Access to the binary installer for the Proteome Discoverer node is available upon request and subject to a beta testing agreement.

### **Please explain why your abstract is innovative for mass spectrometry?**

A complete data processing workflow for protein-RNA/DNA XL-MS.

### **Co-authors:**

*Arslan Siraj, Applied Bioinformatics, Dept. for Computer Science, University of Tübingen*

*Timo Sachsenberg, Applied Bioinformatics, Dept. for Computer Science, University of Tübingen, Institute for Bioinformatics and Medical Informatics, University of Tübingen*

*Luisa M. Welp, Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry*

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*Oliver Kohlbacher, Institute for Bioinformatics and Medical Informatics, University of Tübingen, Applied Bioinformatics, Dept. for Computer Science, University of Tübingen, Institute for Translational Bioinformatics, University Hospital Tübingen, Biomolecular Interactions, Max Planck Institute for Developmental Biology*

*Henning Urlaub, Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, Bioanalytics Group, Institute for Clinical Chemistry, University Medical Center Göttingen*

*Bernard Delanghe, Thermo Fisher Scientific (Bremen) GmbH*

Poster number: **LS-PA-043**

## **CROSS-LINKING MASS SPECTROMETRY ON P-GLYCOPROTEIN**

Abstract ID: **384**

**Presenting author: Gabriella Gellen, MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group, Eötvös Loránd University, Institute of Chemistry**

### **Introduction**

The ABC transporter P-glycoprotein (Pgp) has been found to be involved in multidrug resistance in tumor cells. Conformations of Pgp are extensively investigated, but these studies do not take into account the effect of lipids and cholesterol on Pgp, although they were shown to play important role in its conformations.

### **Methods**

In order to maintain the lipid environment, cross-linking mass spectrometry technology was applied to map Pgp's structure. Experiments were carried out using different cross-linkers on living cells. After membrane protein extraction, complexes were enriched by means of monoclonal anti Pgp antibodies on magnetic beads, followed by on-bead enzymatic digestion. LC-MS/MS measurements were performed on an Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer and on a Waters Select Series Cyclic Ion mobility Mass Spectrometer. Data were processed with ProteinLynx Global Server, Protein Prospector and Merox software tools.

### **Preliminary data (results)**

Results revealed protein-protein interactions of Pgp and proteins that had been known to be in proximity with Pgp. Identified monolinks hold information about solvent accessibility on Pgp while intraprotein cross-links complement 3D structure and aid detection of antibody binding sites.

Acknowledgement :

This project was supported by the Lendület (Momentum) Program of the Hungarian Academy of Sciences (HAS, MTA) and was completed in the Synthesis+ ELTE Thematic Excellence Programme supported by the Hungarian Ministry for Innovation and Technology. Project no. 2018-1.2.1-NKP-2018-00005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2018-1.2.1-NKP funding scheme.

### **Please explain why your abstract is innovative for mass spectrometry?**

Cross-linking on membrane protein human MDR1, followed by LC-MS/MS and LC-IMS/MS

### **Co-authors:**

*Gitta Schlosser, MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group, Eötvös Loránd University, Institute of Chemistry*

*Eva Klement, Single Cell Omics Advanced Core Facility, Hungarian Centre of Excellence for Molecular Medicine*  
*Zsolt Bacso, Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen*

Poster number: **LS-PA-044**

## **BLUE LIGHT PHOTOACTIVATABLE DIAZO COMPOUNDS AS A NOVEL CROSS-LINKING REACTIVE GROUP**

Abstract ID: **407**

**Presenting author: Fabio Gozzo, University of Campinas**

### **Introduction**

Photoactivatable groups are of great interest in chemical cross-linking/mass spectrometry (XLMS) field as they have the potential to create a large number of cross-links when compared to the more commonly used reactive groups like NHS esters. The formation of a carbene intermediate upon light activations makes these groups highly reactive, eliminating the need to have specific amino acid residues within the cross-linker reach to create a cross-link.

Here, we describe a novel photoactivatable cross-linking group based on diazo chemistry that are activated using blue light. The use of blue light instead of UV makes this group very attractive, especially for in cell cross-linking experiments where UV light can be damaging to the cell components

### **Methods**

The diazo compounds were synthesized according to already published procedures. The cross-linkers and analog compounds were tested both in purified proteins as well as cell lysates.

The samples were digested using trypsin and analysed in a Q-Exactive coupled to a Ultimate 3000 nanoLC. Data were processed using Pattern Lab for Proteomics, SIM-XL and MS Scout for crosslinking identifications.

### **Preliminary data (results)**

The new photoactivatable group was tested with a molecule containing a single reactive group. For purified proteins, these molecules presented a relatively fast incorporation (< 10 mins) with a wide range of reactivities, reacting with charged, polar and hydrophobic residues. For BSA, for example, more than 250 residues were modified by the diazo generated carbene. All the detected products correspond to the carbene insertion into O-H, N-H and C-H bonds.

Similar results were obtained for tests in cell lysate, with a large number of modified residues being identified as modified by the diazo reagent.

Preliminary results of these diazo groups incorporated into a cross-linker molecule containing either, a diazo-NHS and a di-diazo structure, generated a good number of cross-links in standard, purified proteins.

### **Please explain why your abstract is innovative for mass spectrometry?**

Use of a novel, blue light activatable cross-linkers for protein-protein interaction studies.

### **Co-authors:**

*Rafael Gallo, University of Campinas*

*Guilherme Reis, Boldrini Child Hospital*

*Wallace de Paula, University of Campinas*

*Giovanna Araujo, University of Campinas*

*Paulo Carvalho, Fiocruz-PR*

*Milan Clasen, Fiocruz-PR*

*Igor Jurberg, University of Campinas*

Poster number: **LS-PA-045**

## **STRUCTURAL REFINEMENT OF THE TUMOR SUPPRESSOR P53 BY MASS SPECTROMETRY-GUIDED COMPUTATIONAL MODELING**

Abstract ID: **885**

**Presenting author: Christian Ihling, Center for Structural Mass Spectrometry, Martin Luther University Halle-Wittenberg, Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin Luther University Halle-Wittenberg**

### **Introduction**

The tumor suppressor p53 is a multidomain transcription factor that plays a central role in DNA repair, cell cycle control, and apoptosis. p53 is a homotetramer, with each monomer consisting of two folded domains, the DNA-binding domain (DBD) and the tetramerization domain. In addition, p53 contains four intrinsically disordered regions (IDRs), the N-terminal transactivation domain, the proline-rich region, the nuclear localization signal (NLS)-containing region, and the C-terminal basic regulatory domain. The DBD binds to sequence-specific response elements that are associated with p53 target gene promoters. The majority of mutations in p53 are located in the DBD and are responsible for cancer triggering. As ~40% of p53's sequence is disordered it is not amenable to X-ray crystallography.

### **Methods**

Therefore, we aim to exploit an integrative structural biology approach combining different mass spectrometry (MS)-based techniques, such as covalent labeling (CL-MS), hydrogen/deuterium exchange (HDX-MS) and cross-linking mass spectrometry (XL-MS) to study changes in p53 upon specific DNA binding in terms of solvent accessibility, conformational changes, as well as H-bond stability and protein dynamics in full-length wild-type p53. XL-MS was applied to different full-length oligomeric p53 variants, the monomeric L344P variant, the dimeric L344A variant, and wild-type tetrameric p53. Specifically, the homobifunctional cross-linkers DSBU and BS<sup>2</sup>G as well as the "zero-length" cross-linker system EDC/sulfo-NHS were employed.

### **Preliminary data (results)**

We obtained information for the p53 monomer, dimer, and tetramer using a variety of chemical cross-linking principles with different spacer lengths and reactivities. The distance constraints imposed by the cross-linkers then served as input for MS-driven computational modeling to generate refined p53 models in the presence and absence of DNA. These models are currently being further validated by complementary HDX-MS data.

### **Please explain why your abstract is innovative for mass spectrometry?**

structural analysis of intrinsically disordered proteins by different structural MS based methods

### **Co-authors:**

*Alessio Di Ianni, Center for Structural Mass Spectrometry, Martin Luther University Halle-Wittenberg, Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin Luther University Halle-Wittenberg*  
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*Panagiotis L. Kastiris, Institute of Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg*  
*Andrea Sinz, Center for Structural Mass Spectrometry, Martin Luther University Halle-Wittenberg, Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin Luther University Halle-Wittenberg*

## Session: Proteomics: Post-translational modifications and their cross-talk

Poster number: LS-PA-046

### PERSONALIZED (GLYCO)PROTEOGENOMIC FEATURES OF THE SERUM HISTIDINE-RICH GLYCOPROTEIN REVEALED BY MASS SPECTROMETRY

Abstract ID: 27

**Presenting author: Yang Zou, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. , Netherlands Proteomics Centre, Padualaan 8, 3584 CH Utrecht, The Netherlands.**

#### Introduction

The histidine-rich glycoprotein (HRG), abundant in serum, has been termed the SwissArmy knife of mammalian serum, as it is involved in many biological processes and can bind many ligands. On HRG, 3 N-glycosylation sites have been identified, namely Asn63, Asn125, and Asn344. Among the human population, multiple allotypes of HRG co-exists, whereby at least 5 variants with allele frequencies of more than 10% have been reported. Among them, the Pro204Ser mutation leads to a novel N-glycosylation site Asn202. The serum levels of HRG in COVID-19 non-survivors were found to be consistently lower than in survivors<sup>1,2</sup>. Moreover, high plasma HRG levels have been claimed to decrease the mortality risk following a bacterial infection<sup>3</sup>.

#### Methods

We developed a rapid and effective strategy for the purification and analysis of serum HRG by IMAC purification, requiring just a 100 mL of serum. Following purification, we analyzed these proteins with (glyco)proteomics. We applied our method to analyze HRG purified from a cohort of 31 severe COVID-19 patients, of which 16 survived, and 15 were non-survivors. As controls, we also analyzed HRG from serum of 13 non-infected individuals.

#### Preliminary data (results)

Using (glyco)proteomics approaches we set out to identify 1) the genetic make-up of HRG in all donors of the cohort, defining their homo-zygotic or heterozygotic allotypes and 2) the glycosylation profiles of each purified HRG sample. We found from the proteomics data strong correlations between specific HRG allotype mutations, with several of these being either mutually exclusive or supportive. These findings could be partly corroborated by data generated from the 1000-genome project. We also observed that some allotypes of HRG were seemingly more frequently observed in the group of survivors *versus* non-survivors (**Figure 1**). For instance, the frequency of Ser204 seemed to be decreased in non-survivors, which also means that these HRG proteins lack the additional N-glycosylation site. We also explored how glycoproteomic variants of HRG act functionally differentially.

#### Please explain why your abstract is innovative for mass spectrometry?

We developed a rapid and effective strategy for the purification and mass spectrometry analysis of serum HRG by IMAC purification, requiring just a 100 mL of serum.

#### Co-authors:

*Bas van Breukelen, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. , Netherlands Proteomics Centre, Padualaan 8, 3584 CH Utrecht, The Netherlands.*

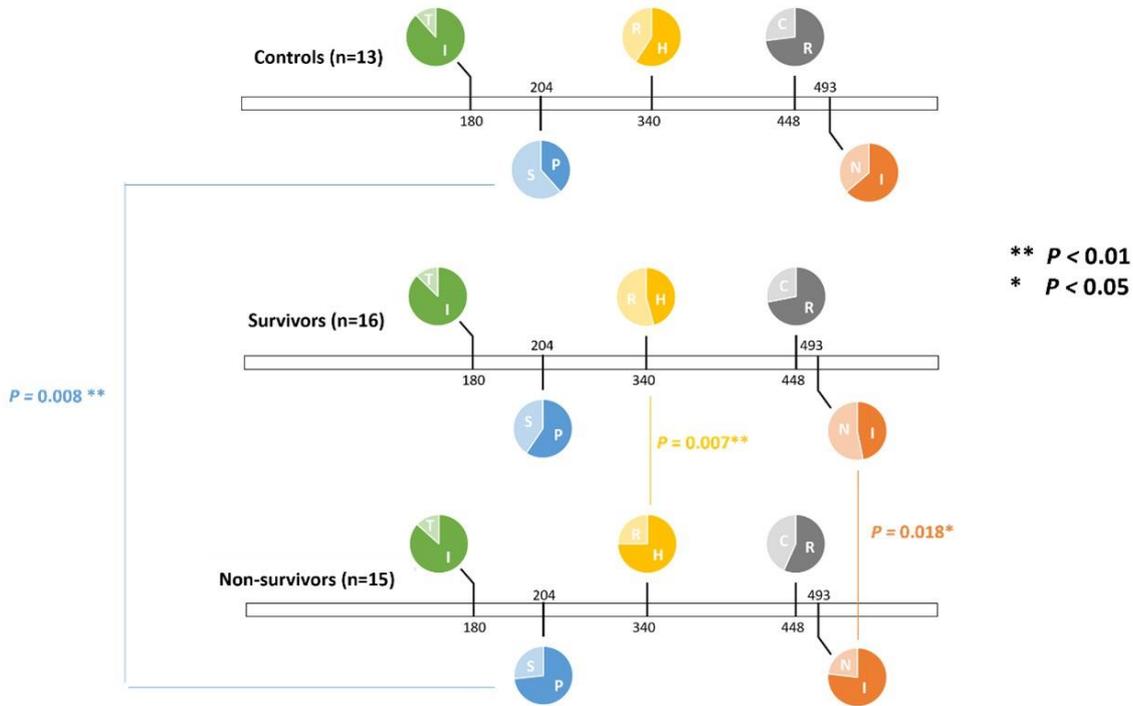
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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours

Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. ,  
 Netherlands Proteomics Centre, Padualaan 8, 3584 CH Utrecht, The Netherlands.



Overview of the frequency of occurrence of mutations in HRG

Poster number: **LS-PA-047**

## IN-DEPTH CHARACTERIZATION OF THE CLOSTRIDIODES DIFFICILE PHOSPHOPROTEOME TO IDENTIFY SER/THR KINASE SUBSTRATES

Abstract ID: **95**

**Presenting author: Mariette Matondo, Institut Pasteur, Université de Paris, CNRS USR 2000, Plateforme Protéomique, Unité de Technologie et Service Spectrométrie de masse pour la biologie, F-75015, Paris, France**

### Introduction

*Clostridioides difficile* (CD) is the leading cause of intestinal post-antibiotic infections in adults. During infection, the bacterium must rapidly respond and adapt to the host environment by using survival strategies. Hanks-type Serine/Threonine kinases (STKs) and phosphatases (STPs) have emerged as important players in bacterial cell signaling and pathogenicity. *C. difficile* encodes two STKs (PrkC and CD2148) and one phosphatase (STP). We established a comprehensive phosphoproteomics strategy to identify STK targets and STP substrates.

### Methods

We optimized a TiO<sub>2</sub> phosphopeptide enrichment approach to determine the phosphoproteome of *C. difficile* a gram-positive bacteria. Enriched peptides were measured by LC-MS/MS on the Orbitrap instrument using data-dependent acquisition (DDA) and data-independent acquisition (DIA) methods. Large-scale comparative phosphoproteomics of the wild-type (WT) strain with several mutants inactivated for  $\Delta$ prkC, CD2148, and isogenic STP was performed to identify STK targets and STP substrates. Data analyses were performed using *in-house* developed bioinformatics and statistical tools.

### Preliminary data (results)

We showed that PrkC is phosphorylated on multiple sites *in vivo* and autophosphorylates *in vitro* while we are unable to detect a phosphorylation *in vivo* and *in vitro* for CD2148. We also observed an enrichment for phosphothreonine among the phosphopeptides less abundant in knock-down mutants of PrkC and/or CD2148, and more abundant in the  $\Delta$ stp mutant. Both kinases target pathways required for metabolism, translation and stress response while cell-division and peptidoglycan metabolism were more specifically controlled by PrkC-dependent phosphorylation in agreement with the phenotypes of the  $\Delta$ prkC mutant. This study provides a detailed mapping of kinase-substrate relationships in *Clostridioides difficile*, paving the way for the development of new therapeutic targets.

### Please explain why your abstract is innovative for mass spectrometry?

- Optimized phosphopeptide enrichment protocol for *C. difficile*
- Combined MS methods
- Dedicated tools for the data analysis
- Identification and characterization of PrkC and CD2148 kinase targets and STP phosphatase substrates

### Co-authors:

Transito Garcia-Garcia, Institut Pasteur, Université de Paris, UMR CNRS 2001, Laboratoire Pathogénese des Bactéries Anaérobies, F-75015, Paris, France

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Quentin Gai Gianetto, Institut Pasteur, Université de Paris, CNRS USR 2000, Plateforme Protéomique, Unité de Technologie et Service Spectrométrie de masse pour la biologie, F-75015, Paris, France, Institut Pasteur, Université de Paris, Hub de bioinformatique et statistique, Département de Biologie computationnelle, F-75015, Paris, France

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Poster number: **LS-PA-048**

## **CHARTING THE PROTEOFORM LANDSCAPE OF THE SERUM PROTEINS BY HIGH-RESOLUTION NATIVE MASS SPECTROMETRY**

Abstract ID: **99**

**Presenting author: Dario Cramer, Utrecht University**

### **Introduction**

The majority of serum proteins are glycosylated, among them several already being utilized as biomarkers in the clinic. So far, methods for the analysis of serum glycoproteins have used either glycan or glycopeptide centric mass spectrometry-based approaches, which provide excellent tools for glycan analysis. However, by definition, they neglect undefined or unknown glycosylation or other co-occurring modifications. High-resolution native mass spectrometry is a new technique for the analysis of intact glycoproteins, allowing qualitative and quantitative observation of all co-occurring modifications present on a glycoprotein. So far, this approach has been used exclusively used on one specific protein at the time. Here we present a method capable of isolating over 20 (glyco)proteins from serum, covering a mass range between 30 and 200 kDa.

### **Methods**

Prior to the analysis about 100 mL of serum is first depleted of 3 of its most abundant proteins, next serum (glyco)proteins are fractionated over tandem anionic and cationic ion-exchange columns. The IEX-based fractionated serum proteins are characterized in-depth using high-resolution native mass spectrometry. Analysis of native mass spectra and the annotation of their glycosylation is performed manually using PMI Intact software.

### **Preliminary data (results)**

Over 20 serum glycoproteins were fractionated and their proteoform profiles analyzed by native mass spectrometry covering a wide variety of masses, modifications and functions. Fractionated proteins include protease inhibitors, complement components, transporter proteins and more. The proteoform profiles of four selected proteins of interest, *i.e.* alpha-1-antitrypsin, ceruloplasmin, hemopexin, and complement protein C3, are characterized further in-depth. This includes, among others, the annotation of N- and O-glycans, protein cysteinylolation, cleavage induced protein activation as well as the detection of gene polymorphism. Additionally, we find that donors with alpha-1-antitrypsin with certain single nucleotide polymorphisms show differential abundance of co-occurring variants in serum. Finally, we expand our approach to a small sample set of serum samples obtained from healthy and diseased individuals. We qualitatively and quantitatively monitor the changes in proteoform profiles of ceruloplasmin and reveal a substantial increase in fucosylation and the extent of N-glycan occupancy in patients with late-stage hepatocellular carcinoma and pancreatic cancer, when compared to healthy controls.

### **Please explain why your abstract is innovative for mass spectrometry?**

The combination of protein fraction by IEX and high-resolution native mass spectrometry analysis allows for a previously unseen exploration and characterization of the serum glycoproteoform landscape.

### **Co-authors:**

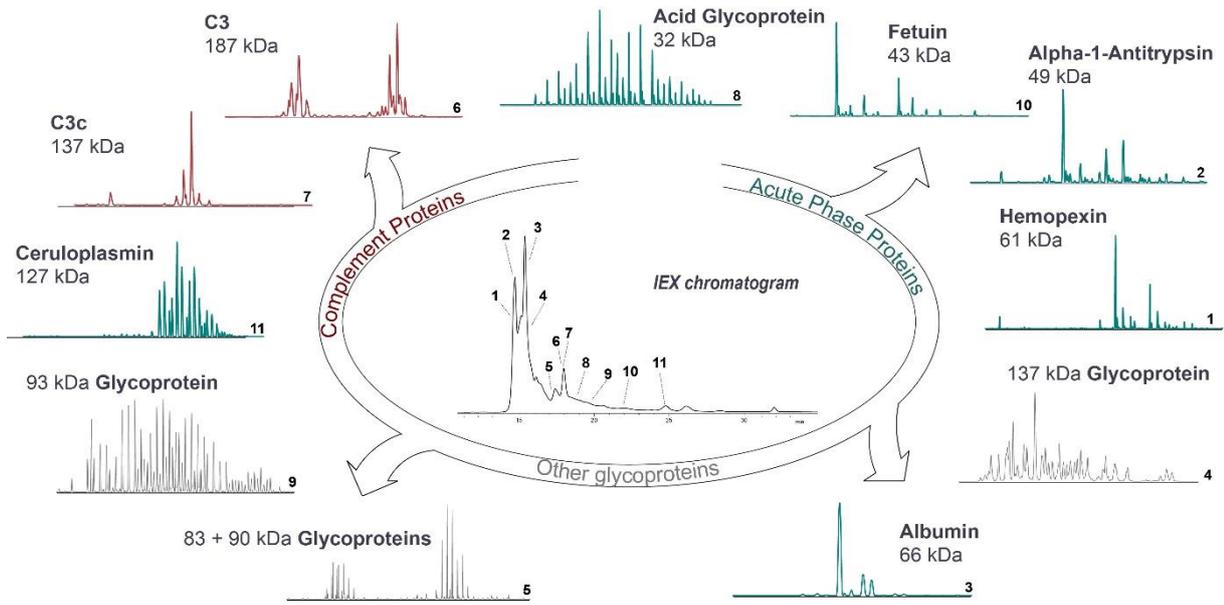
*Albert Heck, Utrecht University*

*Vojtech Franc, Utrecht University*

*Tomislav Caval, Utrecht University*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
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A selection of serum proteins analyzed by IEX & native MS.

Poster number: **LS-PA-049**

## INTEGRATED MASS SPECTROMETRY-BASED PIPELINE FOR CHARACTERIZATION OF TAU IN HUMAN ALZHEIMER'S BRAIN: DEVELOPMENT AND COMPARISON OF SAMPLE PREPARATION METHODS

Abstract ID: **416**

**Presenting author: Rita Azevedo, Département Médicaments et Technologies pour la Santé (DMTS), SPI, Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA)**

### Introduction

Tau protein aggregation into neurofibrillary tangles in the brain is a pathological hallmark of Alzheimer's disease (AD) and related tauopathies [1]. Though the mechanism of tau destabilization is not fully understood, tau protein exhibits several allele-specific isoforms and post-translational modifications (PTMs) specific to tau aggregates [2]. To explore in-depth tau species in brain tissues of AD patients, we developed novel pipelines based on different tau fractions retrieved by buffer solubility, Filter Aided Sample Preparation (FASP) or immunoprecipitation, before high-resolution mass spectrometry (HRMS) analysis.

### Methods

As a proof of concept, we used frozen brain samples from two AD patients (0.6-1.2 g per replicate, middle frontal gyrus). The samples were divided and homogenized, ultracentrifuged at 180.000xg for 30min and solubilized in different buffers successively (2 replicates): (1) Sark method: low-salt buffer, 10% TritonX-100, sarkosyl buffer, urea buffer; (2) RIPA method: high-salt buffer (twice), RIPA buffer, urea buffer; and, (3) GnH method: guanidine hydrochloride and urea buffer. Then, we performed an antibody-free approach by FASP or immunoprecipitation with a panel of commercial tau antibodies and analyzed on reversed-phase capillary liquid chromatography, coupled with a qExactive HRMS.

### Preliminary data (results)

HRMS demonstrated that the RIPA method performed better than the other methods for Tau analysis: coverage of 63% (full-length, 2N4R) and 342 tau peptide-spectrum matches (PSMs) (Sark: 49% and 183 PSMs; GnH: 41% and 169 PSMs). PTM-modified tau species (phosphorylated, methylated, and acetylated) were primarily seen using the RIPA method (59 PSMs; Sark: 19 PSMs, GnH: 21 PSMs). Immunoprecipitation improved tau coverage and PTM detection compared to FASP.

These findings highlight the best performance of the RIPA method followed by immunoprecipitation for the study of AD brain tau allele-specific isoforms and PTMs using HRMS. Better characterization of Tau molecular profile in the brain of AD patients opens the gate for a better understanding of AD pathogenesis and represents an opportunity in the biomarker field.

### Please explain why your abstract is innovative for mass spectrometry?

We developed novel pipelines based on different tau fractions by buffer solubility and high-resolution mass spectrometry for tau analysis.

### Co-authors:

*Nicolas Villain, Institut du Cerveau et de la Moëlle Epinière (Institut du Cerveau – ICM), Pitié-Salpêtrière Hospital  
Susana Boluda, Laboratoire de Neuropathologie Raymond Escourolle, Pitié-Salpêtrière Hospital, APHP, Sorbonne University, 47, Blvd de l'Hôpital*

*François Fenaille, Département Médicaments et Technologies pour la Santé (DMTS), SPI, Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA)*

*Foudil Lamari, Department of Endocrine and Oncological Biochemistry (J.AD), Metabolic Biochemistry (BR, DBR, FI, FL), AP-HP, GH Pitié-Salpêtrière-Charles Foix*

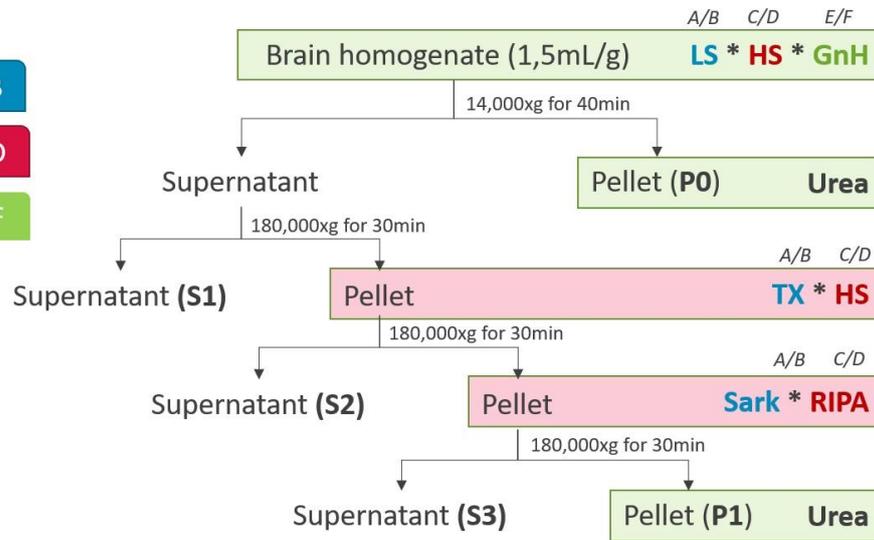
*François Becher, Département Médicaments et Technologies pour la Santé (DMTS), SPI, Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA)*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
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**Method:**

- Sark: replicates A/B
- RIPA: replicates C/D
- GnH: replicates E/F



Workflow of tau extraction using brain tissues from AD patients.

Poster number: **LS-PA-050**

## THE TRYPTOPHAN IMMONIUM ION IS A MARKER FOR INSECT ADIPOKINETIC HORMONES

Abstract ID: **475**Presenting author: **Simone König, University of Münster**

### Introduction

Adipokinetic hormones (AKHs) are important regulators of metabolism in insects and are characterised by a chain length of 8-10 amino acids, an N-terminal pyroglutamate residue, an amidated C-terminus, and favoured positions for some amino acids (L, I, V, F, Y - position 2; N, T - position 3, F, Y - position 4; S, T - position 5; P - 6; W - position 8; G - position 9). AKHs of many species have already been identified. Some are predicted by genomic and transcriptomic work, such as the AKHs of the crambid moth *Ostrinia nubilalis* and of the termite *Kaloterme flavicollis*. We validated the predictions by examining if those AKHs are indeed synthesised in the corpora cardiaca (CC) of these species and a related crambid (*Agriphila straminella*).

### Methods

*Ostrinia* pupae were a gift from F. Marec (Czech Academy of Sciences), *Kaloterme* came from D. McMahon (BAM Berlin), and *Agriphila* was collected near Jena (Germany). CC were dissected from adults and extracted with methanol, centrifuged, the supernatants dried, redissolved in 10 µl methanol followed by 10 µl 0.1% formic acid containing 5% acetonitrile. For LC-MS/MS with Synapt G2 Si coupled to M-Class UPLC, 1 µl was injected. AKH candidate peaks were identified by target-MS for eligible known peptides and by screening for the gas phase fragmentation loss of the tryptophan immonium ion.

### Preliminary data (results)

AKHs are abundant in CCs, but not always easy to identify in the methanol extract. The search for the Trp immonium ion ( $m/z$  159.09) using high vs. low collision energy helped to identify candidates, because this ion is typical for AKHs but otherwise the least abundant amino acid in cells and one of the rarest in the proteome. One other typical signature for Pro-containing 8-amino acid AKHs are their dominant  $y_3$ - $b_4$ - $b_6$  ions due to the Pro-effect. For the crambid moth *Ostrinia*, the predicted AKHs were indeed confirmed (Manse-AKH: pELTFTSSWG amide and Ostnu-AKH: pELTFSTGWGQ amide). The latter peptide, Ostnu-AKH, had never before been found in any other insect. For the other crambid species, *Agriphila*, we detected Manse-AKH plus a second AKH (pELTFSTGWGN amide). This peptide is almost identical to the decapeptide Ostnu-AKH (Q/N replacement at position 10). Moreover, this peptide had been identified earlier by MS in two species of the genus *Chilo* of the family Crambidae and was code-named Chipa-AKH. The termite *Kaloterme* had the predicted octapeptide AKH (pEVNFSPGW amide), which is well known from insects of the order Mantophasmatodea.

### Please explain why your abstract is innovative for mass spectrometry?

Use of the Trp-immonium ion and a Pro-AKH ion signature as gas phase fragmentation markers for AKH elucidation. Identification of predicted AKHs in moth and termite species.

### Co-authors:

Heather Marco, University of Cape Town  
Gerd Gäde, University of Cape Town

Poster number: **LS-PA-051**

## **EFFECTS OF AGEING AND CALORIE RESTRICTION ON ROS-INDUCED PROTEIN DAMAGE IN MITOCHONDRIA OF DIFFERENT TISSUES FROM RATTUS NORVEGICUS**

Abstract ID: **929**

**Presenting author: Ansgar Poetsch, Nanchang University**

### **Introduction**

Ageing is an irreversible, progressive and complex biological process. Despite progress in its understanding, most aspects are enigmatic and the relevance of the myriad of existing theories and contributing factors are a matter of active research and dispute. Several theories attribute mitochondria a key role to ageing given their central function as producer of energy and reactive oxidative species. The prominent mitochondrial free radical theory of ageing (MRFA) postulates an increase of (oxidatively) damaged biomolecules in mitochondria as driver for cellular ageing. Moreover, influencing cellular metabolism and signaling by caloric restriction is known to impact ageing. By leveraging proteomics for global and unbiased identification and quantification of oxidative protein modifications, aims of this project were to scrutinize the relevance of MRFA for mammalian ageing.

### **Methods**

Crude mitochondrial fractions were isolated from the heart and brain tissues of same rat, ages 6.5 months (young) and 27 months (old), fed ad libitum or on a CR diet (CR: intake about 60 % of ad libitum fed animals). Mitochondria from each animal group were digested with Lys-C/Trypsin protease mix using the slightly modified iFASP protocol. iTRAQ-labelled samples and the spike-in internal standard were equally mixed to one experiment. Each experiment was analysed on a LTQ Orbitrap Elite with HCD fragmentation.

Oxidative PTMs were identified and quantified using MaxQuant.

### **Preliminary data (results)**

Tissue-specific mitochondrial proteomes revealed more than 2000 proteins across all four average animal groups (n=3) from cerebrum and more than 1000 proteins from heart. About 200 and 300 proteins with an abundance change in oxidation degree remained after normalisation across all animal groups for cerebrum and heart proteome, respectively. iTRAQ data reveal a high number of different mono-oxidation sites within the detected proteins in all animal groups for both tissues. Other prominent modification sites identified in decreasing order of occurrence are carbonylation of several amino acid residues as well as formation of 2-amino-3-ketobutyric acid and pyrrolidinone. In both tissues methionine oxidation remained the most prominent modification and manifested as sulfoxide. Preliminary data analysis suggests no global increase or decrease in protein oxidation with age or caloric restriction. Instead, oxidation depends on protein, tissue, and caloric intake.

### **Please explain why your abstract is innovative for mass spectrometry?**

Sample processing and data analysis was developed to enable unbiased relative quantification of oxidative protein modifications.

### **Co-authors:**

*Carina Ramallo Guevara, CeMOS*  
*Norbert Dencher, CMM-MIPT*

Poster number: **LS-PA-052**

## **PHOSPHOPROTEOME DYNAMICS IN RESPONSE TO SYNAPTIC UP- & DOWN-SCALING**

Abstract ID: **968**

**Presenting author: Kristina Desch, Max-Planck-Institute for Brain Research**

### **Introduction**

Neuronal networks rely on plasticity mechanisms to adapt dynamically to fluctuations in magnitude and frequency of external stimuli. One such form of plasticity is homeostatic scaling which allows for bi-directional adjustment of the strength of synaptic connections in response to global input changes. The ability of neurons to respond and shape their synapses heavily relies on their molecular repertoire, the proteomic composition. While there have been efforts in elucidating protein synthesis and degradation changes during the homeostatic response, so far no study surveyed the role of the post-translational modifications, in particular phosphorylation, in synaptic scaling in depth. Here we implement a label-free, bottom-up phosphoproteomics strategy tailored to limited sample amounts from primary-cultured neurons while maintaining comprehensive proteomic coverage.

### **Methods**

To investigate phosphorylation in the homeostatic response, we silenced or stimulated primary-cultured cortical neurons with Tetrodotoxin or Bicuculline to induce up- or down-scaling, respectively. To assess responses to immediate activity-changes or following scaling manifestation, we investigated treatment durations of 5min, 15min and 24h. We used affinity-enrichment (TiO<sub>2</sub>) in combination with bottom-up LC-MS/MS analysis to detect alterations in the phosphoproteome and total proteome in an unbiased manner. Peptides were analysed on a Fusion Lumos mass spectrometer (ThermoFisher) coupled to a nano-HPLC (U3000, Dionex) using a 2h or 3h gradient for the phosphoenriched or unenriched peptides.

### **Preliminary data (results)**

Our phosphoproteomics analyses comprised a set of over 40,000 phosphopeptide species (Class I, >75% localization probability) associated with ~26,600 unique phosphorylation sites. We found over 3,300 activity-sensitive phosphorylation events on ~1,200 phosphoproteins. A parallel investigation of the unmodified proteome enabled the integration of alterations of protein abundances at all time points, which resulted in a negligible overlap of the regulated proteome and phosphoproteome. Consistent with the concept of scaling, regulated phosphoproteins were predominantly located in synaptic compartments and involved in cytoskeletal (re-)organization. We identified many early time-limited phosphorylation events that could serve as sensors of neuronal activity offset-difference in a homeostatic system as well as late on-set and/or persistent phosphorylation events that could represent effector mechanisms driving the homeostatic response. Though the majority of regulated phosphorylation events was limited to a specific time point, a quarter of initial events showed sustained regulation over all time points. Persistent phosphorylation was largely reciprocally regulated by up- or down-scaling, suggesting that the mechanisms underlying these two poles of synaptic regulation make use of opposing regulation of the signaling and effector proteins.

Taken together, our experimental strategy allowed for a comprehensive view of protein phosphorylation dynamics in neurons with supplementing information about protein-level regulation. We detected distinct phosphorylation profiles that provide insights into the fundamental processes of the homeostatic response and potentially other forms of synaptic plasticity.

### **Please explain why your abstract is innovative for mass spectrometry?**

Our work provides a strategy to profile activity-sensitive phosphorylation events of neuronal proteins in a comprehensive and time-resolved manner, revealing bi-directional changes that underlie neuronal plasticity.

### **Co-authors:**

*Julian Langer, Max-Planck-Institute for Brain Research, Max-Planck-Institute of Biophysics*  
*Erin Schuman, Max-Planck-Institute for Brain Research*

Poster number: LS-PA-053

## ANALYSIS OF POST-TRANSLATIONAL MODIFICATIONS USING FAST ELECTRON ACTIVATED DISSOCIATION (EAD)

Abstract ID: 1014

Presenting author: Alexandra Antonoplis, SCIEX

### Introduction

In proteomics workflows, collision induced dissociation (CID) is commonly used to fragment peptide ions for analysis. While CID enables identification of many high-confidence peptides, preserving labile post-translational modifications (PTMs) can be challenging. Electron activated dissociation (EAD) is a fragmentation mode that involves capture of electrons by molecular ions to form radicals that dissociate into fragment ions. EAD often preserves PTMs and provides sequence information that is complementary to CID. This work focuses on EAD method development and optimization of fragmentation to localize peptide phosphorylation and glycation sites.

### Methods

A total of 100 µg of digested HeLa cell lysate was fractionated using reverse-phase HPLC. The resulting 44 fractions were separated using a 20-min microflow LC gradient with a 6 µL/min flow rate. For glycated peptide analysis, human serum albumin was used with similar separation conditions. Information dependent acquisition (IDA) was performed using the ZenoTOF 7600 system with Zeno MS/MS and either CID or EAD fragmentation. Parameters for EAD acquisition, including EAD reaction time and electron kinetic energy, were investigated for method development. Mascot was used for database searching and results were imported into Scaffold for analysis.

### Preliminary data (results)

From the CID analysis of HeLa cell lysate, 93,866 peptides were identified at 1% FDR using Mascot as the database search engine. With EAD fragmentation, 52,905 peptides were identified at 1% FDR. While CID identified more peptides due to higher frequency of sampling, EAD provided complementary sequence information. EAD also enabled identification of unique peptides not found using CID, increasing the total number of identified peptides by 11%.

Next, peptide data were mined for PTMs with a specific focus on identifications that are challenging to achieve from CID data. EAD spectra from phosphopeptides identified the sequence and site of phosphorylation using complete c' and z+1 ion series. Phosphorylation sites were localized in peptides containing multiple serines and peptides with modifications near the N-terminus. Additionally, analysis of glycated peptides using EAD enabled site-specific localization of hexose modifications.

Zeno EAD IDA analysis of a fractionated proteome using microflow chromatography enabled large-scale protein identification, with confident identification and automatic site assignment of many peptide modification sites. Further work is planned to investigate the value of EAD for other challenging modified peptides.

### Please explain why your abstract is innovative for mass spectrometry?

Zeno EAD IDA provides speed and sensitivity for large-scale PTM investigation with information-rich MS/MS spectra.

### Co-authors:

Nick Morrice, SCIEX  
Christie Hunter, SCIEX

Poster number: **LS-PA-054**

## **FAIMS ENHANCES THE DETECTION OF PTM CROSSTALK SITES**

Abstract ID: **1045**

**Presenting author: Kish Adoni, University of Birmingham**

### **Introduction**

Protein post-translational modification (PTMs) provide the biological infrastructure from which cells respond to their ever-changing environment. Dysregulation of PTM facilitated signalling stimulates cellular malfunction, initiating diseases such as cancer. With hundreds of different PTMs identified; understanding the mechanisms by which PTMs control cell function is complex. To date, efforts have largely focused on investigating the effect of single PTMs on proteins within signalling networks. Yet, many proteins contain multiple PTMs. Moreover, one PTM can alter the prevalence of another; a phenomenon termed PTM crosstalk. Dysregulated PTM crosstalk has now been identified in a plethora of diseases ranging from cancer to neurodegenerative disorders. As such, understanding PTM crosstalk is critical; however, its detection is challenging since PTMs often occur sub-stoichiometrically.

### **Methods**

Trypsin digested HeLa lysate was subjected to LC-FAIMS-MS/MS using multiple workflows including static compensation voltages (CV) ranging from 0 V to -90 V and internal stepping of four CVs in one LC-FAIMS-MS/MS run. RAW files generated from these experiments were processed and analysed using Proteome Discoverer 2.4. Data generated from these workflows were compared to standard LC-MS/MS. External stepping refers to the sum of the data from all static CV LC-FAIMS-MS/MS runs, and internal stepping refers to the cycling of 4 CVs in one LC-FAIMS-MS/MS run.

### **Preliminary data (results)**

We have developed an enrichment-free, label-free proteomics mass spectrometry method that utilizes high-field asymmetric ion mobility spectrometry to enhance the detection of PTM crosstalk. We show that by searching for multiple combinations of dynamic PTMs on peptide sequences, an enhanced number of peptides containing multiple PTMs were detected that are candidate sites for PTM crosstalk. Our approach showed a 6-fold increase in candidate PTM crosstalk site identification compared with the standard liquid chromatography-mass spectrometry workflow. Of the positive PTM crosstalk candidate sites that we identified, 40 % have not previously been reported.

### **Please explain why your abstract is innovative for mass spectrometry?**

Novel use of LC-FAIMS-MS/MS for the identification of multiple-PTM peptides as candidates for PTM crosstalk.

### **Co-authors:**

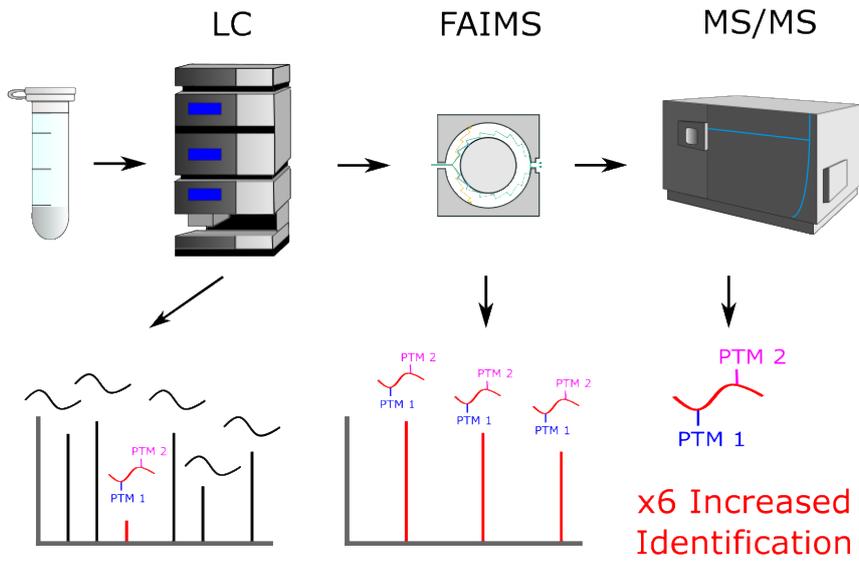
*Debbie Cunningham, University of Birmingham*

*John Heathe, University of Birmingham*

*Aneika Leney, University of Birmingham*

**POSTER SESSION A**

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LC-FAIMS-MS/MS for the identification of multiple-PTM peptides for PTM-crosstalk.

## **Session: Quantification**

Poster number: **LS-PA-055**

### **BENCHMARKING THE QUANTITATIVE ACCURACY OF LABEL-FREE PROTEOMICS BY DIA**

Abstract ID: **123**

**Presenting author: Tobias Jumel, MPI-CBG**

#### **Introduction**

Label-free, quantitative proteomics experiences hallmark improvements, many associated with data-independent acquisition (DIA). To measure differences in performance, the number of identified protein groups and the quantitative precision are readily accessible. However, the quantitative accuracy remains largely unknown, even in cases of performance benchmarks. Our objective is to extend the classical LFQ benchmark principle<sup>1,2</sup> to investigate previously undescribed errors impacting the quantification of fold-changes.

#### **Methods**

We utilized the LFQbench setup with samples consisting of human, yeast, and E.coli digests and expected log<sub>2</sub> fold-changes of 0, +1, and -2, respectively. DIA measurements of 0.9 µg over 90 min gradients were performed in 4 replicates on a Q Exactive HF. Other raw data are from PXD028735<sup>3</sup>. After analysis with DIA-NN 1.8,<sup>4</sup> results were processed with an in-house R script. In short, we detect various error sources with statistical indicators. This process involves subjecting protein groups to differential expression analysis by limma<sup>5</sup> and matching the results against expectations in form of a “confusion matrix”.

#### **Preliminary data (results)**

The in-house R script provides statistical indicators for improved performance assessment and comparison of label-free quantification benchmarks. This includes quantification errors arising from erroneous normalization, identification errors, dispersion, and skewness.

Based on the newly gained insights we simultaneously optimized the proteome coverage and the quantitative accuracy. The resulting overall performance of our Q Exactive HF workflow exceeds the one of a diaPASEF dataset, among others (figure 1). Notably, our results show next to no bias or skewness in form of ratio compression, which is well visible in the diaPASEF data as asymmetry of yeast and E.coli density plots. Furthermore, all types of quantification errors are reduced, including the confusion matrix FDR.

We established a strategy to achieve highly reliable protein quantification while delivering sensitivity on par with benchmark datasets acquired with modern mass spectrometers.

#### **References**

- 1 Kuharev et al., Proteomics 2015
- 2 Navarro et al., Nature biotechnology 2016
- 3 Van Puyvelde et al. bioRxiv 2021 (PXD028735)
- 4 Demichev et al., Nat. Methods 2020
- 5 Ritchie et al., Nucleic Acids Res. 43, e47 (2015).

#### **Please explain why your abstract is innovative for mass spectrometry?**

Providing protein quantification with close to no tendency for over- or underestimation of fold-changes critically improves the utility for sensitive and reproducible differential expression analysis.

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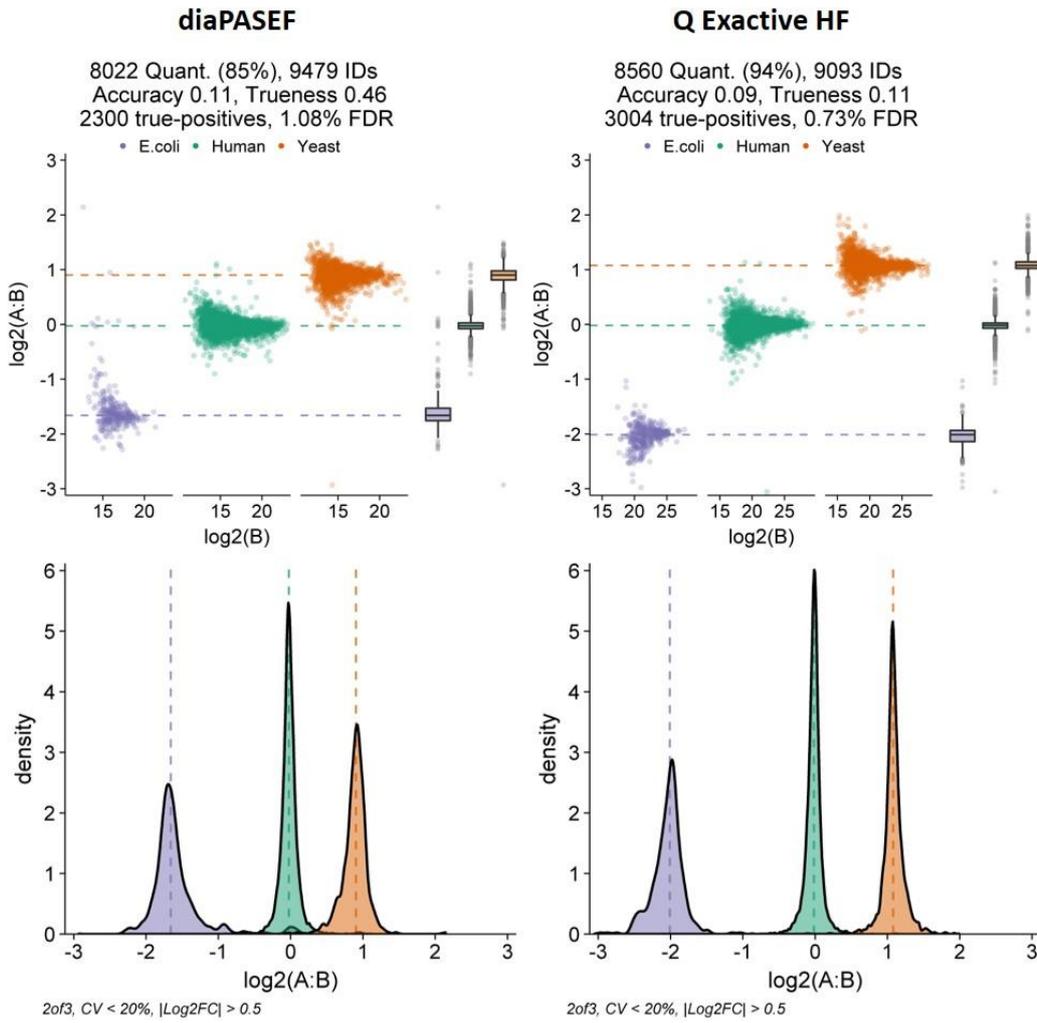


Figure 1: Protein group level visualization of LFQ benchmarks.

Poster number: **LS-PA-056**

## **ABSOLUTE QUANTIFICATION OF 500 HUMAN PLASMA PROTEINS IN COLON CANCER PLASMA SAMPLES BY PRM-PASEF.**

Abstract ID: **158**

**Presenting author: Gunnar Dittmar, LIH**

### **Introduction**

We recently communicated a first evaluation of the newly introduced prm-PASEF approach, which allows multiplexing the acquisition of several targets in a single ion mobility event without compromising sensitivity. We are now applying this approach to the absolute quantitation of 500 blood proteins in colon cancer plasma samples and are comparing it with a broadband dia-PASEF approach

### **Methods**

The plasma sample cohort consisted in 10 patients affected by a colon cancer (adeno carcinoma) and 10 controls. Plasma samples were depleted with a Mars 14 depletion column (Agilent), digested with a trypsin protease and spiked with a mixture of 800 quantified synthetic peptides (PQ500, Biognosys). All samples and controls were separated by nano-HPLC (nanoElute, Bruker Daltonics) on a pulled emitter column (IonOpticks, Australia) using a 100 min gradient. Peptides were analyzed on a timsTOF Pro instrument (Bruker Daltonics) operated both in prm-PASEF and dia-PASEF modes. Data processing has been done with Spectronaut (Biognosys), MaxQuant and Skyline-daily.

### **Preliminary data (results)**

We evaluated the quantification performance of the prm-PASEF in depleted plasma samples by monitoring 370 precursors with a 20 min gradient LC separation. We obtained a median number of 15 data points per elution profile and limits of detection down to 5.5 amole/ $\mu$ l using quantified synthetic peptides as reference. We demonstrated accuracy over more than 3 orders of magnitude of peptides concentrations with a maximum error on the determination of 20%. The median relative standard deviation of the signal of the peptides was of 3%. Using a 100 min gradient with a 25cm chromatography column packed with 1.6  $\mu$ m particles and we monitored 1566 peptides precursors per prm-PASEF acquisition.

### **Please explain why your abstract is innovative for mass spectrometry?**

The prm-PASEF approach has been successfully applied to the analysis of colon cancer plasma samples.

Finally, we will compare these quantitation results to those obtained using a dia-PASEF approach.

### **Co-authors:**

*Antoine Lesur, LIH*

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*Elisabeth Letellier, Luxembourg University*

*Pierre-Olivier Schmit, Bruker Daltonik GmbH*

*Gary Kruppa, BrukerDaltonik GmbH*

Poster number: **LS-PA-057**

## **MASS SPECTROMETRY-BASED PROTEOMICS BIOMARKER DISCOVERY IN A CORONARY ARTERY DISEASE COHORT**

Abstract ID: **179**

**Presenting author: Colleen Maxwell, University of Leicester**

### **Introduction**

Coronary Artery Disease (CAD) is caused by the build-up of obstructive atherosclerotic lesions in the coronary arteries, causing ischaemia, angina, or even myocardial infarction. It is the leading cause of premature death in the developed world and causes 180 deaths every day in the UK alone according to the British Heart Foundation. CAD is diagnosed primarily by CT scan and calcium score which are highly sensitive and accurate, but involve exposure to radiation and are costly compared to blood tests. Establishing a panel of novel protein biomarkers for CAD, specifically calcification, using LC-MS/MS has the potential to lead to a blood test for earlier diagnosis, could lead to precision medicine targets for personalised CAD therapy, and could give novel insight into disease aetiology.

### **Methods**

Novel protein enrichment methods were utilised to isolate areas of the human plasma subproteome in a 100-subject cohort consisting of 50 CAD patients (calcium scores  $\geq 200$ ) and 50 matched controls (calcium scores  $< 10$ ). The isolated proteins were reduced, alkylated, and digested into peptides. Samples were ran using DIA mode on an LC-IMS-ToF-MS/MS platform equipped with a nanoUPLC system. Raw MS/MS data were processed with novel informatics methods and a panel of candidate CAD biomarkers identified using differential expression and pathway analyses. A targeted multiplexed SRM assay was developed and optimised for verification.

### **Preliminary data (results)**

From the shotgun LC-MS/MS analysis of the 100-subject cohort, a total of 4104 proteins were identified from plasma extra-cellular vesicles (ECVs). Figure 1 shows a 3D principal component analysis (PCA) plot illustrating separation between CAD patients vs. healthy controls based on normalised protein abundance profiles. Additionally, 2004 proteins were identified from lipid removal agent (LRA), which enriches for lipoproteins and lipoprotein-associated proteins. This represented a total of 3409 unique quantified proteins for the cohort. Data analysis carried out using the R Studio package Limma revealed a total of 271 differentially expressed proteins between CAD patients and healthy matched controls, 151 from ECV, 128 from LRA, and 8 which overlapped between two protocols. Figure B shows a volcano plot illustrating the  $-\log_{10}(P\text{-value})$  vs.  $\log_2(\text{Fold change})$  of the ECV plasma proteins following differential expression analysis. Pathway analysis with down-weighting of overlapping genes revealed 42 significantly differentially expressed pathways between conditions. A panel of 22 protein candidates which were significantly differentially expressed in CAD patients with calcium score  $>200$  and which were also members of significant pathways were selected to be taken forward to verification.

### **Please explain why your abstract is innovative for mass spectrometry?**

The innovative IMS directed DIA workflow to identify plasma proteins, coupled with novel informatics methods to overcome raw MS data variability in data processing.

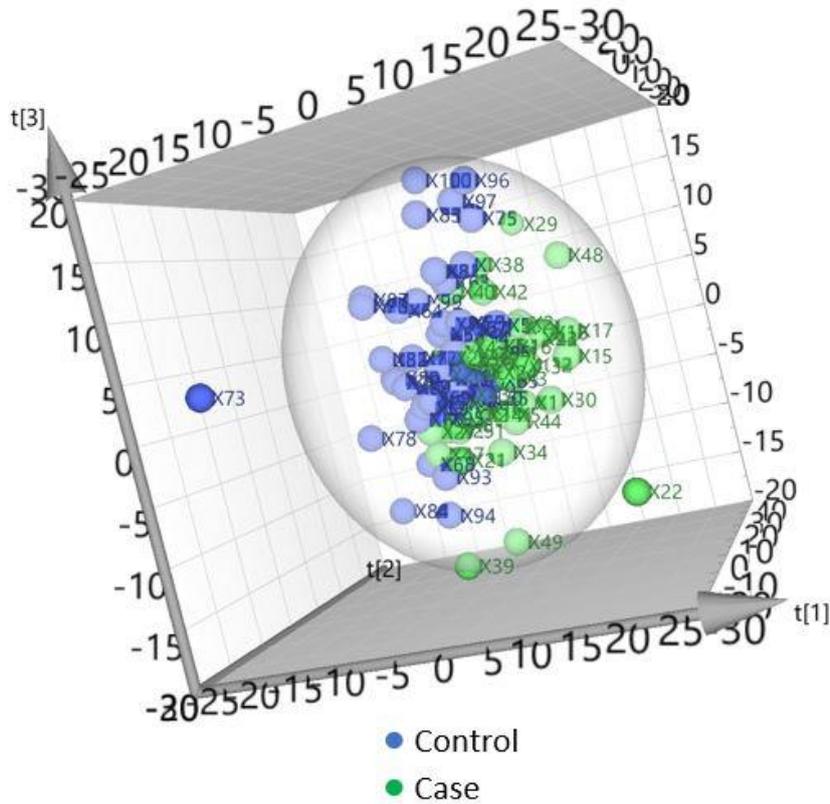
### **Co-authors:**

*Don Jones, University of Leicester*

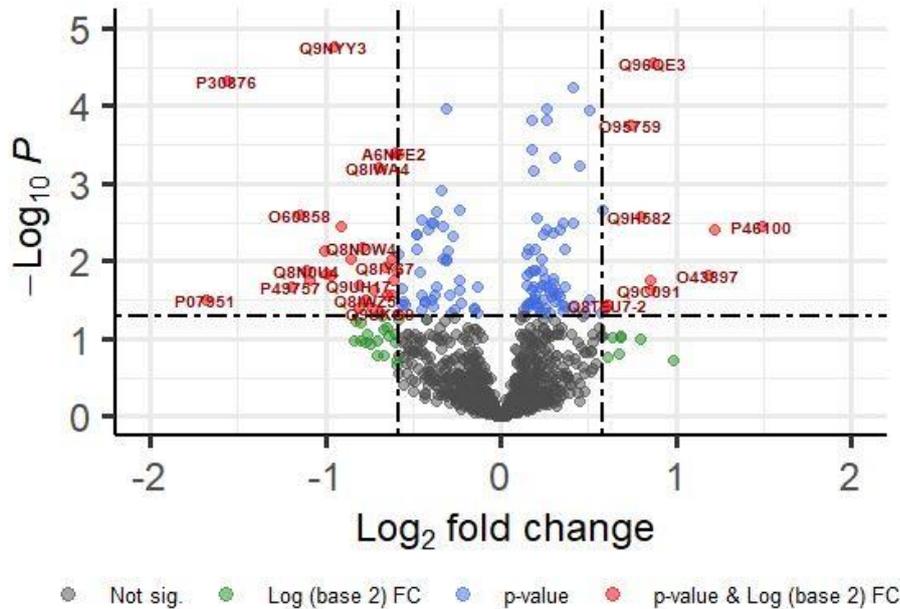
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PCA plot illustrating separation between CAD patients vs. healthy controls.



Volcano plot showing p-value vs. fold change for ECV proteins.

Poster number: **LS-PA-058**

## **SWEAT PROTEOMICS IN CYSTIC FIBROSIS AND COVID-19 – TOWARDS PROTEIN BIOMARKERS FOR NON-INVASIVE, PRECISION MEDICINE**

Abstract ID: **234**

**Presenting author: Gabriel Mazzucchelli, Mass Spectrometry Laboratory, ULiège**

### **Introduction**

In clinical routine, the analysis of sweat composition is used for the determination of drug intake and the diagnosis of cystic fibrosis (CF) based on sweat chloridometry (Gibson & Cooke sweat test). Meanwhile, sweat protein content has already been correlated with disease-specific abundances (e.g. schizophrenia and tuberculosis). The clinical relevance of the sweat proteome relies on the characterization of reference individual physiological profiles under steady state conditions with standardized reproducible methods, for further use in the non-invasive, individualized monitoring of protein biomarkers for biometric tracking, clinical follow-up and precision medicine.

### **Methods**

In light of the potential of eccrine sweat to become a relevant matrix of protein biomarkers for prognosis or diagnosis of disease conditions and clinical follow-up, sweat samples were collected from 28 healthy controls, 14 patients with CF and 10 patients with COVID-19, following the sweat test gold standard guidelines, and analyzed by shotgun proteomics (nanoLC-ESI-Q-Orbitrap MS/MS (ACQUITY M-Class, trap-elute mode, 177-min elution - Q Exactive Plus, DDA, positive ion mode).

### **Preliminary data (results)**

A total of 1071 proteins were identified and quantified at an individual level by a label-free approach, establishing reference eccrine sweat proteome annotations and individual quantitative proteome profiles. The analysis of CF and COVID-19 sweat built upon these references to i) identify candidate companion diagnostic and prognostic biomarkers for CF (CFTR genotype, pancreatic insufficiency, lung function impairment) and COVID-19 (pulmonary and cardiovascular complications), ii) give insights in the pathophysiology of CF (actin bundling) and COVID-19 (hemoglobin (Hb) over-abundance) sweat. For CF and COVID-19, the studies provided candidate biomarkers for companion diagnostics to the sweat test (for CF only), better prognosis evaluation and therapeutic development.

### **Please explain why your abstract is innovative for mass spectrometry?**

From a clinical standpoint, the proteomic analysis of sweat proved to be relevant to improve disease diagnosis or prognosis and better understand pathophysiological mechanisms with a view to precision medicine.

### **Co-authors:**

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Poster number: **LS-PA-059**

## THE TEMPORAL PROTEOME DYNAMICS OF THE MODEL EUKARYOTE YEAST DURING GLUCOSE EXHAUSTION

Abstract ID: **261**

**Presenting author: Maxime den Ridder, Delft University of Technology**

### Introduction

Glycolysis is the central pathway for sugar metabolism in most living organisms. The single celled eukaryote yeast is a widely used model organism for higher eukaryotes in which the regulation of glycolysis is broadly studied. *S. cerevisiae* is one of the few eukaryotic organisms that can efficiently grow under both aerobic and anaerobic conditions. Furthermore, this yeast switches from proliferation into a resting phase when nutrients are exhausted. However, little is known about the proteome dynamics that take place during this transition, particularly under anaerobic conditions. Moreover, like many other organisms including humans, the genome of *S. cerevisiae* contains duplications which result in the expression of so-called 'isoenzymes' in central metabolic pathways including glycolysis. Interestingly, the role of those 'isoenzymes' remains elusive to date.

### Methods

Here we describe a large-scale quantitative proteome study combining shotgun and targeted experiments using a nano-LC coupled to a QE plus Orbitrap mass spectrometer (Thermo, Germany), to capture the proteome dynamics during transition from proliferation into stationary phase, under both aerobic and anaerobic growth. Furthermore, we explore the proteome dynamics of a mutant yeast where the glycolytic isoenzymes are deleted. TMT quantification of the identified yeast proteins was enabled by PEAKS Q (Bioinformatics Solutions Inc. Canada). Moreover, an open source data processing pipeline was developed in Python to streamline processing and visualisation of the large time-series experiments.

### Preliminary data (results)

The majority of essential pathways profoundly changed between aerobic and anaerobic conditions, while anaerobic growth showed substantially less reorganisation during the transition from exponential to stationary phase. This supports the conception that 'anaerobic cells' lack the time and resources to adapt to the changing environment. Notable, aerobic and anaerobic cultures undergo different trajectories into stationary phase, as aerobic cells enter a 'post-diauxic phase' after glucose depletion, while anaerobic cells are not able to respire and enter stationary phase directly. However, the overall protein abundance of glycolytic proteins was significantly higher in the anaerobically cultured cells in all growth phases, suggesting that cells compensate for the decreased ATP yield of strictly fermentative growth (anaerobic) versus respiratory growth (aerobic), by increasing the glycolytic flux. On the other hand, while no phenotypic responses were observed when deleting the isoenzymes (previously), a range of protein-level alterations in glycolysis were observed in the mutant, in particular under anaerobic conditions. These subtle protein-level changes possibly compensate for the loss of the minor isoforms and thereby evading a stronger phenotype.

### Please explain why your abstract is innovative for mass spectrometry?

To the best of the authors knowledge, the study constitutes the to-date most comprehensive proteome dynamics study of yeast, capturing the unique metabolic features of a highly important model eukaryote.

### Co-authors:

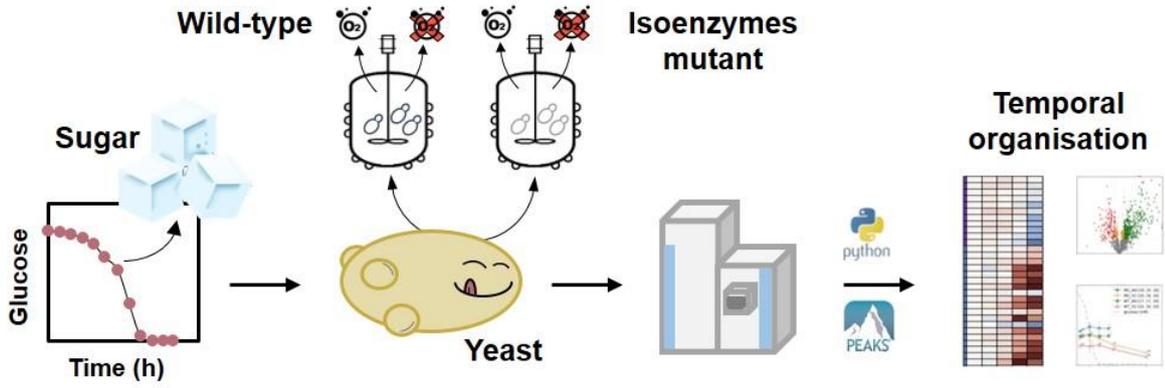
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*Pascale Daran-Lapujade, Delft University of Technology*

*Martin Pabst, Delft University of Technology*

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Temporal proteome dynamics of yeast during aerobic and anaerobic growth.

Poster number: **LS-PA-060**

## MULTIPLEX QUANTIFICATION OF TAU PROTEOFORMS BY MASS SPECTROMETRY FOR THE DIFFERENTIAL DIAGNOSIS OF NEURODEGENERATIVE DISEASES

Abstract ID: 269

**Presenting author: Chloe Jacquemin, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), SPI, 91191 Gif-sur-Yvette, France**

### Introduction

Neurodegenerative diseases (NDDs) are a major public health concern and their diagnosis is still challenging, especially at the early stages. While the identification of tau proteoforms (i.e phosphorylation) in the cerebrospinal fluid (CSF) and plasma has emerged and is now widely used for the diagnosis of Alzheimer's disease (AD), pathophysiological fluid biomarkers are still lacking for non-AD tauopathies such as progressive supranuclear palsy (PSP), Pick's disease, and others. Multiplex liquid chromatography (LC)-mass spectrometry (MS)/MS offers the opportunity to quantify the huge diversity of tau proteoforms in AD and non-AD tauopathies at very low concentrations in fluids. We propose here to test different protocols that allow better detection of tau proteoforms (3R and 4R isoforms, truncations, phosphorylations) using LC-MS-MS on CSF from NDDs patients.

### Methods

Artificial CSF with recombinant tau protein and human CSF samples were analyzed. Fully  $^{15}\text{N}$ -labeled tau was added as an internal standard to the samples. Protein purification was achieved by immunoprecipitation (IP) after evaluating a panel of commercial tau antibodies coated on magnetic beads, or protein precipitation with perchloric acid followed by micro-Solid Phase Extraction (Pc- $\mu$ SPE) using Oasis hydrophilic lipophilic balance sorbent. The purified proteins were resuspended in Rapigest and digested into peptides using trypsin. Multiplex quantitative analysis was performed on reversed-phase capillary LC, coupled with a qExactive high-resolution mass spectrometer operated in the Parallel Reaction Monitoring (PRM) acquisition mode.

### Preliminary data (results)

First, the whole analytical process was optimized, from sample preparation to MS detection, to reach the lowest limits of detection (LOD) in CSF for tau. LC-MS optimizations mainly consisted in fine-tuning the mass spectrometer parameters and testing a nanospray ionization source (NSI). Regarding sample preparation, the enzymatic digestion was thoroughly optimized and two protocols for tau extraction were improved (IP and Pc- $\mu$ SPE).

Secondly, we compared these two protocols in terms of gain in peptide signal intensity. Optimized IP conditions with antibodies targeting the central and N-ter domains offered the best results. IP combined with the NSI source showed a substantial gain in signal intensity (minimum gain factor: 4.5) for peptides from the central domain, compared to previous conditions. The Pc- $\mu$ SPE protocol was hardly compatible with the NSI source (clogging). Surprisingly, Pc- $\mu$ SPE with the ESI source allowed for the detection of more proteoforms in human CSF, notably from the C-terminal domain, whereas IP showed more sensitivity than Pc- $\mu$ SPE on artificial CSF. This could be explained by the important number of tau truncations in human CSF which probably impacted the capture by antibodies.

In total, the Pc- $\mu$ SPE protocol allowed detection of peptides from the N-ter to C-ter of tau, and the best signal for peptides representative of tau proteoforms (e.g 3R and 4R isoforms linked to different tauopathies, and several phosphorylated tau species). This opens the opportunity to use multiplex LC-MS-MS for the detection of new pathophysiological biomarkers in non-AD tauopathies.

### Please explain why your abstract is innovative for mass spectrometry?

Here we show the superiority of an antibody-free sample preparation protocol over IP for CSF tau proteoforms quantification by MS

### Co-authors:

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



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Poster number: LS-PA-061

## MULTIPLEXED LC-HRMS/MS QUANTIFICATION OF NINE SEPSIS PROTEIN BIOMARKERS IN HUMAN SERUM

Abstract ID: 273

**Presenting author: Maxence Derbez-Morin, French National Measurement Laboratory (LNE), Department of Biomedical and Organic Chemistry, 1 rue Gaston Boissier, 75015 Paris Cedex 15, France, Paris-Saclay University, CEA, INRAE, Department of Drugs and Technologies for Health (DMTS), SPI, 91191 Gif-sur-Yvette, France**

### Introduction

In 2017, sepsis led to 11 million deaths worldwide<sup>1</sup>. Its early and accurate diagnosis followed by an appropriate antimicrobial therapy are key to improve patient outcome. Utility of protein biomarkers (PCT, CRP) has been demonstrated for sepsis diagnosis. However, targeting a single protein biomarker may not provide sufficient clinical specificity, thus early diagnosis may be improved by monitoring a panel of serum protein biomarkers. Multiplexed liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) provides reliable protein quantification in human biofluids, but often requires immuno-enrichment when dealing with low abundance proteins. In this work, we evaluated an antibody-free protocol based on LC-HRMS/MS operating in the Parallel Reaction Monitoring (PRM) mode for the robust and sensitive absolute quantification of nine candidate biomarkers of sepsis.

### Methods

A bottom-up strategy was applied for the detection and quantification of the biomarkers<sup>2</sup>. Briefly, human serum was precipitated with methanol, before reduction, alkylation and overnight trypsin-digestion release proteotypic peptides, finally submitted to SPE fractionation. Peptides were separated on a Aeris C18 column with a linear gradient from 5 to 50% of 0.1% formic acid in methanol over 19.5 min at 0.5 mL/min flow rate. Peptides were then analysed on a LC-HRMS/MS system (qExactive) operating in the PRM mode. Three to four peptides were monitored for each protein, using corresponding labelled peptides for quantification by Isotope Dilution Mass Spectrometry.

### Preliminary data (results)

The challenge of this work lies in the large concentration range, above  $10^5$ , of the selected proteins in human serum, consisting in low abundance (e.g. PCT, suPAR) and medium abundance markers (e.g. CRP, SAA1). First, three to four proteotypic peptides were identified for each protein, according to existing recommendations<sup>3,4</sup>. The full protocol, from sample preparation to targeted HRMS analysis, was optimised to achieve absolute quantification of the nine biomarkers in human serum. Mass spectrometry was optimised through both manual testing and design of experiment to identify optimal conditions. Results highlighted the key influence of ion source parameters. LC was also optimised and methanol was used as the mobile phase to enhance the signal of selected peptides and improve chromatographic resolution. These LC-HRMS developments allowed detection of four out of the nine endogenous proteins. In the objective to detect all nine proteins, the development of a robust antibody-free sample preparation workflow at both the protein and peptide levels is necessary. Various protocols were evaluated at the protein level regarding reduction in sample complexity (e.g. overnight ethanol precipitation, isopropanol/trichloroacetic acid depletion, methanol precipitation). At the peptide level, several ion-exchange or reverse phases SPE cartridges for sample fractionation were tested. The most efficient approaches will be confirmed in human serum spiked at concentrations reported in sepsis patients and finally combined in the final assay conditions. Full method validation, and use of primary calibrators of well-characterized purity by peptide impurity corrected amino acid analysis should ensure an accurate quantification in human serum.

### Please explain why your abstract is innovative for mass spectrometry?

A robust antibody-free sample preparation protocol for the multiplex HRMS quantification of sepsis biomarkers over a large concentration range, applicable to cohort of patients samples for early sepsis diagnosis.

### Co-authors:

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## POSTER SESSION A

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Tuesday 30 August 2022 from 14:00 to 15:30 hours

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Poster number: **LS-PA-062**

## **DETERMINATION OF FOOD AUTHENTICITY OF INSECT-BASED NOVEL FOODS BY MASS SPECTROMETRY-BASED IMMUNOASSAYS**

Abstract ID: **296**

**Presenting author: Tobias Meisinger, SIGNATOPE GmbH**

### **Introduction**

Rising global protein demand for animal and human nutrition requires new, safe sources of protein that support healthy, nutrient-rich diets and sustainable food chains. Insects meet these requirements in terms of their high protein content, polyunsaturated fatty acids, vitamins and ability to convert organic side streams into high value protein products, thereby reducing carbon emissions. With the recent approval of first insect products under the EU Novel Food Regulation 2015/2283, food authenticity and safety concerns including allergic risks need to be assessed.

Existing methods for authentication of food products are based on genomic DNA using polymerase chain reaction (PCR) protocols. This qualitative approach is lacking in terms of quantification and can be impeded by food and feed processing.

### **Methods**

The development of protein-based immunoassays for species identification and quantification remains challenging due to highly degree of processing and lacking genomic and proteomic data for most insect species of current interest. For three out of six insect species of interest only a draft genome with no gene annotation is publicly available, genome data from two species are not yet available and only for one species sufficient information on genomic and proteomic level is published. Therefore, conventional immunoassay development approaches are challenging without such data.

### **Preliminary data (results)**

To address this issue, we developed in a first step a bioinformatic workflow based on data-dependent acquisition (DDA) bottom-up proteomics to identify species-specific peptides. The workflow comprises a direct tryptic digestion of insect meal samples in suspension, a LC-MS/MS-based analysis followed by peptide sequence identification via MASCOT searching against in silico translated genomic insect databases and publicly available proteomic Arthropoda databases. Identified peptides were annotated by a homology approach using BLAST and subsequently sorted for uniqueness. The presence of these peptide candidates in their respective insect species samples was confirmed with targeted LC-MS/MS-based analysis.

In a second step, using confirmed species-specific peptides, we developed a multiplex mass spectrometry-based immunoassay capable of insect species identification and quantification in food and feed samples. Following a direct tryptic digestion of sample material and an immunoaffinity enrichment of released peptides, targeted LC-MS/MS-based analysis is performed for peptide quantification using isotope labelled standard peptides.

The developed assay reveals excellent performance in respect of species specificity, sensitivity, and quantification capabilities. The assay will be optimized, validated and applied to screen commercially available food samples prepared by project partners.

### **Please explain why your abstract is innovative for mass spectrometry?**

Development of a LC-MS/MS-based workflow for identification of peptides from insect species with unknown genomes.

Multiplex mass spectrometry-based immunoassay for insect species identification and quantification in food and feed.

### **Co-authors:**

*Andreas Steinhilber, SIGNATOPE GmbH*

*Cristiano Garino, Department of Food Safety, National Reference Laboratory for Animal Protein in Food, German Federal Institute for Risk Assessment (BfR)*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
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Tuesday 30 August 2022 from 14:00 to 15:30 hours



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*Thomas Joos, NMI Natural and Medical Sciences Institute at the University of Tuebingen*

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Poster number: **LS-PA-063**

## **AN AUTOMATED SAMPLE PREPARATION SOLUTION FOR MASS SPECTROMETRY-BASED PROTEOMICS**

Abstract ID: **315**

**Presenting author: Ryan Bomgarden, Thermo Fisher Scientific**

### **Introduction**

The proteomics field lacks standardized reagents and methods for sample preparation as already adopted in genomic and transcriptomic fields. Current methods are tedious, time consuming, highly variable, unsuitable for processing large numbers of samples, and tend to negatively affect the robustness of the LC-MS instrumentation. Therefore, we developed an automated sample preparation solution that simplifies and standardizes mass spectrometry (MS) sample preparation. The new automated sample preparation platform is an intuitive turnkey system (instrument, software, reagents) that enables standardized, hands-off operation and provides robust workflows for label free proteomics and TMT applications.

### **Methods**

The automated platform enables hands off proteins lysis, DNA removal, protein reduction and alkylation, digestion, TMT labeling, pooling and cleanup, providing several workflows and companion reagents to perform several LFQ, TMT11plex and TMTpro workflows at different sample scales. It guides the researchers across the whole workflow using an embedded user interface to easily place reagents and samples and recover processed samples. A SW ecosystem enables researchers to be assisted during the design of their experiments according to the biology changes. The prepared samples are ready to be injected into the external LC-MS system with known concentrations.

### **Preliminary data (results)**

The automated sample preparation platform can process up to 36 samples containing 10-100 µg of initial protein input within 4-6 hours depending on the workflows. The final peptides have minimum missed cleavage rate less than 5% and complete reduction and alkylation. Most importantly, the liquid handler has an integrated UV spectrometer that allows peptides to be readily analyzed by LC-MS with a known concentration.

Using mammalian cells as a model sample, we benchmark the label-free workflow against manual workflows (e.g., EasyPep kits) and other solutions automated/semi-automated commercially available. Our sample preparation solution provides peptide and protein identifications that are equal or greater compared to those solutions benchmark as well as other proteomics quality metrics. Successful MS sample quality and reproducibility for other biological samples (e.g., plasma, tissues, purified proteins) were demonstrated too. Significantly, the system exhibited an extremely high digestion efficiency even for challenging samples. Zero peptide missed cleavages of 95% could be achieved for plasma samples with 1-hour digestion. The sample preparation process is robust and reproducible with less than 5% CVs in terms of the peptide and protein identifications and less than 10% in terms of proteome quantification based on the protein abundances between technical replicates as analyzed by mass spectrometry.

To significantly improve the analysis throughput, samples can be labeled by isobaric labelling (TMT11plex or TMTpro 16plex) approach in an automated fashion without user intervention providing high labeling efficiency of >99%. Using the TMT workflows, proteomes are reproducibly quantified with less than 10% CVs.

### **Please explain why your abstract is innovative for mass spectrometry?**

Overall, this novel automated sample preparation platform for proteomics samples standardizes and simplifies MS sample preparation for proteomics applications having the potential to increase 10X the productivity in proteomics laboratories.

### **Co-authors:**

*Maowei Dou, Thermo Fisher Scientific*

*Steven Reeber, Thermo Fisher Scientific*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

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*Greg Foster, Thermo Fisher Scientific*  
*Sergei Snovida, Thermo Fisher Scientific*  
*Jia Wang, Thermo Fisher Scientific*  
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*Jennifer Toth, Thermo Fisher Scientific*  
*Kristan Bahten, Thermo Fisher Scientific*  
*Kay Opperman, Thermo Fisher Scientific*  
*Daniel Lopez Ferrer, Thermo Fisher Scientific*

Poster number: **LS-PA-064**

## **HIGH-THROUGHPUT PROTEOMIC ANALYSIS OF STORED RED BLOOD CELLS FROM NON-DOMESTIC CAT SPECIES**

Abstract ID: **319**

**Presenting author: Amirmansoor Hakimi, Thermofisher Scientific**

### **Introduction**

Blood transfusions can be a life-saving treatment for animals that have become anemic due to trauma or disease. However, adverse, often life-threatening complications can occur if the donor and recipient blood types are not appropriately matched. In non-domesticated cat species, very little is known about the different blood types found in different species, making transfusions in most situations risky. Additionally, since non-domestic are often found in zoos where only a few individuals of each species are housed, there is a need to be able to either transfuse blood from one species to another or store blood for prolonged periods of times in case of future emergencies

### **Methods**

Blood samples were obtained from 150 non-domestic cats, consisting of 18 different species, housed at AZA (Association of Zoos and Aquariums) accredited institutions across the United States. Samples were aliquoted and stored in a clinical blood transfusion refrigerator for 0, 1, 14, or 28 days, after which the RBC (red blood cells) were pelleted and stored at -80°C. Samples were also cross-matched to determine cross-compatibility. Pelleted RBCs were prepared for bottom-up proteomic analysis using a sample preparation platform for proteomics samples that enables standardized, hands-off operation and allows for protein lysis, reduction, alkylation and digestion without user intervention.

### **Preliminary data (results)**

Trypsin digested red blood cell samples were then cleaned up and analyzed using high-throughput, capillary flow LC-MS/MS analysis on a Thermo Scientific™ Orbitrap Exploris 480™ mass spectrometer, allowing for the analysis of more than 40 samples per day. Such methods allowed for the identification and label-free quantification of more than 400 proteins per sample, despite the high abundance of hemoglobin in the red blood cells. Proteomic profiles were then compared across the different blood storage lengths and across species to investigate the effects of long-term storage of blood. Such information could provide insight as to which species might be less prone to the formation of red blood cell lesions during storage, which would make them more suitable as blood donors.

### **Please explain why your abstract is innovative for mass spectrometry?**

High Throughput Capillary LC-MS

### **Co-authors:**

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Poster number: **LS-PA-065**

## **KIT QUANTA – STANDARDIZATION KIT FOR ABSOLUTE PROTEIN QUANTITATION: CONTROL OF THE LIQUID CHROMATOGRAPHY SETUP-DEPENDENT ARTEFACTUAL METHIONINE OXIDATION OF PEPTIDES**

Abstract ID: **343**

**Presenting author: France Baumans, Mass Spectrometry Laboratory-ULiège**

### **Introduction**

In the world of biomarker discovery, up to now, only a few biomarkers have been validated. The validation remains a crucial step in the development of a biomarker but is quite challenging and lengthy. Mass spectrometry-based methods are high-throughput techniques dedicated to large scale protein quantitation where standardization must be applied. In this context, we are developing a comprehensive standardization strategy aiming to control the entire sample preparation process and easing the performance of longitudinal or large-scale studies of biomarker validation. One aspect of the development is the control of the undesired artefactual methionine oxidation occurring during the sample preparation process and the liquid chromatography separation. Our work focuses on the detection and evaluation of the latter: the on-column oxidation process.

### **Methods**

To evaluate the extent of the on-column methionine oxidation phenomenon, we capitalized on a synthetic methionine-containing peptide and extended the research to complex samples. We first measured the evolution of this oxidation over a 1-year use of the column set. We then evaluated and compared the oxidation levels of methionine-containing peptides with different trapping times and gradient lengths to frame the exact localization of the LC separation. Analyses generated with two other instrumentations were investigated for comparison with the data created with our LC-MS setup. Finally, we tested different sample load to apprehend if it affects the oxidation response.

### **Preliminary data (results)**

Thanks to the synthetic methionine-containing peptide and peptides coming from complex samples, we observed, on our specific instrumentation setup (an ACQUITY UPLC M-Class System composed of a nanoEase M/Z Symmetry C18 Trap Column and a nanoEase M/Z HSS T3 coupled to a Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer) that in addition to solution and electrospray oxidations, methionine-containing peptides can be significantly oxidized inside the analytical column, along the chromatographic separation. On-column methionine oxidation is systematically characterized by a broad chromatographic peak having an intermediate retention time between methionine-containing peptide oxidized in solution and its native counterpart. Similar observation was noticed on another setup from another laboratory based on publicly available data. We showed that the trap column is not the place of methionine oxidation, but the analytical column is. Considering the HSS T3 phase (Waters), our results highlight that the on-column oxidation level significantly increases with the age of the analytical column and the gradient length and reaches 56 % when a 1-year-old column set is used with a 180 min-long LC method. These levels go to 0 % and 18 % for the YMC Triart C18 (YMC Europe GmbH) and the BEH130 C18 (Waters) phases respectively. We also noticed an increase of the on-column oxidation as the sample load decreases. This observation was rationalized by the presence of a limited number of oxidation sites within the stationary phase, being more and more exposed to the mobile phase and to the sample over time.

### **Please explain why your abstract is innovative for mass spectrometry?**

The importance of a quality control for the detection of on-column methionine oxidation and its use for protein quantitation standardization.

### **Co-authors:**

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Poster number: **LS-PA-066**

## MS-BASED ASSAY FOR THE DETERMINATION OF NATRIURETIC PEPTIDES IN SALIVA AND PLASMA SAMPLES

Abstract ID: **359****Presenting author: Alessio Lenzi, Department of Chemistry and Industrial Chemistry - University of Pisa**

### Introduction

Cardiac natriuretic peptides (NPs), i.e., ANP, BNP, and CNP, are hormones initially synthesized from cardiomyocytes, as prohormones. Once they are released from the heart, prohormones are split into two fragments: a longer N-terminal fragment (NT-proBNP, and NT-proANP) and a shorter C-terminal fragment (BNP, ANP). In pathological conditions as heart failure (HF), heart induces the stretching of myocytes due to pressure overload, leading to the release of NPs in blood. Thus, NPs plasma levels increase progressively with the severity of HF and represent the main biomarkers for prognosis and diagnosis of HF. NPs are normally determined in blood using commercial immunoassays kits. Unfortunately, this approach is affected by cross-reactions due to the presence of several NP forms showing homologous structures.

### Methods

In this work we developed and validated a reliable and innovative analytical workflow based on top-down proteomic and UHPLC-MS/MS (triple quadrupole) approach for the determination of NPs in saliva and plasma.

### Preliminary data (results)

Chromatographic separation parameters were optimized by testing different mobile and stationary phases. Results showed that the use of a Zorbax 300SB-C3 column and the use of 0.2% aqueous formic acid (A)/ acetonitrile + 0.2% formic acid (B) mobile phase. Product ion scan mode was used to determine adequate transitions and collision energies for setting up a multiple reaction monitoring (MRM) method for each peptide. MRM considered at least two transitions for each compound, where the most intense was identified as the quantifier and the other transitions as qualifiers. Source parameters (ESI) were optimized for all the analytes to enhance the ionization efficiency and the ion transmission to the mass spectrometer. To enhance ionization efficiency of analytes, the addition of a supercharger agents (e.g., dimethyl sulfoxide and 3-nitrobenzyl alcohol) was tested. In the optimized conditions, the instrumental detection limits were in the range 4-191 pg/mL and, thus, the method resulted suitable for the determination of NPs in biofluids. Particular attention was paid to the evaluation of analyte stability in biological specimens as well as standard solutions. NPs were found to be stabilized at 4°C in solution by the presence of both human serum albumin, as displacement agent, and methionine (45 µg/g) as oxygen scavenger up to 14h. Further details will be extensively discussed in the present work.

### Please explain why your abstract is innovative for mass spectrometry?

Literature lack of a top down LC-MS/MS approach for the determination of a panel of NPs in biofluids.

### Co-authors:

*Denise Biagini, Department of Chemistry and Industrial Chemistry - University of Pisa*

*Silvia Ghimentì, Department of Chemistry and Industrial Chemistry - University of Pisa*

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*Fabio Di Francesco, Department of Chemistry and Industrial Chemistry - University of Pisa*

*Tommaso Lomonaco, Department of Chemistry and Industrial Chemistry - University of Pisa*

Poster number: **LS-PA-067**

## **A HIGH-THROUGHPUT LABEL-FREE QUANTITATIVE PROTEOMICS PLATFORM TO SPEED-UP MICROBIAL STRAIN CHARACTERIZATION**

Abstract ID: **413**

**Presenting author: Nicolas Abello, DSM Science & Innovation, Biodata & Translation, Center for Analytical Innovation, Delft, the Netherlands**

### **Introduction**

DSM is a company active in Health, Nutrition and Bioscience. In our bioprocess and bioproducts R&D, label-free proteomics analysis fulfills an important role in the characterization of microbial strain phenotype, e.g. by allowing to monitor proteome expression during fermentation process conditions, notably for key intracellular enzymes and transporter proteins. In Biotech R&D, analytical throughput and turnaround time of these advanced technologies is often a limiting factor for broad adoption. Therefore, we developed and streamlined the whole workflow from sample preparation to data reporting for high-throughput proteomics of yeast samples. Here we report the upgrade of our label-free quantitative proteomics platform, improving throughput and quality from fermentation sample to data reporting, exemplified with a use case.

### **Methods**

Elements we touched upon are: more reproducible and faster protein digestion, protein/peptide normalization, sample preparation automation, application of quality controls, faster and more reproducible LC-MS analysis, and automation of data handling, visualization and reporting.

LC-MS improvements consisted notably in the adoption of a dual-LC system coupled to Data-Independent Acquisition (DIA) on an Orbitrap Exploris™ 480 mass spectrometer (\*) using the Spectronaut™ (Biognosys) data analysis software, thereby replacing a single-LC system coupled to Data-Dependent Acquisition (DDA) on an Orbitrap Q Exactive™ Plus mass spectrometer (\*), together with the Proteome Discoverer (\*) software.

(\*): Thermo Scientific™

### **Preliminary data (results)**

Overall, we demonstrate a significantly improved Proteomics platform for characterization of yeast strains concomitant with improved data quality and turnaround time.

### **Please explain why your abstract is innovative for mass spectrometry?**

Adoption of a dual-LC system coupled to DIA-MS on an Orbitrap Exploris™ 480 mass spectrometer using the Spectronaut™ software, and automation of data handling, visualization and reporting.

### **Co-authors:**

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*Maurien Olsthoorn, DSM Science & Innovation, Biodata & Translation, Center for Analytical Innovation, Delft, the Netherlands*

*Rob van der Hoeven, DSM Science & Innovation, Biodata & Translation, Center for Analytical Innovation, Delft, the Netherlands*

Poster number: **LS-PA-068**

## **QUALITATIVE AND QUANTITATIVE CHARACTERIZATION OF ELASTIN-LIKE POLYPEPTIDES COMBINING A NOVEL PROALANASE BOTTOM-UP APPROACH AND INTACT PROTEIN ANALYSIS**

Abstract ID: **414**

**Presenting author: André Vente, DSM**

### **Introduction**

Elastin-like polypeptides (ELPs) are elastic and thermoresponsive biopolymers composed of Val-Pro-Gly-Xaa-Gly repeats, used in biomedical applications, e.g. tissue engineering and drug delivery. Different variants of ELP can be produced fermentatively to study structure-property relationships [López Barreiro et al., 2020].

[D. López Barreiro, I.J. Minten, J.C. Thies, and C. M. J. Sagt, ACS Biomat. Sci. Eng., July 2021, <https://doi.org/10.1021/acsbiomaterials.1c00145>]

### **Methods**

Intact protein measurement using QExactive Plus (Thermo Scientific), Exploris 480 (Thermo Scientific) and Synapt G2s (Waters). As well as bottom-up proteomics using a novel ProAlanase digestion approach at low temperature and low pH. A stable isotopically labelled internal standard peptide was added to enhance quantification accuracy and measurements were performed on a QExactive Plus mass spectrometer (Thermo Scientific) operating at high flow LC conditions and Data Dependent Acquisition.

### **Preliminary data (results)**

Despite the lack of amino acids with charged side chains (Arg, His, Lys, Asp, and Glu) in ELP, we demonstrated intact protein analysis using reversed phase LC coupled to ESI-MS. Moreover, truncated protein forms could be chromatographically separated and characterized as well as N-terminal modifications.

Bottom-up proteomics on ELPs poses challenges since standard enzymes will not cleave this polypeptide efficiently and ELPs are poorly soluble at typical digestion temperature and pH. We demonstrate a sensitive and accurate LC-MS quantification method utilizing ELP-specific properties, i.e. proline-rich, soluble at low pH & low temperature. These properties make ELPs an excellent substrate for a proline-specific protease (ProAlanase). The analytical method is based on the detection of Gly-Val-Gly-Val-Pro repeats after ProAlanase-digestion at pH 2 and 10°C. The addition of a stable isotopically labelled internal standard enabled quantification in complex matrices.

### **Please explain why your abstract is innovative for mass spectrometry?**

Both methods combined enabled quantitative and qualitative characterization of fermentatively produced ELPs.

Poster number: **LS-PA-069**

## **COMPARATIVE ASSESSMENT OF QUANTIFICATION METHODS FOR TUMOR TISSUE PHOSPHOPROTEOMICS**

Abstract ID: **664**

**Presenting author: Yang Zhang, Department of Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of 8 Groningen, 9713 AV Groningen, The Netherlands , Institute of Biochemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck, 6020 10 Innsbruck, Austria , Laboratory of Pediatrics, Section Systems Medicine of Metabolism and Signaling, University of Groningen, University Medical Center Groningen, 9713 AV, Groningen, The Netherlands**

### **Introduction**

Continuous improvements in the speed and sensitivity of mass spectrometry systems are bringing the phosphoproteome of human tumors into reach. However, the choice of the most appropriate quantitative proteomic strategy for a particular biomedical problem or diagnostic requirement remains largely on a trial and error decision. We compared label-free quantification (LFQ), spike-in-SILAC (stable isotope labeling by amino acids in cell culture), and tandem isobaric mass tag (TMT) technology for quantitative phosphosite profiling in tumor tissue.

### **Methods**

Samples with known ratios of phosphopeptide abundance were generated from cancer tissues and cell cultures. IMAC-enriched phosphopeptides were analyzed by nanoLC-MS/MS using an Orbitrap Lumos Fusion instrument.

### **Preliminary data (results)**

TMT provided the lowest accuracy but the highest precision and robustness to different phosphosite abundances and matrices. Spike-in-SILAC provided the best compromise regarding accuracy, precision, and robustness, but suffered from low phosphosite coverage. LFQ offered the lowest precision, but this approach provided the highest number of identifications. Both spike-in-SILAC and LFQ were susceptible to matrix effects. Match between run (MBR) analysis improved phosphosite coverage in LFQ and spike-in-SILAC, but decreased precision and robustness of quantification.

**BioRxiv link: DOI: 10.1101/2022.02.07.479414**

### **Please explain why your abstract is innovative for mass spectrometry?**

The first comparative study of the quantitative performance of LFQ-, spike-in-SILAC-, and TMT-based tissue phosphoproteomics offers a resource for systems oncology proteomics and data analysis.

### **Co-authors:**

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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



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*Peter L. Horvatovich, Department of Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of 8 Groningen, 9713 AV Groningen, The Netherlands*

Poster number: **LS-PA-070**

## **FURTHER STEPS IN MINIATURIZED TISSUE SAMPLING WITH A NANOSECOND INFRARED LASER (NIRL) FOR QUANTITATIVE PROTEOMICS**

Abstract ID: **683**

**Presenting author: Manuela Moritz, University Medical Center Hamburg-Eppendorf**

### **Introduction**

In the last years, ultrashort pulsed infrared (IR) and ultraviolet (UV) lasers, operating at high peaks in the absorption spectrum of water, can efficiently be used for sample homogenization and subsequent mass spectrometric (MS) omics analysis. In our latest publication we have shown 3D tissue sampling with a nanosecond infrared laser (NIRL) in combination with differential quantitative proteomics, revealing a relative high number of identified proteins for a low ablation volume of about 500 nL.

In this study, we demonstrate further steps in miniaturization, including optimization of sample preparation for MS proteome analysis.

### **Methods**

Different murine tissue types were ablated directly from the sample with a wavelength of 2940 nm by a nanosecond infrared laser (NIRL). The aerosol was condensed on glass slides with different coatings and dissolved in buffer solution. Also different protocols for tryptic digestions were tested. Afterwards, proteome analysis with liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed. The proteins were identified with a database search and statistically analysed with Perseus. Based on quantitative protein data the reproducibility was determined through principal component analysis (PCA) and the Pearson correlation between different samples.

### **Preliminary data (results)**

First, we were able to reduce our ablation volume from 700 nL down to 23 nL, based on volume measurements with optical coherence tomography (OCT), which corresponds to 3000 and 100 laser pulses, respectively. To confirm the applicability of this reduced sample volume, different mouse organs were ablated. Based on quantitative proteome analysis, it could be shown that organ specific patterns can be found with a high reproducibility.

As further steps we increased the numerical aperture (NA) of the laser optics, resulting in an higher intensity. By utilizing hydrophobic coated microscopy slides and tryptic digestion in a one-pot sample preparation step we aim to improve the number of identified proteins reducing surface adsorption losses and avoiding all sample transfer steps. With higher reproducibility, the ablation volume can be further decreased, resulting in a higher spatial resolution for 3D tissue sampling directly from the sample.

### **Please explain why your abstract is innovative for mass spectrometry?**

Quantitative proteome analysis could be performed on volumes in the lower nanoliter scale, sampled as voxel directly from tissue.

### **Co-authors:**

*Antonia Gocke, University Medical Center Hamburg-Eppendorf*  
*Hartmut Schlüter, University Medical Center Hamburg-Eppendorf*  
*Jan Hahn, University Medical Center Hamburg-Eppendorf*

Poster number: **LS-PA-071**

## **ADDRESSING THE CHALLENGES OF PROTEOMICS DATA PROCESSING TO SUPPORT DRUG DISCOVERY AND DEVELOPMENT**

Abstract ID: **689**

**Presenting author: Anna Quagliari, Mass Dynamics, C/O Hub Southern Cross, Level 2, 696 Bourke Street, Melbourne, Victoria 3000, Australia.**

### **Introduction**

More than 1000 tools for proteomics mass spectrometry (MS) data processing exists<sup>1</sup>, which highlights the emphasis placed on solutions that can appropriately mine data and translate this to relevant biological knowledge. For protein-based drug discovery and development, robust and scalable data processing workflows are essential for proteomic studies and need to be combined with objective quality control reporting and reproducible downstream analysis. Here, we present our Mass Dynamics web-based analysis environment (MD 1.0) that analyzes and visualizes bottom-up proteomics data<sup>2</sup>. Cloud-based architecture enables researchers to store data for automated data processing and visualization for annotation and sharing of findings with collaborators. MD 1.0 provides a seamless interactive workflow for differential expression analyses and protein characterisation workflows, including bottom-up peptide mapping and multi-attribute monitoring.

### **Methods**

MD 1.0 is composed of a javascript and rails app and open-source R and python packages utilized via Amazon Web Services (AWS) for a reproducible yet scalable workflow. MD 1.0 is able to quantify complex proteomics data and expand biological insights by leveraging existing knowledge bases such as the GO ontologies and Reactome using pre-existing datasets. We have evaluated MD 1.0 using well-characterised iPRG2015, a dynamic range dataset (PXD000279)<sup>3</sup>, HER2 and recombinant therapeutic (monoclonal antibody) peptide mapping datasets<sup>4</sup>, and by comparison to existing platforms.

### **Preliminary data (results)**

The MD 1.0 discovery service analyses of ground truth datasets were comparable to existing tools using both discrete and continuous measures. MD 1.0 architecture currently allows evaluation of various data inputs; from raw data to pre-processed data, such as MaxQuant output for LFQ and TMT datasets. MD 1.0 facilitates analysis, annotation and sharing of LFQ results and provides interactive and downloadable quality control reports, an automatic Reactome integration for Over Representation Analysis (ORA) and a GO/Reactome enrichment feature via the CAMERA algorithm from the LIMMA package. This is highlighted through re-analysis of existing LFQ studies, including the investigation of proteomic mechanisms for Her2 resistance. The MD 1.0 protein characterisation analysis of peptide mapping results from a digest of a monoclonal antibody therapeutic was comparable to what's previously reported<sup>4</sup>, assessing amino acid sequence and post-translational modification identification and quantitation.

### **References:**

1. Tsiamis, V.; et. al. One Thousand and One Software for Proteomics: Tales of the Toolmakers of Science. *J. Proteome Res.* 2019, 18, 3580–3585
2. Bloom, J.; et. al. Mass Dynamics 1.0: A Streamlined, Web-Based Environment for Analyzing, Sharing, and Integrating Label-Free Data. *J. Proteome Res.* 2021, 20, 11, 5180–5188
3. Cox J.; et. al. Accurate Proteome-wide Label-free Quantification by Delayed Normalization and Maximal Peptide Ratio Extraction, Termed MaxLFQ. *Mol Cell Proteomics.* 2014 Sep;13(9):2513-26,
4. Jakes, C.; et. al. Tracking the Behavior of Monoclonal Antibody Product Quality Attributes Using a Multi-Attribute Method Workflow. *J. Am. Soc. Mass Spectrom.* 2021 32 (8), 1998-2012

### **Please explain why your abstract is innovative for mass spectrometry?**

The MD 1.0 platform is built on open-sourcing practices and collaborative tools, providing a benchmarked, sustainable and robust proteomics MS tool to support drug discovery and development.

### **Co-authors:**

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

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Poster number: **LS-PA-072**

## **COMPARING THE PROTEOMES OF SPATIALLY RESOLVED EPITHELIAL CELL REGIONS AND WHOLE LUNG TISSUE TO STUDY ACUTE EPITHELIAL LUNG INJURY IN MICE**

Abstract ID: **787**

**Presenting author: Eva Griesser, Drug Discovery Sciences, Boehringer Ingelheim Pharma GmbH & Co. KG**

### **Introduction**

Epithelial injury is one of the major drivers of acute and chronic pulmonary diseases. Preclinical animal models are required to study early disease-driving mechanisms. Several models are already available, e.g. the diphtheria toxin receptor/diphtheria toxin (DTR/DT) cell depletion model allowing targeted injury. The human DTR (hDTR) is sensitive to DT inducing cell death by blocking protein synthesis, whereas the murine DTR is  $10^5$  times more resistant. However, generation of cell-specific DTR transgenic mice is time-consuming and costly. Therefore, a novel and flexible DTR/DT model of acute epithelial lung injury driven by adeno-associated virus (AAV) variant 6.2 mediated hDTR expression was recently established. AAV6.2 vector transduces specifically into bronchial epithelial and alveolar epithelial type II cells leading to their depletion after DT administration.

### **Methods**

Using laser-capture microdissection different epithelial cell regions (bronchial epithelium and normal and infiltrated alveolar tissue; ~3000 cells each) were isolated from formalin-fixed and paraffin-embedded lung tissue of AAV-stuffer (control) and AAV-hDTR mice, which were administered intratracheally with 100 ng DT for 24 h. Microdissected samples were analysed using tandem mass tagging and high-resolution mass spectrometry. In addition, we analysed the proteome of whole lung tissue originating from the exact same animals and compared the bulk data with the spatially resolved dataset.

### **Preliminary data (results)**

The LCM data showed upregulation of proteins involved in immune and inflammatory response in all epithelial cell regions. Interestingly, this response was stronger in the bronchial epithelium. However, most deregulations were specific to the lung region. Downregulation of marker proteins, such as SP-C, SP-A and Muc1 for alveolar epithelial type II cells, and Scgb1a1 for club cells, indicated death of these cell types in the corresponding region. Extracellular matrix proteins were increased in the infiltrated alveolar tissue, which promotes cell migration to the site of injury, while proteins involved in pulmonary surfactant synthesis, alveolar fluid clearance and alveolar-capillary barrier were downregulated in the alveoli. Overall, the LCM data provides spatially resolved insights into repair mechanisms in upper and lower airways after acute epithelial injury.

Analysis of the whole lung proteome revealed a strong interferon mediated immune signature as demonstrated by upregulation of cGAS-STING and interferon pathways. Moreover, increase of proteins involved in proliferation, DNA replication and provisional scaffold composition was shown.

Comparison of bulk and spatially resolved proteomes revealed a large overlap of differentially regulated proteins, but also remarkable differences. Together our data confirms the broad usability of bulk proteomics and highlights the benefit of proteomic analyses of specific tissue regions or single cell types.

### **Please explain why your abstract is innovative for mass spectrometry?**

Spatially-resolved proteomics of epithelial cell regions was performed using limited sample amount (3000 cells per sample).

### **Co-authors:**

*Martin Gesell, Drug Discovery Sciences, Boehringer Ingelheim Pharma GmbH & Co. KG*

*Hannah Wyatt, Drug Discovery Sciences, Boehringer Ingelheim Pharma GmbH & Co. KG*

*Daniel Veyel, Drug Discovery Sciences, Boehringer Ingelheim Pharma GmbH & Co. KG*

*Birgit Stierstorfer, Non-clinical Drug Safety, Boehringer Ingelheim Pharma GmbH & Co. KG*

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Poster number: **LS-PA-073**

## **SINGLE CEREBRAL ORGANOID MASS SPECTROMETRY ANALYSIS: TOWARDS NEURODEGENERATIVE DISEASES**

Abstract ID: **833**

**Presenting author: Marketa Nezvedova, The Recetox, Faculty of Science, Masaryk University**

### **Introduction**

Recent advances in analytical mass spectrometry and cell biology opened new avenues to investigate neurodegeneration. Cerebral organoids (COs) are three-dimensional cell cultures representing an emerging model system to study biological processes causing neurological diseases. COs recapitulate the brain tissue's cytoarchitecture, mimicking the brain development in vivo. However, the limited batch-to-batch reproducibility of COs requires tools to characterize the cellular composition and neuronal maturation. Immune-based assays are frequently used to characterize cell-specific protein markers in COs. However, they suffer selectivity issues have limited throughput and multiplexing capacity. Sample pooling is often required, and developing a new immunoaffinity assay is time-consuming and costly. Our study profiled a panel of protein markers in a single CO using selected reaction monitoring (SRM) quantitative proteomics.

### **Methods**

The system of ultra-high performance liquid chromatography (UHPLC with C18 Peptide CSH column) coupled to tandem mass spectrometry in the mode of selected reaction monitoring (SRM-MS/MS) was used for targeted protein analysis.

### **Preliminary data (results)**

SRM protein assays allowed us to simultaneously quantify up to 50 proteins per analysis in a single CO. The total protein content in individual COs ranged between 7 and 140 µg, while the minimum amount of 1 µg total protein equivalent is required per SRM analysis. Thus, each CO can be analyzed repeatedly to quantify hundreds of target proteins. We quantified 37 markers to characterize organoids' cell composition (i.e., neural stem cells, radial glial cells, neurons, astrocytes) and 13 proteins involved in neurodegeneration. The single organoid analysis avoids a bias due to sample pooling and offers more accurate data with the possibility of removing the outliers. We found COs a suitable in vitro model system that recapitulates in vivo developmental features (Figure 1). We discovered an age-dependent variance in cytoarchitecture and protein marker levels in COs.

### **Please explain why your abstract is innovative for mass spectrometry?**

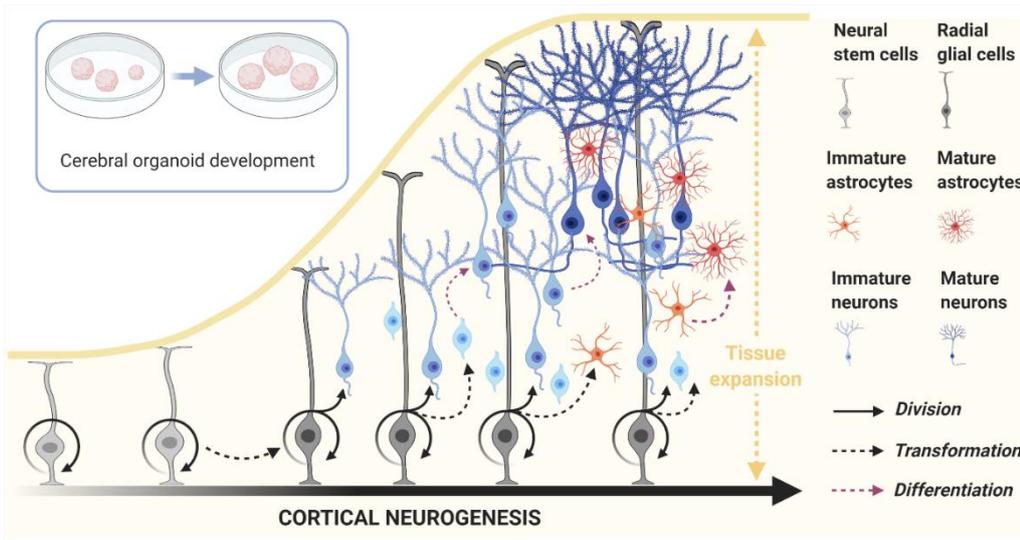
Quantification of 50 protein targets using SRM assay in a single cerebral organoid.

### **Co-authors:**

*Tereza Vanova, Dept. of Histology and Embryology, Faculty of Medicine, Masaryk University*  
*Dasa Bohaciakova, Dept. of Histology and Embryology, Faculty of Medicine, Masaryk University*  
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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



Cerebral organoid development. Cell types during neurogenesis.

Poster number: **LS-PA-074**

## **MASS SPECTROMETRY-BASED DIFFERENTIAL QUANTITATIVE PROTEOMICS OF INFLAMED MURINE AND HUMAN BLADDERS FOR THE IDENTIFICATION OF BIOMARKERS AND NOVEL TARGETS TO TREAT URINARY TRACT INFECTIONS**

Abstract ID: **853**

**Presenting author: Bente Siebles, Section for Mass Spectrometric Proteomics, Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany**

### **Introduction**

Urinary tract infections (UTIs) are common diseases, affecting over 150 million people each year worldwide. Potential consequences are severe symptoms including sepsis and frequent recurrences. The current treatment is based on antibiotics, which can alter the gut microbiome and result in the development of antibiotic-resistant bacteria. Hence, new, antibiotic-independent treatment strategies are needed. UTI-related research on human tissue is extremely limited, as biopsies are not commonly performed in routine diagnostics for UTIs. To our best knowledge, we present for the first time, proteomic data on human UTI samples as an attempt to identify new potential biomarkers and therapeutic targets. To overcome sample number limitations in the future, we additionally investigate the suitability of a mouse model infected with uropathogenic *E. coli* to mimic human UTIs.

### **Methods**

Our samples consisted of 12 bacterial-infected and healthy formalin-fixed mouse bladders as well as 20 inflamed and healthy formalin-fixed paraffin-embedded (FFPE) human bladders. LC-MS/MS-based bottom-up proteomics was performed on a nano-UPLC coupled to a quadrupole-ion-trap-orbitrap mass spectrometer using an in-house workflow for proteomic data processing and analysis.

### **Preliminary data (results)**

We identified significantly differential abundant proteins that could serve as potential UTIs biomarkers. Among those proteins, potential therapy target candidates for UTI patients were identified. Gene set enrichment analysis furthermore disclosed functional changes in UTI tissue. Following these results, a high resemblance between human and the murine UTI model was disclosed, proposing the used model as a potential way to overcome sample number limitations in UTI-related proteomic research in the future.

### **Please explain why your abstract is innovative for mass spectrometry?**

Mass spectrometry enables the identification of therapeutic targets and biomarkers for UTI patients and related mice models, towards the development of antibiotic independent therapy options.

### **Co-authors:**

*Hannah Voß, Section for Mass Spectrometric Proteomics, Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*  
*Olga Shevchuk, Department of Immunodynamics, Institute for Experimental Immunology and Imaging, Medical Research Centre, University Hospital Essen, Essen, Germany*  
*Jenny Dick, Department of Immunodynamics, Institute for Experimental Immunology and Imaging, Medical Research Centre, University Hospital Essen, Essen, Germany*  
*Sibylle von Vietinghoff, Nephrology Section, Medical Clinic 1, University Hospital Bonn, Rheinische Friedrich-Wilhelms University, Bonn, Germany*  
*Jessica Schmitz, Nephropathology Unit, Institute for Pathology, Hannover Medical School, Hannover, Germany*  
*Jan Hinrich Bräsen, Nephropathology Unit, Institute for Pathology, Hannover Medical School, Hannover, Germany*  
*Daniel Robert Engel, Department of Immunodynamics, Institute for Experimental Immunology and Imaging, Medical Research Centre, University Hospital Essen, Essen, Germany*  
*Christoph Krisp, Section for Mass Spectrometric Proteomics, Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*  
*Hartmut Schlüter, Section for Mass Spectrometric Proteomics, Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Poster number: **LS-PA-075**

## **SIMULTANEOUS DETECTION AND QUANTIFICATION OF ANGIOTENSIN I, II, 1-7 AND 1-9 BY LC-MS/MS IN HUMAN PLASMA**

Abstract ID: **858**

**Presenting author: Loreen Huyghebaert, CHU Liège**

### **Introduction**

Recent studies showed that angiotensin-converting enzyme 2 (ACE2) is used by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as a cellular entry receptor. SARS-CoV-2 causes downregulation of ACE2 leading to renin-angiotensin-aldosterone system (RAAS) major imbalance. This is an essential element of unfavorable evolution in patients with COVID-19. With lower level of ACE2, cleavage of Ang I and Ang II is decrease and therefore, Ang 1-7 and Ang 1-9 levels are decreased. The development of a quantitative method for these angiotensins is particularly interesting in the context of the prognosis/follow-up of patients with COVID-19.

### **Methods**

Plasma samples have been extracted by solid phase extraction with an OASIS MAX 96-well pelution plate, evaporated, reconstituted and then filtered.

Separation and quantification were achieved on a Nexera X2 UPLC (Shimadzu Corporation, Kyoto, Japan) coupled to a QT5500 mass spectrometer (Sciex, CA, USA) fitted with an IonDrive™ Turbo V ion source and using electrospray ionization in positive mode (ESI +). Chromatographic separation was performed on a Luna Omega® C18 100Å core-shell column (100 × 2,1 mm, 1.6 µm) from Phenomenex (Torrance, CA, USA). The mobile phase composition was water and ACN both containing 0,4 % FA.

### **Preliminary data (results)**

For each angiotensin, a Q1-scan experiment as well as a product ion experiment were performed in order to defined the transitions. Parameters compounds were obtained by MRM experiment while source parameters were optimized by post-column flow injection. Mobile phases were chosen based on a flow injection analysis.

Once the inlet and MS method were optimized, the sample preparation was developed. An melution MAX plate was selected to optimize the SPE in order to have a chemistry orthogonal to the one of the column. Recoveries rates ranged between 45% and 65% and no matrix effect was observed.

A calibration curve was realized in cow plasma with calibrators going from 6 to 5.10<sup>4</sup> pg/mL. At 24 pg/mL, the peaks were intense enough with a good and symmetrical peak shape.

### **Please explain why your abstract is innovative for mass spectrometry?**

Simultaneous detection and quantification of peptides relevant in clinical application by LC-MS/MS in human fluids.

### **Co-authors:**

*Justine Demeuse, University of Liège*

*Philippe Massonet, CHU Liège*

*Elodie Grifnée, CHU Liège*

*Stéphanie Peeters, CHU Liège*

*Caroline Le Goff, CHU Liège, University of Liège*

*Etienne Cavalier, CHU Liège, University of Liège*

Poster number: LS-PA-076

## IMPROVING THE QUANTIFICATION OF HEAVY ISOTOPE-LABELLED HISTONE SPECIES

Abstract ID: 875

**Presenting author: Thomas Mair, Center for Diagnostics, Institute of Clinical Chemistry and Laboratory Medicine, Medical Center Hamburg-Eppendorf (UKE)**

### Introduction

Acetylation is a crucial post-translational modification, particularly on histones, where reversible acetylation regulates a wide range of processes including gene expression. Recently, progress has been made in analyzing histone acetylation dynamics by the development of combined metabolic and chemical labelling (CoMetChem). However, accurate quantification of acetylated histone isotopologue species is still a challenge. The aim of this work was thus to investigate if chemical labelling of unmodified histone lysine residues using  $^{13}\text{C}_4\text{D}_6$ -acetic anhydride ( $^{13}\text{C}_4\text{D}_6$ -AA) takes place quantitatively, whether hydrogen-deuterium exchange (HDX) occurs under the experimental conditions of labelling and tryptic digest, and to which extent label impurities impact the quantification of acetylated histone isotopologue species. In this context, a method to correct for M-1 isotopes created by label impurities was developed.

### Methods

First, to investigate chemical labelling yields, histones were isolated, fractionated, derivatized by  $^{13}\text{C}_4\text{D}_6$ -AA, digested using trypsin, and analyzed by LC-MS/MS. In this context, the potential effect of a side reaction between  $^{13}\text{C}_4\text{D}_6$ -AA and dithiothreitol (DTT) was determined by performing the digest both with and without using DTT for protein denaturation.

Furthermore, potential occurrence of HDX was investigated by treating unmodified BSA lysine residues with  $^{13}\text{C}_4\text{D}_6$ -AA for various timespans followed by LC-MS/MS analysis.

Lastly, an R-script to correct for M-1 isotopes caused by label impurities was developed to improve the quantification of acetylated histone isotopologues.

### Preliminary data (results)

Regarding chemical labelling yields, it was discovered that some histones were highly derivatized (H3.1 (with/without DTT: 85.89%/99.18%), H3.3 (with/without DTT: 95.95%/90.64%), and H4 (with/without DTT: 99.13%/88.45%)), while derivatization yields of other histones (H1 (with/without DTT: 62.16%/62.44%), H2A (with/without DTT: 0.00%/0.00%), and H2B (with/without DTT: 47.54%/32.41%)) were comparatively lower. Low yields are caused by low sequence coverages due to the lack of detectable peptides generated by tryptic digest. DTT usage does not have a big impact on derivatization yields, but it generally increases sequence coverages.

Time-resolved quantitative analysis of acetylated BSA peptides revealed, first, that no HDX occurs under the conditions of derivatization and tryptic digestion, and second, that the M-1 isotopic peaks were caused by label impurities of  $^{13}\text{C}_4\text{D}_6$ -AA.

Subsequently, a method to correct for M-1 isotopes caused by label impurities was developed to accurately quantify monoisotopic peaks (M0) of acetylated histone isotopologues. It was used to determine half-lives, turnover rates, and acetylation/deacetylation reaction rates of acetylated isotopologue species with and without M-1 correction to investigate the effect of label impurity correction on the quantification of acetylation dynamics. For isotopologue species which did not overlap with M-1 isotopes of other species, differences in calculated dynamics were mostly within standard deviation. For isotopologue species where overlap with M-1 isotopes of other species occurred, it was shown that calculation of turnover rates and half-lives was considerably improved by M-1 correction.

### Please explain why your abstract is innovative for mass spectrometry?

This work gives insights on how to analyze chemical histone acetylation and how determination of acetylation dynamics can be improved by correcting for M-1 peaks caused by label impurities.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### **Co-authors:**

*Philipp Kobler, Department of Biochemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck*  
*Anna-Sophia Egger, Department of Biochemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck*

*Alienke van Pijkeren, Department of Biochemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Department of Analytical Biochemistry and Interfaculty Mass Spectrometry Center, Groningen Research Institute of Pharmacy, University of Groningen*

*Marcel Kwiatkowski, Department of Biochemistry and Center for Molecular Biosciences Innsbruck, University of Innsbruck*

Poster number: **LS-PA-077**

## **LONGITUDINAL PROTEOTYPING OF PLASMA FROM ACUTE COVID-19 PATIENTS USING PARALLEL MRM AND DIA METHODS**

Abstract ID: **886**

**Presenting author: Yassene Mohammed, Leiden University Medical Center, University of Victoria**

### **Introduction**

Exact mechanisms of acute COVID-19 disease are not fully understood. Although it is known to cause widespread changes in host immune, inflammatory and coagulation responses, there has been limited investigation in changes to the plasma proteome during the first weeks of hospitalization. Our objective was to compare plasma protein abundances in COVID-19 patients with healthy controls, and to determine COVID-19-associated longitudinal changes in protein levels during first two weeks of hospitalization. Secondly we investigated differences between survivors and non-survivors, and effects of being exposed to angiotensin receptor blockers (ARBs) and ACE-inhibitors (ACEi). We used quantitative targeted proteomics with internal standards for absolute quantification, and compared this to analysis of the same samples by a data independent acquisition approach.

### **Methods**

Samples were collected and processed within four hours upon admission from 46 confirmed COVID-19 patients, and on days 2, 4, 7, and 14; 33 males, 13 females; mean age 64.5 years (IQR 52-79). We used targeted proteomics with internal standards for 270 proteins, detecting 192 of which 172 were quantifiable in the range of sub-fmol/ $\mu$ l to sub-nano/ $\mu$ l. RPLC interfaced to a QQQ Agilent 6490 via a standard-flow ESI source operated in positive ion mode were used for MRM. For DIA, a library was created from pooled aliquots. RPLC EASY-nLC 1000 and Orbitrap Fusion Tribrid were used.

### **Preliminary data (results)**

The absolute quantitative proteomics method detected 192 proteins, of which 172 were quantified, while the DIA method detected levels for 204 proteins. 133 proteins were shared between the two methods and these showed excellent correlations with a mean Spearman's correlation per protein for all samples at 0.867 (median 0.872). Of all proteins quantified, 29 were significantly higher in abundance in acute COVID-19 and two proteins were significantly lower (BH p-value < 0.05 and two-fold change). Functional enrichment analysis of these 31 proteins showed them to be from acute inflammatory response, complement activation, regulation of inflammatory response, and regulation of protein activation cascade.

Longitudinal analysis showed distinct abundance profiles including: steady increase, rapid decrease followed by recovery, sudden decrease followed by an increase, and steady levels maintained over time. Analysis of these profiles revealed increased levels of multiple lipid associated functions. Complement activation, humoral immune response, and general protein activation cascade were associated with proteins showing a rapid decrease followed by recovery. Proteins associated with acute inflammatory response showed similar profile, but to a lesser extent. Significant difference between survivors and non-survivors was limited to S100A-9, S100-A12 and apolipoprotein A. Differences between ARB inhibitor users and non-users included increased complement factor B, Fructose-bisphosphate aldolase B, IgGFC-binding protein, and decreased Vascular cell adhesion protein. Two proteins differentiated in their abundance between ACE inhibitor users and non-users; Fructose-bisphosphate aldolase B and Protein S100-A9 both showed decreased abundance in the ACE users.

### **Please explain why your abstract is innovative for mass spectrometry?**

Longitudinal mass spectrometry-based proteomics study of acute COVID-19 patients and comparison of DIA to MRM on same samples.

### **Co-authors:**

*Yassene Mohammed, Leiden University Medical Center, University of Victoria*

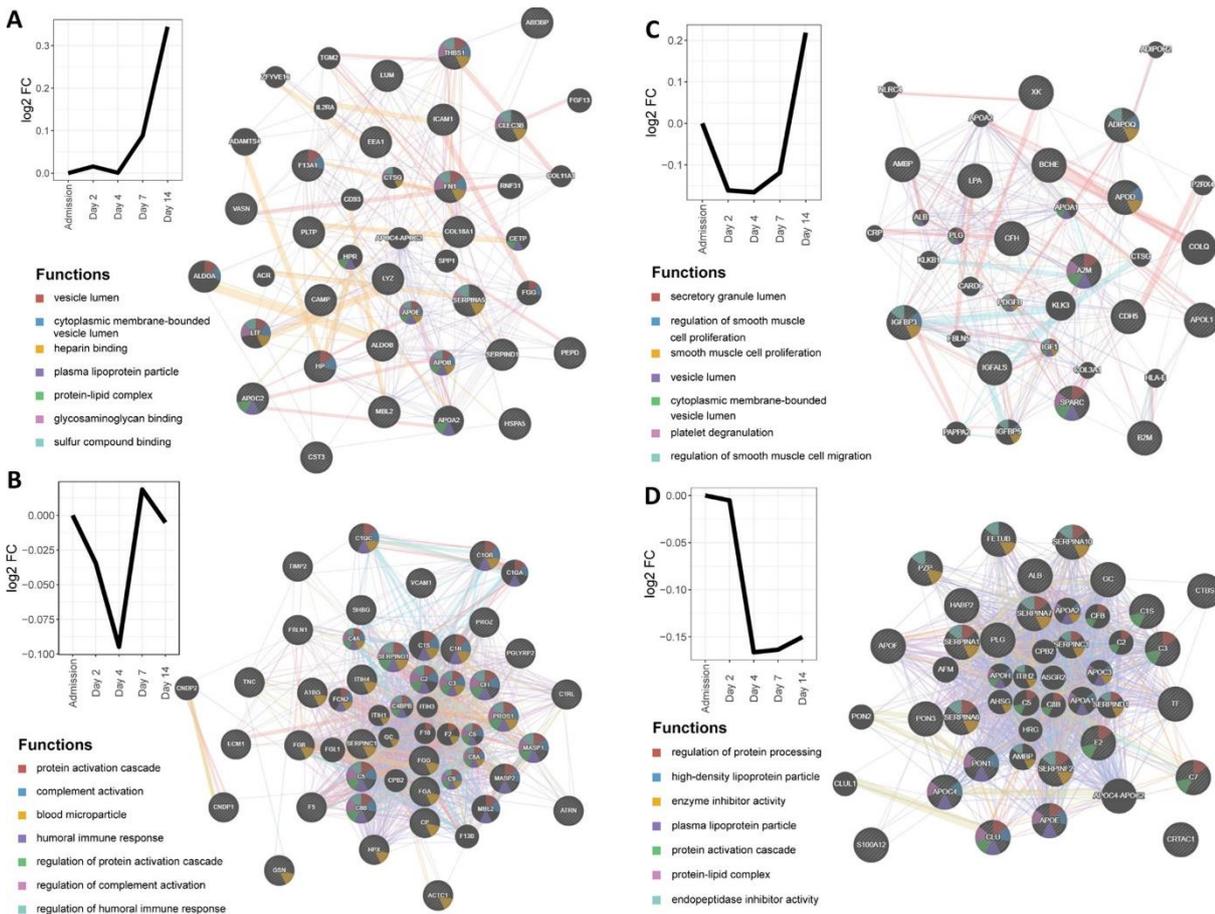
*David R. Goodlett, University of Victoria, University of Gdansk*

*Matthew Cheng, McGill University*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours

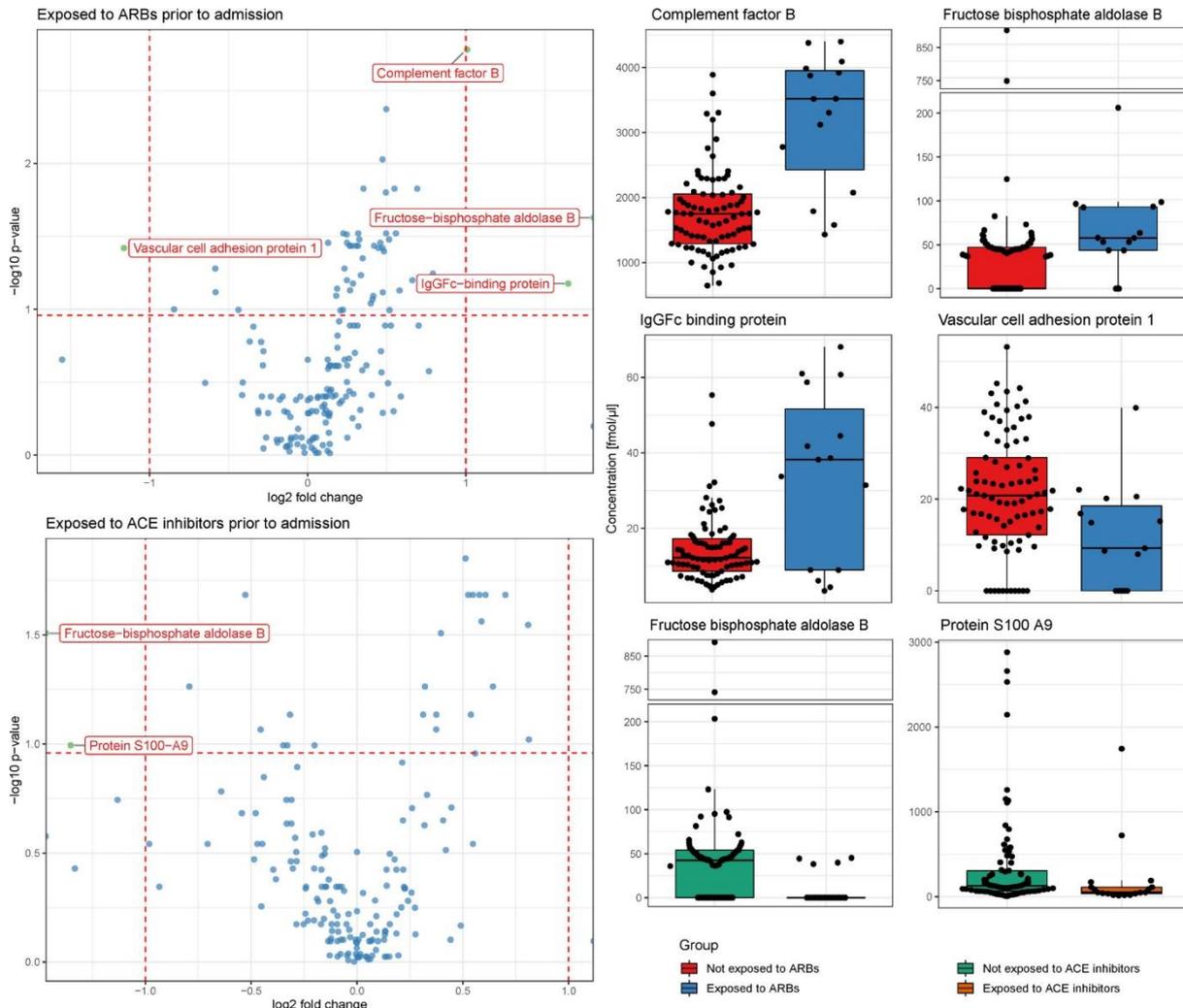
Donald C. Vinh, McGill University  
 Todd C. Lee, McGill University  
 Alison Mcgeer, Mt. Sinai Hospital, University of Toronto  
 David Sweet, Vancouver General Hospital, University of British Columbia  
 Karen Tran, Vancouver General Hospital, University of British Columbia  
 Terry Lee, University of British Columbia  
 Srinivas Murthy, University of British Columbia  
 John H. Boyd, University of British Columbia  
 Joel Singer, University of British Columbia  
 Keith R. Walley, University of British Columbia  
 David M. Patrick, University of British Columbia  
 Curtis Quan, McGill University  
 Sara Ismail, McGill University  
 Laetitia Amar, McGill University  
 Aditya Pal, McGill University  
 Rayhaan Bassawon, McGill University  
 Lara Fesdekjian, McGill University  
 Karine Gou, McGill University  
 Francois Lamontagne, University of Sherbrooke  
 John Marshall, St. Michael's Hospital  
 Greg Haljan, Surrey Memorial Hospital  
 Robert Fowler, Sunnybrook Health Sciences Centre  
 Brent W. Winston, University of Calgary  
 James A. Russell, University of British Columbia



Time course of protein levels in the COVID-19 patients.

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Protein levels in acute COVID-19 ARBs/ACEi-exposed patients.

Poster number: **LS-PA-078**

## QUANTIFICATION OF ASIALOGLYCOPROTEIN RECEPTOR SUBUNITS IN HBV INFECTED AND NON-INFECTED MICE LIVER USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

Abstract ID: **893**Presenting author: **Emmanuel Njumbe Ediage, Janssen Pharmaceutica**

### Introduction

The conjugation of siRNA to Triantennary N-acetylgalactosamine (Tris-GalNAc) has enabled highly selective, potent, and long-lasting knockdown of target genes and down regulation of target proteins in the liver. The expression level of asialoglycoprotein receptor (ASGPR) on hepatocytes is an important determinant driving the cellular pharmacokinetic (PK) and pharmacodynamic (PD) properties of GalNAc-siRNAs. Inclisiran, an GalNAc-siRNA, showed a 2-fold increase in plasma exposure ( $C_{max}$  and AUC) among participants with moderate hepatic impairment relative to those with normal hepatic function [1]. Hypothetically, an increase in plasma concentrations of an GalNAc-siRNA may imply reduced hepatic uptake due to potential downregulation of ASGPR expression. Qualitative assays for ASGPR have been reported but these techniques showed limited specificity and selectivity compared LC-MS/MS.

### Methods

A bottom-up proteomics-based hybrid LC-MS/MS method was set up for the quantification of ASGPR proteins in mouse liver and kidney tissues homogenized in lysis buffer at a 1:9 ratio. Calibration standards (prepared in dog liver homogenate) and tissue homogenates were subjected to immunoaffinity (IP) pulldown using anti-ASGPR1/2 antibodies. The IP purified proteins were trypsinized to generate at least two proteotypic peptides for each ASGPR subunit. The proteotypic peptides were chromatographically separated by gradient elution on a Peptide BEH C18 column and detected using electrospray MS in positive ion mode with at least two MRM transitions per proteotypic peptide.

### Preliminary data (results)

Calibration curves prepared in dog liver homogenate showed good linearity in the range of 0.1-20  $\mu$ g protein per gram tissue. The ratio of ASGPR1 to ASGPR2 was on average 1:2. The protein expression levels expressed per gram of mouse liver were 14.9–71.5  $\mu$ g/g of liver (equivalent of 0.27–1.32 nmol/g liver) for ASGPR1 and 29.7–132  $\mu$ g/gram of liver (equivalent of 0.85–3.79 nmol/g) for ASGPR2. Preliminary data (from very limited sample size) showed complete knockout of ASGPR2 and a 20-60-fold reduction of ASGPR1 expression on protein level in ASGPR2 knockout mice compared to wildtype. Furthermore, no traces of either ASGPR1 or ASGPR2 were detected in kidney homogenates, confirming tissue specific abundance of ASGPR proteins. The protein expression levels for both ASGPR1 and ASGPR2 was similar between the HBV infected and non-infected groups.

### Please explain why your abstract is innovative for mass spectrometry?

This is the first quantitative hybrid LC-MS/MS assay for the quantification of ASGPR in mouse liver and kidney using bottom-up proteomics approach

Poster number: **LS-PA-079**

## **PHARMACOLOGICAL INFLUENCE ON THE NEURONAL PROTEOME AS A PATHOPHYSIOLOGICAL TRIGGER FOR THE DEVELOPMENT OF MENTAL ILLNESS, ESPECIALLY DEPRESSION**

Abstract ID: **897**

**Presenting author: Sam Thilmany, Center for Preventive Doping Research – Institute of Biochemistry, German Sport University Cologne, Cologne, Germany, Federal Institute for Drugs and Medical Devices, Bonn, Germany**

### **Introduction**

Depression is a common disorder with a worldwide prevalence of 3.8 %, according to the World Health Organization (WHO). Little is known about the risk factors for the development of depression. However, it seems clear that it is multifactorial and requires a complex interaction of social, psychological, and biological factors. A possible influence by the intake of exogenous sex hormones (e.g., ethinylestradiol and levonorgestrel) has been controversially discussed for years. It is of high social relevance due to the popularity of hormonal contraceptives. Furthermore, this potential interaction would be a significant aspect of preventive doping education for professional athletes due to the misuse of sex hormones and sex hormone receptor ligands (e.g., selective androgen receptor modulators (SARMs)) for performance enhancement.

### **Methods**

We investigate the influence of different steroid hormones and steroid hormone derivatives by incubating neuronal cells with these substances and subsequently analyzing the proteome. For proteomic analysis, the trypsinized proteins of the different incubation experiments are each labeled with an isobaric marker and then pooled. Pre-fractionation is performed at high pH to reduce sample complexity. The individual fractions are then analyzed by nanoscale liquid chromatography coupled to an Orbitrap mass spectrometer. Subsequently, the differentially expressed proteins found in the data will be further contextualized with transcriptomics data and compared to known biomarkers of depression to elucidate possible relationships.

### **Preliminary data (results)**

In the initial stage of this research project, an optimal methodology is being developed. Literature research has shown that membrane and transmembrane proteins may be interesting biomarkers for mental disorders. Isolation of these proteins is usually done with strong detergents or high-salt buffers. However, these substances are incompatible with mass spectrometry, requiring careful sample preparation. In addition, these proteins are usually present in low concentrations, requiring optimized LC-MS analysis to maximize detection. We are currently testing different possibilities to optimize LC-MS analysis using standard trypsinized protein extracts by Promega (P/N V6951). Parameters such as the gradient length, the type of ionization (nESI, MnESI), and the column play an essential role. Our initial setup with a Waters Acquity UPLC BEH C18 column (P/N 186007485) is not optimal due to the long capillary after the column and the necessary connection to the emitter that leads to significant peak broadening. A new approach is using in-house manufactured columns with an integrated emitter, effectively eliminating any dead volume.

To select a suitable cell line for the experiments, we cultured several neuroblastoid cell lines (SH-SY5Y, Kelly, and SK-N-BE(2)) and the neuronal progenitor cell line ReNcell VM and isolated the RNA. The individual RNA isolates were analyzed by DNA microarray and can now be compared with isolates from different brain regions of healthy adult humans (obtained from TaKaRa and Amsbio). Here, the focus is on the genes that, according to the literature, may be potential biomarkers for mental disorders.

### **Please explain why your abstract is innovative for mass spectrometry?**

To the best of our knowledge, this is the first time that the influence of sex hormones on the neuronal proteome is investigated by means of mass spectrometry-based bottom-up proteomics.

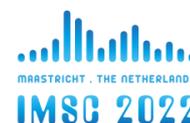
### **Co-authors:**

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours



*Andreas Thomas, Center for Preventive Doping Research – Institute of Biochemistry, German Sport University Cologne, Cologne, Germany*

*Matthias Vogel, Federal Institute for Drugs and Medical Devices, Bonn, Germany*

*Catharina Scholl, Federal Institute for Drugs and Medical Devices, Bonn, Germany*

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Poster number: **LS-PA-080**

## **GLOBAL ANALYSIS OF INTEGRATED ACYLOME PROFILING IN SEPSIS**

Abstract ID: **923**

**Presenting author: Annyae Na, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University**

### **Introduction**

In 2016, sepsis is one of the representative life-threatening diseases in the world that is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Despite the continuous progress in medicine, the specific pathogenesis mechanism of sepsis is still unclear. Recent studies have shown that a key strategy of many pathogens is to use posttranslational modification (PTMs) to modulate host factors critical for infection including inflammations and sepsis. However, their other important roles in mechanism of sepsis, have not been well characterized.

### **Methods**

In present study, we used Kacy-antibody bead including Kac, Ksu and Kmal to enrich highly sensitive immune-affinity purification and combine high-resolution LC-MS/MS to perform the first global lysine acylome analysis in CLP-surgery induced sepsis in mice, leading pathogenic mechanism of integrative Kacy and revealing their crosstalk of change in modification site.

### **Preliminary data (results)**

A total of 2230 site in 1,235 Kac proteins, 1886 site of 599 Ksu proteins and 455 site of 241 Kmal proteins were quantified and normalized by their proteins level. We focused on 379 sites in 219 up-regulated proteins as integrative PTM of Kac, Ksu, Kmal, according to decreased expression levels of sirtuin family as a deacetylase in CLP group. Importantly, KEGG pathway of integrative Kacy in 219 up-regulated proteins revealed three central metabolic pathways: glycolysis/gluconeogenesis, pyruvate metabolism and TCA cycle. The enzymatic analysis in TCA cycle pathway was performed to support bioinformatic results, and we also revealed the up-regulated protein site of Kacy modifications in CLP group.

### **Please explain why your abstract is innovative for mass spectrometry?**

These findings point to key pathogenic mechanism by integrative PTMs alteration in sepsis, thus provides an important foundation for in-depth study of the biological function of lysine acylation in sepsis.

### **Co-authors:**

*So Young Choi, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University*

*Jong-Sup Bae, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Kyungpook National University*

Poster number: **LS-PA-081**

## **PHOSPHOPROTEOMICS PROFILING OF BLADDER CANCER FOR THE DISCOVERY OF ANTI-METASTASIS PATHWAY**

Abstract ID: **988**

**Presenting author: Eunji Sung, College of Pharmacy, Kyungpook National University,**

### **Introduction**

Protein phosphorylation is the most essential post-translational modification that dominates signaling transduction, and it is involved in major regulatory mechanisms of cell signaling networks. Cancers characterized by these signaling pathways remain a major challenge in cancer therapy. The Warburg effect, a metabolic shift to aerobic glycolysis, is one of the potential causes of chemical resistance. By knock down genes associated with aerobic glycolysis, we investigated phosphorylated proteomic changes in bladder cancer.

### **Methods**

In this study, we performed quantitative proteomic analysis coupled with TiO<sub>2</sub> enrichment by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to investigate the differential phosphoproteome expression in J82 and PDK4-knockdown (KD) J82 cells.

### **Preliminary data (results)**

A total of 209 differentially phosphorylated proteins (DPPs) with 2,743 phosphosites were identified in PDK4 knockdown compared to controls. KEGG pathway network analysis identified five statistically significant signaling pathways, include Ribosome biogenesis, adherens junction, proteoglycans in cancer, bladder cancer and gap junction. According to GO analysis, these DPPs were involved in a variety of cellular components (CCs), biological processes (BPs), and molecule functions (MFs). The kinase network analysis identified kinase including EGFR, ERK and CDK and the substrate of these kinases could provide new ideas for signaling pathway involved in cancer growth.

### **Please explain why your abstract is innovative for mass spectrometry?**

Down substrates related to PDK4 have been discovered in bladder cancer, and it is assumed that these factors play a role in cancer metastasis.

### **Co-authors:**

*Eun Hye Lee, Regenerative Medicine, Kyungpook National University*

*Tae-Hwan Kim, Department of Urology, School of Medicine, Kyungpook National University*

*Yun-Sok Ha, Department of Urology, School of Medicine, Kyungpook National University*

Poster number: LS-PA-082

## QUANTIFICATION OF THERAPEUTIC MONOCLONAL ANTIBODIES IN HUMAN PLASMA BY MEASURING THE INTACT LIGHT CHAIN USING 2D-LC ORBITRAP HRMS

Abstract ID: 1004

Presenting author: Magnus Olin, Thermo Fisher Scientific

### Introduction

Quantification of monoclonal antibodies (t-mAbs) by liquid chromatography - tandem mass spectrometry (LC-MS/MS) is commonly done using a bottom-up approach targeting a 'signature' peptide. After sample purification, trypsin digestion is performed, and signature peptides are analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). This approach is laborious and sometimes it is not feasible due to a lack of unique signature peptides. Here we present a simpler middle-up quantification approach, where the t-mAb is quantified using high resolution mass spectrometry (HRMS) targeting the intact light chain of the t-mAb. The method is based on two-dimensional liquid chromatography in combination with high resolution mass spectrometry (2D-LC-HRMS). As a proof of principle, the method was validated for the fully human monoclonal antibody Adalimumab (ADM) in human plasma.

### Methods

Sample preparation was performed using Thermo Scientific™ Melon™ Gel Purification Kit followed by reduction of the disulfide bonds using TCEP. 20 µL sample was injected onto a 2D-LC-HRMS consisting of 2 Thermo Scientific™ Vanquish™ Horizon UHPLC System pumps, a valve, and a Thermo Scientific™ Q Exactive™ Orbitrap™ Mass Spectrometer. A heartcut was transferred from the first separation dimension (WCX) to the second separation dimension (RP) and the exact  $m/z$  of the charge states (+10, +11, +12) were monitored using t-SIM at 140000 resolution.

### Preliminary data (results)

The method performance was evaluated following the EMA guideline for bioanalytical method validation. Within-run and between-run precision and accuracy, linearity, LLOQ, selectivity, matrix effect, carry-over, and stability were evaluated, all found to be within acceptable limits.

The method was cross-validated to an LC-MS/MS method based on signature peptides by using remnant EDTA plasma samples from 37 patients on ADM therapy. The middle-up 2D-LC-HRMS method results were on average 1.5 times lower compared to the bottom-up signature peptide LC-MS/MS method. These differences were only seen in t-mAb treated patients and not in spiked plasma samples. An explanation for these findings may possibly be due to the formation of a charge variant of ADM in patients over time. 2D-LC-HRMS can quantify native ADM concentrations, whereas the bottom-up signature peptide LC-MS/MS method can only quantify total ADM concentrations. The bottom-up LC-MS/MS method targets a much smaller portion of the entire t-mAb, which has a lower probability of being modified.

Since it is unclear how active the modified ADM is during the treatment, the presented 2D-LC-HRMS method, measuring only the native ADM form, provides a conservative measure of active ADM concentrations in patients on ADM treatment.

### *For Research use Only. Not for Use in Diagnostic Procedures*

### Please explain why your abstract is innovative for mass spectrometry?

A method for the determination of adalimumab in human plasma using 2D-LC-HRAM MS is presented. Compared to traditional LC-MS/MS methods this method is faster, simpler, and possibly more accurate.

Chromatogram of Adalimumab at LLOQ overlaid blank human plasma

## Session: Proteomics: Top down

Poster number: **LS-PA-083**

### **MULTIDIMENSIONAL MULTIPLE-STAGE TOP-DOWN ANALYSIS OF INTACT NON-REDUCED ANTIBODIES IN THE OMNITRAP PLATFORM COUPLED TO ORBITRAP MASS SPECTROMETRY**

Abstract ID: **49**

**Presenting author: Athanasios Smyrnakis, Fasmatech Science & Technology**

#### **Introduction**

Multidimensional multiple-stage top-down experiments are performed in the Omnitrap platform for in-depth characterization of intact non-reduced antibodies (mAbs). Intact mAbs are activated and dissociated in MS<sub>n</sub> workflows involving resonance excitation in pulsed argon gas, external injection of variable energy electron and interactions with UV photons. High quality information-rich top down mass spectra are generated in MS<sub>3</sub> and MS<sub>4</sub> experiments enabled by operating the Omnitrap platform in ion-accumulation mode. The ion concentration in these experiments ranges across six orders of magnitude. Dissociation of intramolecular disulfide bonds is observed in MS<sub>4</sub> CID and MS<sub>4</sub> UV experiments with radical ions. Superior sequence coverage obtained in a top down MS<sub>4</sub> is demonstrated experimentally.

#### **Methods**

Experiments are performed on an Omnitrap connected to a Q Exactive Plus mass spectrometer (Thermo Fisher). Herceptin was diluted to 5 μM in water:acetonitrile in 50:50 ratio (v/v) with 0.1% formic acid. Static nanoelectrospray was performed using pulled borosilicate glass coated emitters. A tantalum disk was used to inject 1 μA of electron current into the ion trap. Electron capture dissociation (ECD) was performed with near 0 eV electrons. UV light produced by a deuterium lamp was focused by a MgF<sub>2</sub> lens and an electro-mechanical shutter was installed to integrate photo-dissociation in MS<sub>n</sub> workflows.

#### **Preliminary data (results)**

The extreme versatility of the Omnitrap technology is highlighted by a new set of MS<sub>3</sub> and MS<sub>4</sub> experimental workflows developed specifically for the analysis of intact mAbs. Integration of a multitude of ion activation methods with the desired order enables new levels in protein characterization to be accomplished. It is shown that resonance excitation CID leads to the dissociation of intermolecular disulfide bonds connecting the heavy and light chains of Herceptin, while the effect is observed under both denatured and native electrospray ionization conditions. In addition, resonance excitation CID produces the least congested spectrum where high intensity dissociation products can be isolated for subsequent analysis. A ten-fold increase in the intensity of Herceptin CID fragments is accomplished by operating the Omnitrap platform in ion-accumulation mode to counterbalance the effect of signal partitioning into an excessive number of dissociation pathways and to enable MS<sub>3</sub> and MS<sub>4</sub> experiments to be performed with high efficiency. MS<sub>3</sub> ECD and MS<sub>4</sub> CID experiments are performed with Herceptin light chain ions. Dissociation of intramolecular SS bonds is observed only at the MS<sub>4</sub> level, raising sequence coverage to >85%. The complex fragmentation patterns originating from the dissociation of intermolecular and intramolecular SS bonds are carefully identified and highlighted. Heavy chain MS<sub>2</sub> CID dissociation products are subjected to MS<sub>4</sub> CID and MS<sub>4</sub> VUV analysis and the sequence coverage obtained for N-terminus fragments is >50%. All spectra are processed manually in PeakFinder, a new software developed specifically to accelerate manual processing of heavily congested mass spectra.

#### **Please explain why your abstract is innovative for mass spectrometry?**

MS<sub>4</sub> top-down workflows with ion accumulation applied to intact mAbs and involving collisions with buffer gas molecules and interactions with electrons and UV photons.

#### **Co-authors:**

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*Roman Zubarev, Karolinska Institutet*

Poster number: **LS-PA-084**

## **TISSUE-BASED PROTEOMIC PROFILING IN HYPERPLASIA AND ENDOMETRIAL CANCER PATIENTS**

Abstract ID: **64**

**Presenting author: Hicham Benabdelkamel, Proteomics Resource Unit, Obesity Research Center, College of Medicine, King Saud University, P.O. Box 2925 (98), Riyadh 11461, Saudi Arabia**

### **Introduction**

Cancers arising from the uterus are among the most prevalent gynecological malignancies affecting women. Endometrial cancer (EC) is the most common among this group. The present study was aimed at tissue-based proteomic profiling analysis in endometrial cancer patients, hyperplasia, and control patients.

### **Methods**

The present study used a conventional 2D in Gel electrophoresis followed by a mass spectrometry approach with bioinformatics including a network pathway analysis pipeline to identify differentially expressed proteins and associated metabolic pathways between the study groups. Thirty-six patients (12 Endometrial Cancer, 12 Hyperplasia, and 12 controls) were enrolled. The mean age of study participants was 46-75 years. Baseline characteristics and laboratory evaluation were carried out (Figure 1).

### **Preliminary data (results)**

Eighty-seven proteins were found with statistically significant changes in abundance between the study groups. Among the 87 proteins identified, 53 were significantly differentially regulated (28 up-regulated, 25 down-regulated) in tissue samples of EC patients compared to Control (Ctrl). Further, 26 proteins were significantly dysregulated (8 up-regulated, 18 down-regulated) in tissue samples of hyperplasia (HY) patients compared to Ctrl. Furthermore, 32 proteins were significantly differentially regulated (19 up-regulated, 13 down-regulated) in tissue samples of EC patients compared to HY. The proteins identified in our study are known to regulate cellular processes (36%), followed by biological regulation (16%). Ingenuity pathway analysis (IPA) found that these differentially expressed proteins between EC and HY are linked to AKT, ACTA2, and other signaling pathways.

Number of biomarkers candidates in blood or tissue for EC detection have been reported. Unfortunately, none of them have been translated into routine clinical practice. Our current work has enhanced knowledge for discovering novel biomarkers in EC patients.

### **Please explain why your abstract is innovative for mass spectrometry?**

In our data, positional biomarkers were identified by proteomics approaches (2D-DIGE and mass spectrometry) and might be used for diagnosis, prognosis and as therapeutic target in Endometrial cancer EC patients.

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**POSTER SESSION A**

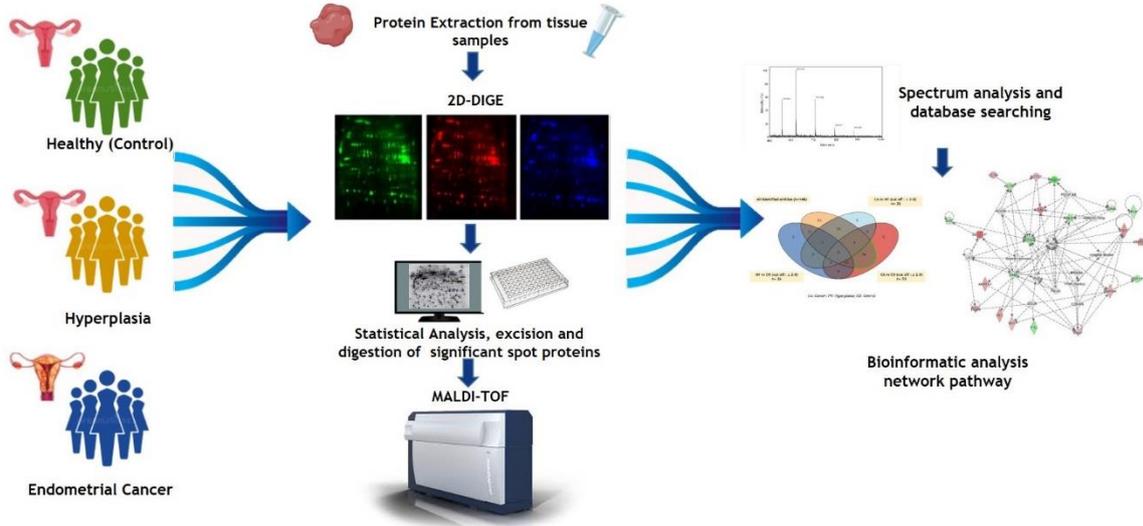
Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

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Workflow of proteomics analysis of the endometrial cancer tissue.

Poster number: LS-PA-085

## PERSONALIZED LONGITUDINAL MONITORING OF SERUM IGG1 AND IGA1 REPERTOIRES IN SEVERE COVID-19 PATIENTS PROVIDES A VIEW AT PATIENT SPECIFIC IMMUNE RESPONSES

Abstract ID: 87

**Presenting author: Max Hoek, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Padualaan 8, 3584 CH, Utrecht, The Netherlands, Netherlands Proteomics Center, Padualaan 8, 3584 CH, Utrecht, The Netherlands**

### Introduction

Recently, we introduced sensitive methods to monitor serum IgG1 and IgA1 repertoires and showed that these repertoires are simple, albeit unique for each individual. The recorded Ig profiles provide a qualitative and quantitative measure of the 50-500 most abundant antibodies in the serum of the donor. We recorded the IgG1 and IgA1 repertoires from 17 individuals, admitted to intensive care due to COVID-19. Samples were collected shortly after admission and at two later time points. These patients received various (different) treatments including transfusions and antibody therapies. By monitoring both the IgG1 and IgA1 profiles in these 17 patients, cumulatively leading to over 100 Ig profiles, we observed striking unique features and responses for each patient.

### Methods

First, the total IgG1 and IgA1 levels in each donor diverge notably from 0.5 to 14 mg/mL for IgG1 and 0.06 to 4.5 mg/mL for IgA1. The ratio between IgG1 and IgA1 do not necessarily correlate per donor and can vary immensely, with several donors having higher IgA1 levels than IgG1. Monitoring longitudinally and comparing between and within donors, the Ig repertoires reveal that they are unique per individual but do correlate very well when evaluated over time in a single donor.

### Preliminary data (results)

Still, during disease development and in response to various treatments, we observe very substantial changes in either IgG1 or IgA1 repertoires, and sometimes in both. In some donors, we observe and monitor the treatment with Tocilizumab, a recombinant IgG1, which appears as an abundant clone in the IgG1 repertoires of several donors. Our data provide a direct measurement of the serum concentration of this therapeutic molecule and we can monitor how its concentration changes over time and relates to the concentration of endogenous clones in the IgG1 repertoire. In some donors, the recombinant administered Tocilizumab is by far the most abundant IgG1 clone detected in the repertoire. We argue that by knowing this beforehand the administered doses might have been adjusted. Some donors respond by producing a single very abundant IgG1 or IgA1 clone over the course of hospitalization. Top-down *de novo* sequencing of these abundant clones revealed the most likely V-gene alleles of these clones, which are suggested to be COVID neutralizing alleles.

Overall, our longitudinal monitoring of the serum and IgG1 and IgA1 repertoires of individual donors over time reveals that serum immunoglobulin features and antibody responses are personalized traits of each patient and may also be heavily affected by the distinct treatment administered to each patient while hospitalized.

### Please explain why your abstract is innovative for mass spectrometry?

The impact of these findings argues for a more personalized approach in patient's diagnostics, both in serum proteomics as well as in monitoring immune responses.

### Co-authors:

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## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

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Poster number: LS-PA-086

## MANIPULATING CHARGE STATE AND CHARGE SITES TO IMPROVE TOP-DOWN FRAGMENTATION

Abstract ID: 98

**Presenting author: Tanja Habeck, Clemens-Schöpf-Institute, Department of Chemistry, Technical University of Darmstadt**

### Introduction

Electrospray ionization (ESI) is a key enabling technology in protein mass spectrometry, and the presence of multiple charges is especially useful for dissociation. Using supercharging reagents to manipulate charge states in protein ESI is a common strategy to enhance fragmentation efficiency. Supercharging has been extensively studied in the past; however, manipulation of protonation sites without changing the net charge state has only emerged very recently as an alternative approach to improve fragment yield in top-down MS. Furthermore, there are few in-depth studies that systematically link physicochemical properties of (co-)solvents to selective protomer formation, especially at the level of intact proteins. The existence of these protomer species has been described before and also distinct differences in the fragmentation behavior were observed.

### Methods

In this study, we used a Waters Synapt XS ion mobility – mass spectrometer with electron-transfer and collision-induced dissociation capabilities. Protein ions were generated with nano-ESI and injected with direct infusion. To examine the effect of different charge locations under both denaturing and near-native conditions, co-solvents were added in low concentrations (down to around 1%) to avoid inducing conformational changes in solution.

### Preliminary data (results)

We performed a systematic screening of co-solvents with different physicochemical properties (dielectric constants, boiling points, etc.) including propylene carbonate, sulfolane, and dimethyl sulfoxide. We added these to both native and denaturing protein solutions and were able to shift the ESI protonation sites of a range of model proteins. This resulted in a significantly different fragmentation pattern, enhancing the total fragment yield and sequence coverage. Ion mobility was used to investigate any conformational effects. Moreover, electron transfer dissociation was used to determine the charge sites in the presence and absence of solvent additives. Overall, this study helps to correlate the mechanism of charge manipulating chemicals in protein ESI and their effects on fragmentation and conformational characteristics. This is beneficial for classical top-down protein analysis through achieving higher cleavage coverage and combines this with insights into conformation-sensitive analysis techniques such as ion mobility spectrometry and electron-based fragmentation.

### Please explain why your abstract is innovative for mass spectrometry?

Physicochemical properties of charge modifiers were linked to selective protomer formation in order to expand the insights of a top-down MS experiment.

### Co-authors:

*Kevin Kretschmer, Clemens-Schöpf-Institute, Department of Chemistry, Technical University of Darmstadt*

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Poster number: **LS-PA-087**

## **NATIVE LESA MASS SPECTROMETRY OF INTACT PROTEINS AND PROTEIN COMPLEXES DIRECTLY FROM LIVING BACTERIAL COLONIES**

Abstract ID: **152**

**Presenting author: Yuying Du, University of Birmingham**

### **Introduction**

We have previously confirmed LESA MS for the analysis of denatured intact proteins directly from living colonies of bacteria. In order to breach the peptidoglycan cell wall and outer membrane, the use of denaturing solvents is necessary. While this approach enables analysis of intact proteins, those proteins are unfolded and structural information is lost. By coupling LESA MS with electroporation which can rupture the thick cell wall with minimal heating, it is possible to analyse intact proteins directly from yeast. Here, we have combined electroporation with LESA MS using native-like solvents to perform native MS of proteins directly from bacteria.

### **Methods**

Colonies were grown on LB agar and subjected to electroporation using a home-built device. Electroporation parameters were: voltage applied = 3 kV, number of pulses = 35, pulse length = 20us. LESA was performed using a TriVersa NanoMate (Advion) with a solvent comprising 200 mM ammonium acetate. Mass spectrometry experiments were performed using an Orbitrap Eclipse (Thermo Fisher Scientific). Data were acquired in the 900-4000 m/z range at a resolution of 120000 at m/z 400. HCD was performed using 5-40% normalised collision energy. MS/MS spectra were searched using the ProSight software.

### **Preliminary data (results)**

Proteins from E.coli K12 were detected using native-like solvents by LESA MS. Although the same proteins were detected both with and without electroporation, a much higher mass spectral reproducibility was achieved with electroporation. The success rate for observing proteins with electroporation was 77%, significantly higher than the 14% without electroporation. A total of 25 proteins were observed following native LESA MS. Among them, acyl carrier protein was detected with a range of different post-translational modification. Thirteen proteins were identified by top-down mass spectrometry of which 7 were previously undetected including Csp E, YibT, HPr, PSiF, YgiW, Antigen 43 alpha chain and superoxide dismutase. Protein antigen 43 alpha chain (~49.8 kDa) is the highest molecular weight protein detected following LESA of bacterial colonies so far. Most significantly, three multimeric proteins were identified – the HDE A, HDE B and superoxide dismutase homodimers. The superoxide dismutase dimer was observed with 2 Mn<sup>2+</sup> ions bound. Our results confirm that electroporation followed by LESA MS using ammonium acetate is a suitable approach for direct native mass spectrometry of bacteria.

### **Please explain why your abstract is innovative for mass spectrometry?**

This work is the first demonstration of native LESA MS of intact proteins and protein complexes in living bacterial colonies.

### **Co-authors:**

*Emma Sisley, University of Birmingham*  
*Oliver Hale, University of Birmingham*  
*Robin May, University of Birmingham*  
*Helen Cooper, University of Birmingham*

Poster number: **LS-PA-088**

## **UTILIZATION OF FAST PHOTO-OXIDATION OF PROTEINS AND TOP DOWN MASS SPECTROMETRY FOR STRUCTURAL CHARACTERIZATION OF PROTEINS**

Abstract ID: **197**

**Presenting author: Petr Novak, Institute of Microbiology of the Czech Academy of Sciences, Vestec, Czech Republic**

### **Introduction**

Protein footprinting coupled to mass spectrometry is commonly applied for protein structural studies, providing information on protein conformations and dynamics. Traditional mass spectrometry approaches for structural elucidation include hydrogen deuterium exchange, chemical cross-linking, ion mobility and covalent labeling. Among these, hydroxyl radicals are a perspective probe for the fast protein footprinting as introduced two decades ago. There are different methods to generate them including Fenton reaction, radiolysis of water and fast photochemical oxidation of proteins (FPOP); Bottom up mass spectrometry is the dominant method to identify modified amino acids and determine the solvent accessible area of proteins. Here, we present utilization of the Top-down sequencing for localization of modified residues within the protein structure.

### **Methods**

An excimer laser (248nm KrF) was used to generate hydroxyl radicals in a quench flow set-up. The extent of the hydroxyl radical incorporation was checked by high-resolution mass spectrometry (solarix XR 15T, Bruker Daltonics). When a significant oxidation of protein was observed, intact, singly and doubly modified protein ions were isolated in quadrupole and the ions of interest were fragmented by a broad repertoire of techniques. CID and ETD were performed in the hexapole, while ECD and IRMPD in the ICR cell. Ms2links algorithm was used for annotation, and the lab-built software for calculation of the extent of modification.

### **Preliminary data (results)**

Careful analysis of Ubiquitin fragment spectra revealed the sequence information at residue level and unambiguously identified the sites of oxidation. Different ion series provided complementary information for the structural characterization of protein and improved the spatial resolution of the presented method. Subsequently, CID and ECD fragment ion signals were utilized to calculate the extent of oxidation per amino acid. The extent of oxidation of a given sequence ion was defined as the ratio of the oxidized sequence ion intensity to the sum of the intensity of the modified and unmodified sequence ion ( $\text{modified} / (\text{unmodified} + \text{modified})$ ). Only side chains of solvent accessible reactive amino acid residues were found oxidized by hydroxyl radicals on Ubiquitin molecule. Experiments were performed in triplicates and standard deviation was determined to be less than 2%. Once the top-down based method was developed using Ubiquitin, it was successfully applied to FOXO4, a protein twice as large, and on apo and holo forms of Myoglobin. These proteins were labeled by hydroxyl radicals, intact and singly oxidized products were isolated and dissociated using CID and ECD, and extent of modification was calculated. Detail quantification of modifications obtained from fragment spectra allowed determining which amino acids are more exposed to the solvent when the heme (in case of myoglobin) or DNA (in case of FOXO4) is removed. The results of top-down analysis were compared to bottom-up data. The top-down and bottom-up data were found to be in a good agreement.

### **Please explain why your abstract is innovative for mass spectrometry?**

Addressing the benefits/limits of different dissociation techniques for Top down analysis of FPOP protein samples.

### **Co-authors:**

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Poster number: **LS-PA-089**

## **THERE BE DRAGONS: THE DANGERS ASSOCIATED WITH ASSIGNING INTERNAL FRAGMENT IONS OF PROTEINS**

Abstract ID: **207**

**Presenting author: David PA Kilgour, Nottingham Trent University**

### **Introduction**

There is a great interest in the potential for internal fragments, generated during top-down tandem mass spectrometry of intact proteins, to provide information inside gaps in terminal fragment sequence coverage. But, there are many risks associated with attempting to make use of these internal fragment assignments for samples that are either potentially impure or are not already very well characterised, and, as there are so many more possible internal fragments, the value in the assignments appears to be orders of magnitude lower than for terminal fragments – even for high-resolution data. Additionally, there are differences between the various nomenclatures used to describe internal fragment ions, and this inconsistency makes it harder for us, as a community, to discuss the issues, as this potentially exciting area develops.

### **Methods**

We have investigated the internal fragment ions produced from top-down experiments on both FT-ICR MS and Orbitrap instruments, assigned using our in-house developed software tool, AutoSeequer, part of the AutoVectis Suite. We have then compared these assignments to those produced by other software.

We have also repeated the same assignment process using spurious protein sequences to demonstrate the issues that can result.

### **Preliminary data (results)**

We describe how both terminal and internal fragment ions are assigned in AutoVectis (full distribution fit and not deconvolved) versus other packages (e.g. Peak-by-Peak and ClipMS and others). We will demonstrate the potentially large numbers of problematic internal fragment assignments that will be seen in top-down spectra: e.g. peaks that can be assigned to both terminal and internal fragments, to multiple different internal fragments from the same protein or to internal fragments from different proteins or proteoforms. We also demonstrate some new machine learning algorithms (Cookie Cutter and Prosaic); these two new algorithms intelligently retrain themselves on your data to help remove problematic assignments; because the average mass errors or the combination of the mass errors coupled to the signal-to-noise ratios are statistically anomalous.

### **Please explain why your abstract is innovative for mass spectrometry?**

Novel confidence metric algorithms for filtering top-down internal fragment assignments. Exposition of risks associated with use of internal fragments in top-down characterisation.

### **Co-authors:**

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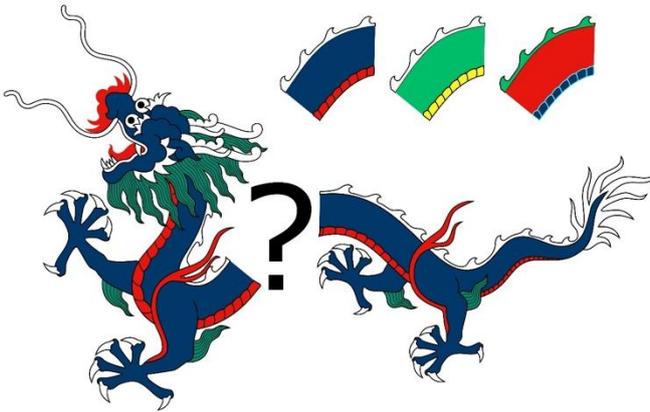
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*Logan Mackay, University of Edinburgh*

*Luca Fornelli, University of Oklahoma*

POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



? D R A G O N ✓  
 ? D R A R O N ✓  
 ? D R E L O N ✓

Internal fragments may match others, illustrated by the dragon analogy.

Internal fragments - assigned to the same peak list

	1	M	Q	I	F	V	K	L	L	T	G	K	T	I	T	L	L	E	V	E	S	S	57
True	21	D	T	I	D	N	V	K	A	K	I	Q	D	K	E	G	I	P	P	D	Q		37
Sequence	41	Q	R	L	I	F	A	G	K	Q	L	E	D	G	R	T	L	A	D	Y	N		17
	61	I	Q	K	E	S	T	L	H	L	V	L	R	L	R	G	G						1
	1	M	Q	I	F	V	K	L	L	T	G	K	T	I	T	L	L	E	V	E	S	S	57
False	21	D	K	F	K	H	L	K	T	E	A	E	M	K	A	S	E	D	L	K	K		37
Sequence	41	H	G	T	V	V	L	T	A	L	G	G	I	L	K	K	K	G	D	L	Y	N	17
	61	I	Q	K	E	S	T	L	H	L	V	L	R	L	R	G	G						1

Sequence coverage for the **False** sequence, based on internal fragments, is almost identical

What extra confidence do internal fragments really provide?

Poster number: **LS-PA-090**

## SQUEEZE EVERY DROP

Abstract ID: **289**

**Presenting author: Bruno Bellina, Medicines Discovery Catapult**

### Introduction

It is finally 2022 and all the technological advances have made mass spectrometry (MS) better, faster, and stronger. With high throughput mass spectrometry (HTMS) method reaching sub-second per sample, it is now capable of driving forward drug discovery programmes from biochemical screening to *in vivo* work. MS has become a plug & play platform, easy-to-use, robust that delivers high-content and high-quality data.

### Methods

In order to keep-up with the HT aspect, we need to be innovative from the sample collection stage up to the final MS-ready plate. There are 3 key points that we consider being essential: 1-reduce costs, time and wastes, 2-using standardised equipment and of course 3-the ease of use. We will be presenting novel workflows focusing on the sample preparation for the analysis of human plasma and serum using proteomics methods.

### Preliminary data (results)

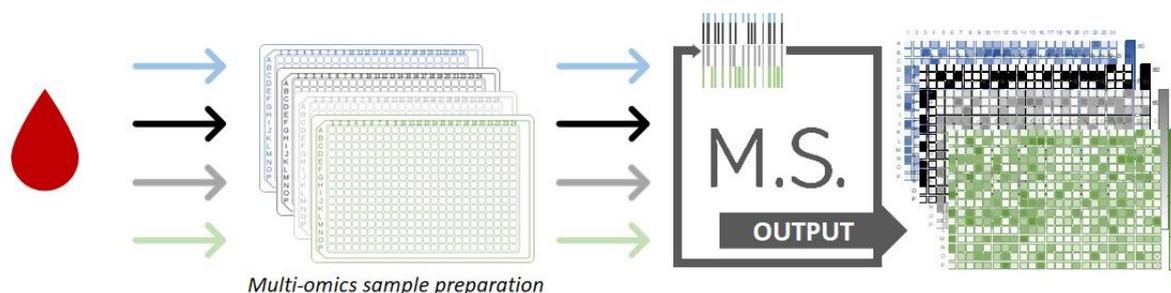
We have built miniaturised and automated protocols for microsampled plasma analysis (>500 nl). Using well-plate format and contactless dispensing, we have increased the throughput, drastically reduced the cost/sample without compromising on the data quality and richness. We will also present how this method can be transferred to another type of biological sample from cell lysates to tissues or organs but also to a single protein analysis.

### Please explain why your abstract is innovative for mass spectrometry?

We have developed a novel miniaturised workflow for the preparation of human sample (plasma, serum, tissue, etc..) that is compatible with high throughput mass spectrometry.

### Co-authors:

*Martin Bachman, Medicines Discovery Catapult*



Poster number: LS-PA-091

## ENABLING THE HIGH-THROUGHPUT ANALYSIS OF LARGER PROTEOFORMS FROM MAMMALIAN CELLS USING A REFINED TARGETED PROTON TRANSFER CHARGE REDUCTION (TPTCR) DATA ACQUISITION STRATEGY

Abstract ID: 326

Presenting author: Luca Fornelli, University of Oklahoma

### Introduction

Traditionally, top-down proteomics has been applied only to the analysis of mixtures of proteins <30 kDa. Using a modified Tribrid Orbitrap mass spectrometer of previous generation (Orbitrap Fusion Lumos), we demonstrated that the spectral congestion that characterizes broadband mass spectra of mixtures of 30-60 kDa bacterial proteoforms could be overcome through a new data acquisition method termed targeted proton transfer charge reduction (tPTCR). This method combines gas-phase fractionation (GPF) and proton transfer charge reduction (PTCR) to interrogate small portions of the mass-over-charge space and generates new, deprotonated charge state envelopes starting from highly-charged electrosprayed protein cations. Using the latest generation of PTCR-enabled tribrid Orbitrap instruments we can now expand the mass limit for the characterization of post-translationally modified mammalian proteoforms up to 80 kDa.

### Methods

Whole protein extracts from HEK293 cells and various human cancer cell lines were fractionated based on molecular weight (MW) by electrophoretic techniques and by liquid chromatography. High MW fractions were analyzed on both a PTCR-enabled Orbitrap Eclipse and on a modified Orbitrap Tribrid instrument (Thermo Scientific). The tPTCR method was applied using 2-4 quadrupole-selected narrow  $m/z$  windows (typically 1.5  $m/z$  unit-wide) in a single liquid chromatography – mass spectrometry (LC-MS) experiment, depending on the protein MW. Gas-phase sequencing was obtained using multiple ion activation techniques available on the Orbitrap Tribrid instruments. Data analysis was performed using ProSight PD.

### Preliminary data (results)

Mammalian protein mixtures >30 kDa demonstrated an incredible degree of complexity compared to *P. aeruginosa* proteins. Even by reducing the  $m/z$  region of the spectrum subjected to PTCR to  $\leq 1$   $m/z$  unit, the majority of resulting spectra showed the contemporary presence of multiple proteoforms. While ProSight PD can handle the presence of chimeric spectra by running distinct database searches for each deconvoluted precursor mass, we applied extensive pre-fractionation through high-resolution GELFrEE or PEPPI-MS and ion exchange chromatography prior to reversed-phase LC on-line coupled to MS.

For protein fraction >50 kDa, we attempted a modified tPTCR data acquisition strategy where PTCR was applied not only for precursor detection but also for reducing signal overlap in fragmentation spectra.

The Orbitrap Eclipse mass spectrometer allowed a substantial reduction in the number of microscans required for the acquisition of both intact (at low resolving power) and fragmentation (at high resolving power) mass spectra compared to the originally employed Orbitrap Fusion Lumos. This allowed for up to 4 distinct  $m/z$  regions to be investigated in a single LC-MS experiment, with that reducing the total number of LC-MS runs to be performed on each sample in order to analyze the required  $m/z$  window (about 25  $m/z$  unit in total). To further increase the signal-to-noise in PTCR spectra of intact proteoforms up to 80 kDa we are now applying tPTCR on a modified Orbitrap Tribrid instrument with improved ion transmission to the Orbitrap mass analyzer and capable of faster data acquisition cycles.

### Please explain why your abstract is innovative for mass spectrometry?

Ion-ion reactions in the gas-phase and gas-phase fractionations are utilized on state-of-the-art Tribrid Orbitrap mass spectrometers to obtain high-throughput analysis of whole proteoforms >50 kDa.

### Co-authors:

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Jake Kline, University of Oklahoma*  
*Romain Huguet, Thermo Scientific*  
*Christopher Mullen, Thermo Scientific*  
*Graeme McAlister, Thermo Scientific*  
*John Syka, Thermo Scientific*  
*Michael Goodwin, Thermo Scientific*  
*Xiao Wang, Thermo Scientific*  
*Raman Mathur, Thermo Scientific*  
*David Bergen, Thermo Scientific*  
*Jesse Canterbury, Thermo Scientific*  
*Mike Senko, Thermo Scientific*  
*Kenneth Durbin, Proteinaceous Inc*

Poster number: **LS-PA-092**

## **DEVELOPMENT AND OPTIMIZATION OF ON-TISSUE DIGESTION PROTOCOLS AS A TOOL TO INCREASE THE NUMBER AND SEQUENCE COVERAGE OF TISSUE PROTEINS**

Abstract ID: **538**

**Presenting author: Julia T. Kulpa, The Maastricht MultiModal Molecular Imaging (M4I) institute, Division of Imaging Mass Spectrometry (IMS), Maastricht University, 6229 ER Maastricht, The Netherlands**

### **Introduction**

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is a technique used for the investigation of spatial distribution of molecules in tissues. Numerous enzymes have been used in published proteomics protocols, however, when it comes to the MALDI MSI focus was primarily on the trypsin on-tissue digestion. This is due to many challenges encountered during on-slide digestions. The use of a metalloendopeptidase LysN has shown to provide datasets complementary to the one acquired with trypsin which leads to the increase in the number of protein identifications. Alternatively, enzymes such as AspN, GluC, LysC, or chymotrypsin can be used to improve the sequence coverage. However, the use of aforementioned enzymes has not yet been reported in published on-slide digestion protocols.

### **Methods**

Here, we present a developed protocols for on-tissue digestion and subsequent matrix application protocols. Analyzed samples include mouse spleen, kidney, and brain tissues. Enzymes were applied with the HTX M3+ sprayer (HTX Technologies LLC). Afterwards, CHCA and DHB matrices were applied with either HTX M3+ sprayer or HTX Sublimator (HTX Technologies LLC). All of MALDI MSI measurements were done in positive mode on RapifleX and timsTOF (Bruker). While the Orbitrap QExactive HF (Thermo Fischer Scientific) was used for high mass accuracy measurements.

### **Preliminary data (results)**

First, we present novel protocols for on-tissue digestion for enzymes such as LysN and  $\alpha$ -chymotrypsin. Additionally, the existing on-slide digestion protocols such as LysC and trypsin were optimized. Our results show that combining data acquired from an on-tissue digestion with two different complementary enzymes increases the total number of identified proteins. Furthermore, we demonstrate that serial on-tissue enzymatic digestion improves the certainty of protein identification. Finally, we show that the choice of matrix and matrix application method are both important factors influencing the number of identified proteins from tissue in MALDI and MALDI-2 MSI experiments.

### **Please explain why your abstract is innovative for mass spectrometry?**

Development of novel on-tissue enzyme digestion protocols that led to an increase in the sequence coverage and number of identified tissue proteins in MALDI MSI experiments

### **Co-authors:**

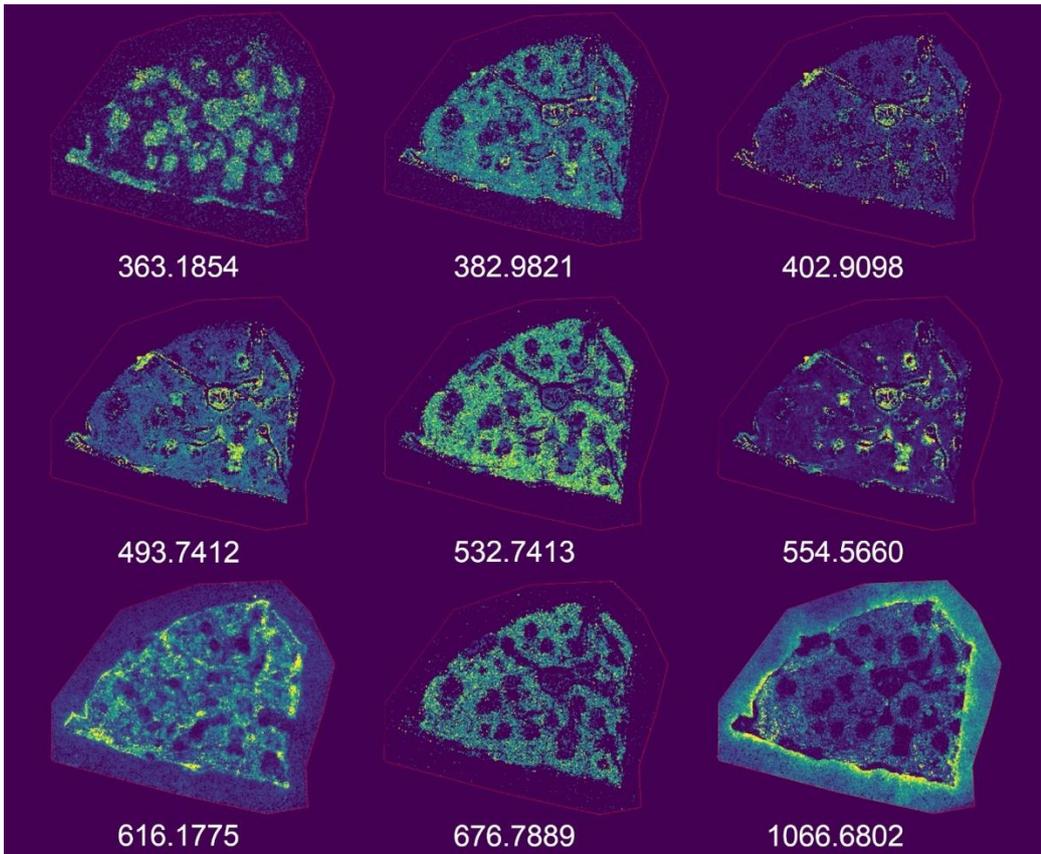
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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



The spatial distribution of peptides obtained with MALDI-2 MSI

Poster number: **LS-PA-093**

## **TWO DIMENSIONAL MASS SPECTROMETRY AS A TOOL FOR BIOTHERAPUTICS ANALYSIS USING ULTRAVIOLET PHOTODISSOCIATION AND ELECTRON-BASED DISSOCIATION TECHNIQUES**

Abstract ID: **539**

**Presenting author: Meng Li, University of Warwick**

### **Introduction**

Bioactive proteins in venom from scorpions has been widely used as alternative medicines for the treatment of a great array of conditions, such as cancers and blood coagulations. However, only partial genome sequences are available for these venom proteins; which is challenging to determine biomarkers from the crude venom. To fully cover the sequence of each protein in the venom, herein, we applied a 2DMS technique coupled with ultraviolet photodissociate (UVPD) and electron-based dissociation (ExD) fragmentation methods. 2DMS allows for an unbiased separation and detection of proteins which facilitates the detection of proteins with different physical properties; while the combination of UVPD and ExD fragmentation methods resulted in high protein coverages, hence a more confident determination of the biomarker sequence.

### **Methods**

Crude scorpion venom sample was obtained through electrical stimulation of the scorpion *Mesobuthus Martensii*, fractionated using reverse phase C18 column and tested for in-vitro inhibition of coagulation factor Xa. 2DMS analysis of the bioactive fraction was acquired using electron capture dissociation (ECD), electron induced dissociation (EID), and UVPD as fragmentation methods. Statistical methods was used to determine possible pharmaceutical targets. All spectra were collected on 12 Tesla and 15 Tesla Bruker Solarix FTICR MS. Sequence and modification information of the potential pharmaceutical targets was obtained using both top-down and bottom-up approaches.

### **Preliminary data (results)**

2DMS is a data independent analysis technique which has been shown to be applicable in the characterisation of complex mixtures without the need for prior separation. It allows for the correlation of all fragments to its respective precursors, without requiring quadrupole isolation or chromatographic separations. Based on our results, several unique proteins were solely observed with 2DMS compared to nano-LC tandem MS experiment due to the unbiased protein separation and identification in 2DMS, indicating 2DMS is a complementary experiment to nano-LC experiment which results in the ability to assign proteins of various properties. In addition, vertical precursor lines from a 2DMS plot allows the identification of the precursors that are producing common fragments which allows a fast screening and grouping of proteins with same backbone but various PTMs.

Novel potential biotherapeutics was sequenced using both top-down and bottom-up approaches using 2DMS with UVPD, ECD and EID. These fragmentation methods provided complimentary information to allow for the increased accuracy during de novo sequencing. With the insufficient genomic sequence information for the scorpion venom species, there is a need for UHR-MS/MS for de novo sequencing efforts. Fragments obtained from EID has shown UVPD-like cleavages, which enhance the cleavage coverage of these proteins. The unparalleled mass accuracy and resolving power of the FTICR-MS has allowed for the highest possible confidence during analysis which is essential in the sequencing study of these scorpion venoms proteins. This method is highly applicable to identifying potential pharmaceutical leads from other natural products.

### **Please explain why your abstract is innovative for mass spectrometry?**

Using multiple tandem techniques coupled to 2DMS to interrogate bioactive proteins obtained from scorpion venom.

### **Co-authors:**

*Yuko P. Y. Lam, University of Warwick*

*Christopher A. Wootton, University of Warwick*

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Callan Littlejohn, University of Warwick  
Francesca Bellingeri, University of Warwick  
Alina Theisen, University of Warwick  
Mark P. Barrow, University of Warwick

Poster number: **LS-PA-094**

## ION MOBILITY ASSISTED GLYCOPROTEOMICS ON TIMS-TOF

Abstract ID: **613**

**Presenting author: Andris Jankevics, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands, Netherlands Proteomics Center, Padualaan 8, 3584 CH Utrecht, The Netherlands**

### Introduction

Protein glycosylation plays diverse roles in biology, influencing processes such as cell-cell adhesion, receptor-recognition, immunity and signaling. Characterization of intact glycopeptides is however challenging due to their inherent microheterogeneity (multiple glycans can modify the same glycosylation site). Advances over the past decade have made detection of glycopeptides more routine. However, accurate identification of glycopeptides from complex datasets remains a bottleneck. Optimized methods utilizing the biochemical properties and fragmentation features of glycopeptides are therefore crucial for detecting glycopeptides in complex samples. *N*-Glycopeptides have been shown to physically separate in the ion mobility dimension from unmodified peptides. Here, we investigated in samples of varying complexity (recombinant proteins as well as human plasma), if ion-mobility separation of glycopeptides can improve the identification performance.

### Methods

Sialylglycopeptide ( $\alpha$ 2,6-SGP), and tryptic digests of tissue non-specific alkaline phosphatase (TNAP) and histidine-rich glycoprotein (HRG) were separated on Ultimate 3000 nanoUHPLC (Thermo Fischer Scientific) coupled on-line to a timsTOF Pro mass spectrometer (Bruker Daltonics, Bremen, Germany). Peptides were separated on an Ion Optics nanoUHPLC column (75  $\mu$ m x 25 cm, 1.6  $\mu$ m, C18; Ion Optics, Australia), heated at 50 °C. For complex glycoproteomes, we used 200 ng of a pooled human plasma digest. We developed and tested several data pre-processing techniques to export files to MGF for searching through MSFragger for glycopeptide identification.

### Preliminary data (results)

Trapped Ion mobility separation (TIMS) aided parallel accumulation serial fragmentation (PASEF) provides a unique capability to separate and accurately sequence peptides at very fast scan rates. We first optimized the TIMS-PASEF method on two synthetic glycopeptide standards. Glyco-oxonium ions 204.087 (HexNAc), 274.092 (NeuAc-H<sub>2</sub>O), 366.140 (HeNAcHex) and 657.23 (HexNAcHexNeuAc) were selected as glycopeptide diagnostic ions to provide a view on the location of the glyco-peptides inside the mobiligram. Data on the glycoproteins TNAP and HRG containing complex glycosylation, e.g. sialylated (N<sub>4</sub>H<sub>5</sub>S<sub>2</sub>) and core fucosylated (N<sub>4</sub>H<sub>5</sub>S<sub>2</sub>F<sub>1</sub>) glycans, demonstrated that glycopeptides could be physically separated in the ion mobility dimension from unmodified peptides. We observed glyco-oxonium ion clustering in the ion mobility space, while peptides without these diagnostic ions were outside this ion mobility region. This allows for efficient targeting of the mass spectrometer to sequence only ions of interest. Additionally, we found that the standard PASEF conditions were not optimal to identify all ions of interest and resorted to fragmenting the same precursor with collision energies (CEs) ranging from 20 to 120 eV. After combining the fragmentation spectra from different CEs into a single spectrum and combining this with focusing of the mass spectrometer on the ion mobility space where the glyco-oxonium ions clustered, we were able to increase the identification rate 7-fold for this platform. In the next step, we are working this novel approach into a standardized acquisition approach using the API provided for the mass spectrometer.

### Please explain why your abstract is innovative for mass spectrometry?

Method development on Trapped Ion Mobility Separation (TIMS) of glycopeptides sequencing for enhanced glycoproteomics on TIMS-TOF Pro mass spectrometer.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### **Co-authors:**

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*Richard A Scheltema, Netherlands Proteomics Center, Padualaan 8, 3584 CH Utrecht, The Netherlands, Netherlands Proteomics Center, Padualaan 8, 3584 CH Utrecht, The Netherlands*

Poster number: **LS-PA-096**

## **SMALL PROTEIN DISCOVERY IN PROKARYOTES BY COMBINED TOP-DOWN & BOTTOM-UP SEQUENCING AND PROTEOGENOMICS**

Abstract ID: **967**

**Presenting author: Jakob Meier-Credo, Max-Planck-Institute of Biophysics**

### **Introduction**

Small proteins, also termed sProteins or microproteins, are short ORF-encoded polypeptides of up to 100 amino acids that comprise a divergent and ubiquitous family of proteins that play essential roles in virtually all organisms. Even so, they represent a group of analytes that is often missed in conventional bottom-up proteomics workflows due to incomplete genome annotations and methodological bias towards detecting larger proteins. Here we set out to develop novel strategies for the extraction, purification and identification of these proteins in *Pseudomonas stutzeri*; a wide-spread, gram-negative bacterium that can grow under aerobic and low oxygen conditions which serve as a model system for studying the pathogenicity of its close homolog *Pseudomonas aeruginosa* during lung infections associated with impaired oxygen diffusion.

### **Methods**

Cells were grown under aerobic and oxygen limiting conditions. DNA was prepared and subjected to PacBio long read sequencing to create an iPTgxDB database for protein identification. For proteomics experiments, cells were lysed by ultrasonication in water with benzonase and protease inhibitors. For bottom-up, proteins were solubilized in SDS, digested on S-TRAPs and desalted. For top-down experiments, proteins were extracted by ultrasonication in Urea-Acetonitrile buffer, pre-cleared through MW-cutoff membranes and purified using SPE. All LC-MS experiments were run on an Ultimate 3000 coupled to a Fusion Lumos operated in target-optimized DDA methods. Analysis was performed in PEAKS and bioinformatic tools.

### **Preliminary data (results)**

We performed a comprehensive genomic and proteomic analysis of *P. stutzeri* in aerobic and oxygen-limiting conditions. We combined state-of-the-art *de novo* genome assembly to report the first complete *P. stutzeri* ATCC14405 genome along with bottom-up and top-down proteomics analyses to report the most detailed study of proteome remodeling in response to oxygen limitation in *P. stutzeri*. Using the conventional NCBI genome annotation we identified more than 2900 proteins (71.3% of the predicted proteome), including 116 small proteins out of which 33 were uniquely identified by the top-down methods. We employed label-free quantification for both conditions and found many known key metabolic pathways as well as yet uncharacterized proteins being regulated or exclusive. For oxygen limitation these included a significant up-regulation of well-established enzymes involved in the denitrification cascade, i.e. proteins belonging to the nar, nir, nor and nos gene clusters as well as a metabolic adaptation to the anaerobic arginine deiminase pathway. Under normoxic conditions we observed up-regulation of the aerobic respiratory cbb3 oxidase isoforms as well as of enzymes involved in asparagine metabolism that feed into the TCA cycle, exopolysaccharide biosynthesis and biofilm formation. We then focused on identifying novel proteins by using the integrated proteogenomics search database (iPTgxDb) that captures the entire protein coding potential of the genome. After stringent PSM-level filtering we obtained more than 30 novel proteins where bottom-up data provides evidence for even long missed proteins as well as expressed pseudogenes and top-down measurements exclusively contribute 9 novel sProteins with large sequence coverage.

### **Please explain why your abstract is innovative for mass spectrometry?**

Novel sample preparation and combined top-down/bottom-up data acquisition strategies for sProtein discovery in prokaryotes.

### **Co-authors:**

*Benjamin Heining, Agroscope & SIB Swiss Institute of Bioinformatics*  
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Poster number: LS-PA-097

## USING TOP-DOWN MASS SPECTROMETRY TO STUDY THE PROTEOFORM CHANGES CAUSED BY VPS35-D620N PROTEIN MUTATION OF PARKINSON'S DISEASE

Abstract ID: 979

Presenting author: Yuko, Pui Yiu Lam, University of Dundee

### Introduction

The D620N mutation in the Vacuolar Protein Sorting 35 (VPS35) protein is associated with Parkinson's Disease. VPS35, VPS26, and VPS29 are the subunits of the cargo selective complex of the retromer, which functions in selective membrane transport of protein cargo from the endosome to Golgi or plasma membrane. Currently, the signalling pathways of VPS35 mutation associated with Parkinson's are poorly understood. Top-down mass spectrometry (MS) is an emerging tool to study proteoforms in proteomics samples; this method, however, is challenged by MS sensitivity, resolution, and ion suppression in the instrument. Thus, sample preparation and separation are the most critical steps for top-down proteomics. Herein, we have optimised various sample preparation methods to target proteins with molecular weight <30 kDa for proteoforms studies.

### Methods

Littermate matched Mouse Embryonic Fibroblasts harbouring either wild-type VPS35 or a homozygous knock-in of the Parkinson associated VPS35[D620N] mutant were generated. For VPS-35 immunoprecipitation experiments, samples were incubated and eluted with 0.5% by vol TFA solution, 1% by mass SDS, or 8M urea. Detergents were removed using an HiPPR detergent removal spin column kit. High molecular weight proteins were removed using MWCO filters or acetonitrile precipitation methods. Offline fractionation was applied to reduce the complexity of the samples. Samples were then loaded into an Orbitrap Exploris 240 MS. Data was analysed using the MASH Suite software.

### Preliminary data (results)

Acetone or chloroform precipitation and MWCO filter washes with high molarity of urea solution are the most common methods to remove detergents from top-down proteomics samples. These methods are effective in removing all types of detergents, but also results in a significant amount of protein loss, especially low molecular weight proteins which are soluble in high organic solvents and easily trapped in the membrane of MWCO filters. We found that using an HiPPR detergent removal spin column kit can reduce the low molecular weight protein lost by at least 20%.

Previous publications have demonstrated the combined use of high pH extraction solutions, followed by chloroform precipitation and finally concentration using MWCO filters to perform SDS gel extraction. This method can enhance the intact protein recovery but results in dramatic loss of low molecular weight proteins. In our experiments, acetonitrile mixed with 100 mM ammonium bicarbonate solution was found to be the most effective extraction buffer for intact protein. Extracted samples can directly be concentrated using MWCO filters without chloroform precipitation which reduces the loss of low mass proteins.

With the optimised sample preparation methods, we can easily observe over 150 proteins with more than 400 different proteoforms in a single-shot experiment. The most common modifications observed are oxidation, acetylation, and phosphorylation. Surprisingly, a modification with mass shift of +52.9 Da was also commonly observed in the mutant samples which we believe it is a modification caused by iron metal binding. Further detailed analysis is required to validate this hypothesis.

### Please explain why your abstract is innovative for mass spectrometry?

New, simple sample preparations are optimised for top-down proteomics which enhance the detection of low molecular weight (<30 kDa) proteins.

### Co-authors:

*Matthew Taylor, University of Dundee*

*Gloria Shi, University of Dundee*

*Dario R. Alessi, University of Dundee*

Poster number: **LS-PA-098**

## **HIGHLY EFFICIENT AND REPRODUCIBLE SAMPLE HOMOGENISATION FOR PROTEOMIC ANALYSES OF VARIOUS ORGANS UP TO 50 MG**

Abstract ID: **999**

**Presenting author: Ina Aretz, PreOmics**

### **Introduction**

Efficient protein extraction is a crucial and challenging step in tissue sample preparation for mass spectrometry. The PreOmics BeatBox is a revolutionary technology for reliable and reproducible throughput of tissue/cell samples. The BeatBox is a fast and easy-to-use instrument that completes sample homogenization in as little as 10 minutes, without cross contamination and minimal heat induction. The BeatBox has a surprisingly small footprint and quiet operation compared to traditional tissue processing instruments.

Here, we present the BeatBox Tissue Kit 24x, enabling efficient homogenization of various tissue types from 5 to 50 mg wet weight and up to 24 samples in parallel. BeatBox tissue homogenization can be seamlessly integrated into the PreOmics iST sample preparation workflow, enabling LC-MS ready samples in less than 3 hours.

### **Methods**

Two different homogenization techniques in combination with the iST protein digestion technology for LC-MS ready samples were compared; the BeatBox technology and conventional bead beating. Various tissue types (brain, liver, and cardiac muscle) were prepared to evaluate the homogenization efficiency.

Obtained peptides were analyzed on an EASY-nLC™ 1200 system (ThermoFisher Scientific) coupled to a timsTOF Pro mass spectrometer (Bruker Daltonics) in DDA-PASEF mode with a 45 min gradient. The data were analyzed by MaxQuant software, version 2.0.1.0 (*Mus musculus* canonical version UniProt database, February 2022), and Perseus software, version 2.0.3.0.

### **Preliminary data (results)**

The BeatBox technology integrated with the iST workflow enables efficient proteomics sample preparation for challenging tissue samples. Additionally, FFPE samples can successfully be homogenized using the BeatBox and applied to the iST workflow for subsequent LC-MS analysis. Simultaneous processing of 24 samples within 10 minutes followed by the iST workflow enables the preparation of LC-MS ready samples in 3 hours.

The BeatBox workflow enables the reliable processing of different tissue input amounts (wet weight) from 5 to 50 mg, with a Pearson correlation coefficient >0.9, and increased protein extraction efficiency for specific tissues. From intact tissue samples to complete DDA acquisition in less than 4 hours, more than 2500 protein identifications for mouse liver and more than 3000 protein identifications for mouse brain were achieved. Compared to a common bead beating workflow, the amount of identified proteins increased to 10% using the BeatBox.

Overall, applying the BeatBox for sample homogenization enables exceedingly reproducible protein extraction and identification across different starting amounts and tissue types.

### **Please explain why your abstract is innovative for mass spectrometry?**

Highly efficient homogenization of various tissue types from 5 to 50 mg wet weight and up to 24 samples in parallel in only 10 minutes using the innovative BeatBox platform.

### **Co-authors:**

*Zuzana Demianova, PreOmics*

*Patrick Schreiner, PreOmics*

*Nils A. Kulak, PreOmics*

Poster number: **LS-PA-099**

## DETECTION OF INTERMEDIATES IN THE HYDROXYL RADICAL DERIVED OXIDATION OF PEPTIDES BY MS AND LC-MS

Abstract ID: **1003**

Presenting author: **Nik Vagkidis, University of York**

### Introduction

Proteins are targets for radical oxidants in biological systems due to their abundance and reactivity.<sup>1</sup> The HO<sup>•</sup>-mediated oxidation of proteins generates many radical intermediates, including carbon-centred (P<sup>•</sup>) and oxygen-derived radicals such as alkoxy (PO<sup>•</sup>) and peroxy (POO<sup>•</sup>).<sup>2</sup> POO<sup>•</sup> can participate in hydrogen atom abstraction (HAA) reactions yielding hydroperoxides (POOH) as the main intermediate. Direct evidence of the intermediate radicals is lacking as they are short-lived and can produce different products.<sup>2</sup> Our aim is to collect evidence for their formation and reactivity, by analysing them *via* mass spectrometry (MS) thus offering new mechanistic insights.

1. M. J. Davies, *Biochem. J.*, 2016, **473**, 805–825

2. C. L. Hawkins, P. E. Morgan, M. J. Davies, *Free Radic. Biol. Med.*, 2009, **46**, 965-988

### Methods

We have recently developed a method for trapping and MS detection of short-lived radical intermediates (Figure 1). The method is based on rapid addition of intermediate radicals **2** to alkene-based traps **1** possessing a good radical leaving group **3**. The trapping results in the formation of a stable product **4** which can be studied by either direct-injection mass spectrometry (DI-MS) or liquid chromatography – mass spectrometry (LC-MS). Detection and identification of intermediate radicals and products such as hydroperoxides is further supported by well-established techniques such as isotope exchange and tandem MS (MS/MS).

### Preliminary data (results)

In this study we use *N*-protected tyrosine and phenylalanine peptides as a model system, and we elucidate the HO<sup>•</sup>-derived oxidation of peptides, by trapping radical intermediates, as well as identifying oxidation products by DI-MS and LC-MS. HO<sup>•</sup> reaction with tyrosine and phenylalanine is shown in Figure 2.<sup>1</sup> It reacts either by direct addition to the aromatic ring yielding hydroxylated **5** and doubly hydroxylated **6** products or by HAA to yield carbon-centred radicals **7**, which in aerobic environments react with O<sub>2</sub> in a diffusion-controlled rate to produce peroxy radicals **8**, that are converted to hydroperoxides **9**. However, O<sub>2</sub> addition can be slow for highly delocalised radicals such as tyrosine phenoxyl radicals ( $k < 10^3 \text{ M}^{-1} \text{ s}^{-1}$ ).<sup>1</sup> As such, we believe **7** and **8** can react with our alkene-trap to afford non-radical products. Detection of **7** as a trapped-adduct has been successful by DI-MS for both the tyrosine and the phenylalanine containing peptides. Hydroperoxides **9** and doubly-hydroxylated **6** species share identical *m/z*, and therefore, are indiscernible by MS. By carrying out deuterium exchange followed by DI-MS studies we have been able to differentiate between **9** and **6** as they differ in the number of labile protons. Hydroperoxides **9** undergo rapid two-electron reduction in presence of various catalysts (UV light, metal ions, elevated temperatures), to yield alkoxy radicals (PO<sup>•</sup>) and HO<sup>•</sup>.<sup>1</sup> PO<sup>•</sup> participate in rapid HAA reactions to afford alcohols **10**. Formation of hydroperoxides was further supported by fragmenting alcohols **10** that likely result from their decay.

### Please explain why your abstract is innovative for mass spectrometry?

The recently reported new trapping method allowed MS detection of radical intermediates formed during the aerobic HO<sup>•</sup>-induced peptide oxidation. Isotope exchange MS studies helped to differentiate between oxidation isomers.

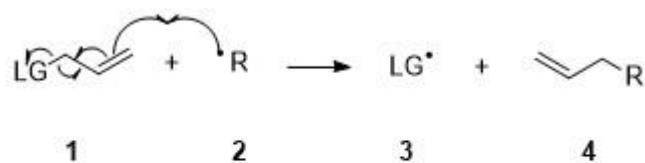


Figure 1. Novel radical trap design and trapping mechanism

Novel radical trap design and trapping mechanism

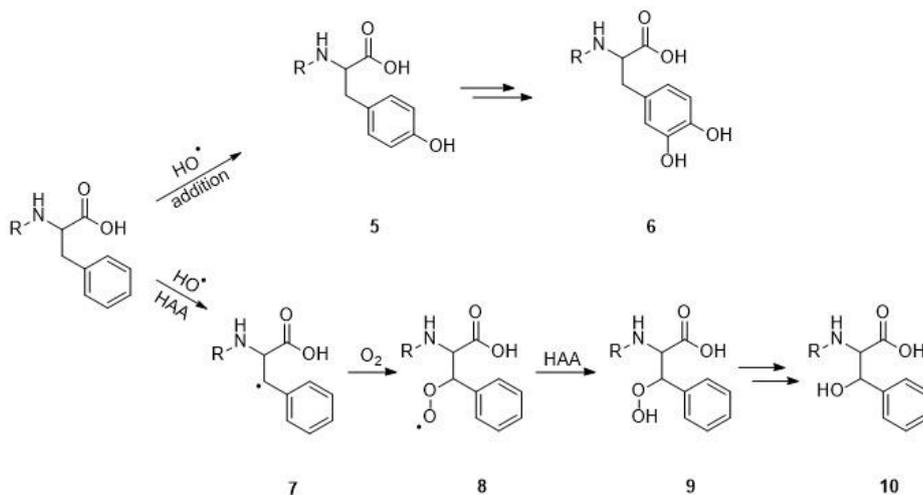


Figure 2. Hydroxyl radical-derived oxidation of phenylalanine in presence of O<sub>2</sub>

Hydroxyl radical-derived oxidation of phenylalanine in presence of O<sub>2</sub>

## **Session: Single cell MS / in cell MS**

Poster number: LS-PA-100

### **LABEL-FREE SINGLE CELL ANALYSIS WORKFLOW ON THE TIMSTOF SCP MASS SPECTROMETER USING THE CELLENONE PLATFORM**

Abstract ID: 703

**Presenting author: Christoph Krisp, Bruker Daltonics GmbH & Co. KG**

#### **Introduction**

Single cell proteomics is a rapidly developing field with the potential to make important contributions to the understanding of cellular heterogeneity. Recent enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry established in the timsTOF SCP paired with automated single cell sorting and sample preparation realized with the cellenONE platform, a liquid-dispensing instrument for cell isolation and picolitre dispensing, allows for sensitive proteome analyses at the single cell level. Coupled to developments in processing of data independent acquisition (DIA) mode data files using deep learning with neuronal networks (e.g. DIA-NN) further improves detectability and quantifiability of proteins from minimal input samples such as single cells.

#### **Methods**

20, 10, 5, 2 and 1 HEK 293 and HeLa cells were sorted, lysed, and digested using the cellenONE® platform. After single cell deposition and reagent dispensing, samples were directly incubated at 50°C with high humidity on deck of the instrument. Tryptic peptides were transferred into autosampler vials and injected onto an Aurora C18 column (IonOptics) using a nanoElute. Peptides were separated via a 30 min ACN gradient and eluted into a timsTOF SCP (Bruker). Data were acquired in dia-PASEF (data independent acquisition in parallel accumulation serial fragmentation) mode and analyzed with DIA-NNv1.8 in predicted library mode.

#### **Preliminary data (results)**

Commercially available HeLa cell protein digests (Pierce) were used for gradient versus peptide load assessments in a range from 100 pg up to 50 ng peptide loads onto the column, analyzed with 15, 30, and 60 min acetonitrile gradient lengths. The 30 min gradient performed best for peptide loads up to 5 ng, the 60 min gradient was more beneficial for loading > 5ng, the 15 min gradient only performed at par with the 30 min in picogram peptide loads.

The cellenONE platform sorted and prepared HEK and HeLa cells were run with the 30 min gradient and data acquired in dia-PASEF mode on a timsTOF SCP. Data processing in DIA-NN using an ion mobility and retention time predicted library of human protein sequences without match between runs identified for reproducibly more than 1000 protein groups with up to 5,000 peptide sequences from single HEK and single HeLa cells and close to 4,000 protein groups with up to 25,000 peptide sequences for 20 HEK or HeLa cells.

Quantitative comparison using principal component analysis clearly separated single HEK cells from single HeLa cells while single cells from one cell type clustered closely together. Several secretoglobulins and desmosomal proteins were either present at a higher abundance or were exclusively found in HEK cells, typical proteins in early embryonal kidney development. HeLa cells showed higher abundance of cell cycle control, cell growth stimulating, and DNA damage repair proteins as well as several oncogenes, hallmark proteins for cancerous cells.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Label-free single cell analysis workflow identifying > 1000 proteins from single cells using the cellenONE platform and the timsTOF SCP mass spectrometer

**Co-authors:**

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Anjali Seth, Cellenion*

*David Hartlmyr, Cellenion*

*Tharan Srikumar, Bruker Ltd.*

*Pierre-Olivier Schmit, Bruker Daltonique S.A*

*Guilhem Tourniaire, Cellenion*

*Markus Lubeck, Bruker Daltonics GmbH & Co. KG*

*Gary Kruppa, Bruker s.r.o.*

Poster number: **LS-PA-101**

## **IMPROVED DATA-INDEPENDENT ACQUISITION (DIA) AND DATA-DEPENDENT ACQUISITION (DDA) PERFORMANCE ON LOW-LEVEL PROTEOMIC SAMPLES USING A NOVEL ZENO TRAP**

Abstract ID: **1015**

**Presenting author: Ihor Batruch, SCIEX**

### **Introduction**

Identification and quantification of large numbers of proteins is important for the characterization of biological systems to gain insight into their composition and function. The ZenoTOF 7600 system is equipped with a Zeno trap that improves the duty cycle to more than 90% at the MS/MS level, enabling gains in sensitivity of 5 to 20x. Improved MS/MS sensitivity is important for single-cell and other applications in which samples are present at low-nanogram levels, as it enables more peptide and protein identifications. Here, we evaluated the performance of data-dependent acquisition (DDA) at 200 to 400 ng of commercial digest loads and data-independent acquisition (DIA) approaches on a novel ZenoTOF 7600 system by testing loads ranging from 0.25 ng to 400 ng.

### **Methods**

A ZenoTOF 7600 system with an OptiFlow Turbo V ion source was coupled to a Waters M-Class LC system equipped with a Waters Acquity HSS-T3 (25 cm x 75 µm ID) column or EvoSep EV-1106 (15 cm x 150 µm ID) nano columns. Protein identifications of K562 cell digest (SCIEX), ranging from 0 to 400 ng load, were assessed following 45- or 180-min LC gradients with DDA or Zeno SWATH DIA mode. DDA data were processed with the ProteinPilot application within OneOmics suite (SCIEX Cloud) and Zeno SWATH DIA data were processed in DIA-NN software.

### **Preliminary data (results)**

We used a Zeno SWATH DIA mode with a 45-min gradient to test cell digest loads that were within the single-cell regime, such as 0.25, 0.5 and 1 ng loads. From these experiments, over 900-1100, 1400-1500 and 2100-2300 protein groups were identified, for 0.25, 0.5 and 1 ng loads, respectively, and 45-55% of these identifications had a CV less than 20% when searched against a spectral library. At the precursor level for the same loads, there were 3000-4000, 5000-5600 and 8700-12000 corresponding precursors for the 0.25, 0.5 and 1 ng loads. We tested higher loads and identified 4200, 5000 and 6100 protein groups for 5, 10 and 25 ng loads, respectively, and 64-83% of these identifications satisfied the 20% CV cutoff. For a 50 and 200 ng load, more than 6300 and 7000 protein groups were identified, respectively, of which over 90% had a CV of under 20%. There were over 55000 and 66000 precursors mapping to 50 and 200 ng loads. When the data were searched against a FASTA library in library-free mode, the number of identifications at 20% CV cutoffs approach those achieved when processed using the spectral library approach.

A 200 ng and 400 ng load of K562 tryptic digest was tested in DDA mode on a Waters Acquity column, using a 180-min gradient. From these experiments, we were able to identify over 4500 and 5100 protein groups for the two loads, respectively, with over 43000 and 56000 peptides for each load.

### **Please explain why your abstract is innovative for mass spectrometry?**

High duty cycle for MS/MS on ZenoTOF 7600 system provides high number of identifications at single-cell level loads.

### **Co-authors:**

*Stephen Tate, SCIEX*

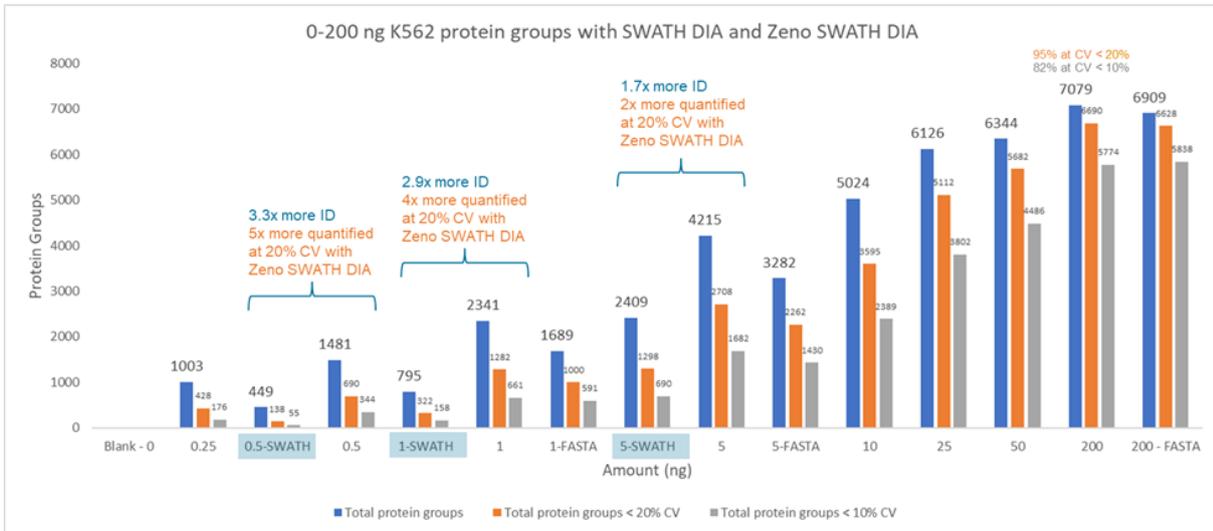
*Nic Bloomfield, SCIEX*

*Anjali Chelur, SCIEX*

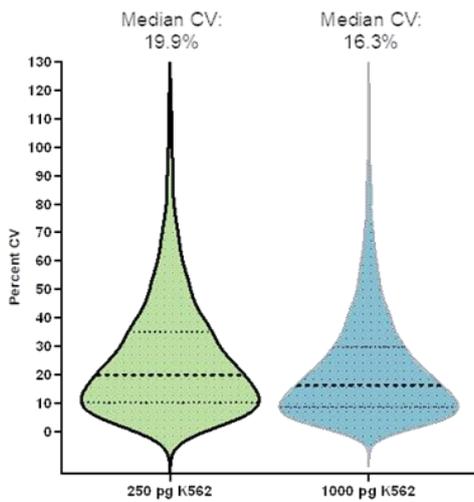
*Jose Castro-Perez, SCIEX*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
 Monday 29 August 2022 from 14:00 to 15:30 hours  
 Tuesday 30 August 2022 from 14:00 to 15:30 hours



Protein groups with SWATH (0.5-1 ng) and Zeno SWATH DIA



Median protein CV with Zeno SWATH DIA

Poster number: **LS-PA-102**

## **ROBUST AND HIGH-THROUGHPUT SINGLE CELL ANALYSIS WITH THE EVOSEP ONE**

Abstract ID: **1022**

**Presenting author: Nicolai Bache, Evosep**

### **Introduction**

Single cell proteomics is a rapidly growing field where cutting edge technologies are pushed to the limit. One of the greatest challenges is to maintain sensitivity throughout the workflow by reducing sample handling during preparation and enable ultra sensitive MS analysis. The Evosep One represents a powerful platform for high sensitivity work including single cell proteomics. Recent advancements and optimization of Whisper™ Flow technology combined with a new generation Evtip provide efficient capture and recovery of peptides from especially low sample amounts down to the single cell level. Additional optimizations include a new 15 cm column from IonOpticks (75 µm ID with 1.7 µm beads, Generation 3 Aurora Elite), which is perfectly suited for disposable trap columns with a slightly more hydrophobic material.

### **Methods**

HeLa tryptic digest was purchased from Pierce and dilutions were loaded on Evtip Pure in six replicates from 62.5 pg to 32 ng. HeLa cells were sorted, harvested and digested using the cellenONE, where 1, 5 and 10 cells were collected in separate proteoCHIP wells. Samples were analyzed with an Aurora Elite column operated at 50 °C using the Whisper 40 SPD method and a timsTOF SCP mass spectrometer operated in dia-PASEF mode. Data was analyzed with DIA-NN v1.8 with a predicted library.

### **Preliminary data (results)**

We initially assessed the chromatographic improvement of the 15 cm IonOpticks column against the recommended 15 cm Evosep performance column (EV1112). From 5 ng HeLa (Pierce), the FWHM is on average reduced from 4.5 seconds to 3.2 seconds. This increased peak performance is resulting in 50% higher peptide coverage leading to 20% more proteins with nearly 5,000 proteins identified with dia-PASEF® on a timsTOF SCP mass spectrometer.

We then analyzed a dilution series ranging from 62.5 pg to 32 ng HeLa digest in six replicates to establish sensitivity. From the 125 pg load, corresponding to single cell level, we identified 7,000 peptides leading to 1,600 proteins, whereas the high load of 32 ng resulted in just more than 55,000 peptides and leading to close to 7,500 proteins. The reproducibility was excellent throughout the dilution series, so we challenged the sensitivity further by analyzing single sorted HeLa cells. These were prepared using the cellenONE in separate proteoCHIP wells and directly loaded on Evtips. Preliminary experiments showed good coverage with more than 10,000 peptides and 2,000 proteins identified from a single cell. Finally, we explored the opportunity to develop an even faster Whisper method with a throughput of 80 samples per day to enable single cell analysis of larger cohorts. Altogether, the combination of IonOpticks columns, Whisper methods on the Evosep One and dia-PASEF on the timsTOF SCP provides an excellent combination for robust and high-throughput single cell analysis using the cellenOne instrument from Cellenion.

### **Please explain why your abstract is innovative for mass spectrometry?**

Robust and scalable workflows are needed to gain biological insight from single cell proteomics, which is efficiently achieved by the Evosep One combined with the timsTOF SCP.

### **Co-authors:**

*Dorte Bekker-Jensen, Evosep*  
*Christoph Krisp, Bruker*  
*David Hartlmayr, Cellenion*

Anjali Seth, Cellenion  
Ole B. Hoerning, Evosep  
Moritz Heusel, Evosep  
Magnus Huusfeldt, Evosep  
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Jarrod Sandow, IonOpticks  
Gary Kruppa, Bruker  
Nicolai Bache, Evosep

Poster number: LS-PA-103

## SINGLE-CELL EQUIVALENT SENSITIVITY ON TIMSTOF PRO WITH 35 MINUTES GRADIENT TIME ENABLED BY LABEL-FREE REFERENCE RUN APPROACH

Abstract ID: 1025

Presenting author: Goran Mitulović, Bruker

### Introduction

Single-cell proteomics requires both high sensitivity and high sample throughput, i.e. rapid LC gradients with high scanning speed mass spectrometry. Besides the dedicated timsTOF SCP (Single Cell Proteomics) instrument, which is described in a recent preprint (Brunner A-D et al, *Molecular Systems Biology* (2022)18:e10798), the versatile timsTOF Pro instrument, which was designed for shotgun proteomics experiments using standard sample amounts, also offers a “high sensitivity” mode. We present a method that enables single-cell proteomics on this instrument, reasoning that concomitant search of reference samples could increase the sensitivity of peptide and protein detection in DIA (Data Independent Acquisition) measurements by the machine learning algorithms implemented in current DIA analysis software.

### Methods

A U3000 RSLC system (Thermo Fisher Scientific, Bremen, Germany) was equipped with a BrukerTen analytical column and operated in direct injection mode. LC gradient time was 35 minutes. Data acquisition on the timsTOF Pro instrument was performed in DIA (Data Independent Acquisition) versus DDA (Data Dependent Acquisition) mode. Low sample amounts of 10ng and 1ng (the latter comparable to the single cells) were measured by enabling the “high sensitivity” mode on the timsTOF Pro instrument. In addition, samples of 200ng were measured and searched concomitantly. Spectronaut 16 and Spectromine 3.2 (Biognosys, Schlieren, Switzerland) were used for data analysis.

### Preliminary data (results)

So-called “Direct DIA” analyses with Spectronaut identified on average 307 protein groups in 1ng of HeLa sample. When 10ng and 200ng HeLa LC runs acquired with the same gradient were searched concomitantly, protein identifications increased to 1458 protein groups on average for the 1ng samples. In addition, on average 4377 protein groups could be identified with 10ng and on average 4693 groups with 200ng in combined searches.

Our initial method detected a considerable number of protein groups in wash runs between samples. We, therefore, established measurements to curb carry-over, e.g., by injecting a small amount of TFE to wash the autosampler and the analytical column. This strongly reduced spurious identifications in wash runs, suggesting that the large increase in protein identifications by our approach is real. We also searched data against a combined *fasta* database comprising sequences from Yeast and E.coli in addition to human and contaminant sequences. The minimal fraction of false discovered protein and peptide sequences from the two organisms not being present in the sample provides further support that the identifications achieved by our reference run approach are authentic. We, finally, compared figures of merit to the alternative approach of DDA (data dependent acquisition). While 4219 identified protein groups for 200ng with DDA were similar to DIA, 499 groups for 1ng and 2129 groups for 10ng with DDA show that for small sample amounts, our DIA reference run approach increased the number of identified protein groups more than twice. Data completeness was also generally superior with DIA.

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours

Monday 29 August 2022 from 14:00 to 15:30 hours

Tuesday 30 August 2022 from 14:00 to 15:30 hours

### Please explain why your abstract is innovative for mass spectrometry?

Establishment of a high-throughput method for sensitive single-cell proteomics on a timsTOF Pro instrument using DIA and reference runs.

#### Co-authors:

*Peter Pichler, Research Institute of Molecular Pathology*

*Gary Kruppa, Bruker*

*Manfred Hacking, Medical University of Vienna*

*Karl Mechtler, Research Institute of Molecular Pathology*

Poster number: **LS-PA-104**

## TAKING PROTEOMICS DOWN TO THE SINGLE CELL LEVEL

Abstract ID: **150**

**Presenting author: Christoph Krisp, Bruker Daltonik GmbH**

### Introduction

The predominant aim of single cell analysis in clinical research proteomics discovery, companion diagnostics, or personalized medicine research is to decipher the cellular heterogeneity in samples, e.g. tumor tissue. Therefore, dozens to hundreds of individual single cells are required to be analyzed in a relatively short timeframe. Due to recent improvements in mass spectrometry platforms and proteomics sample preparation for single cell as well as fast and robust liquid chromatographic separation, the analysis of single cell proteomes has become an achievable goal.

### Methods

Human cervical cancer cell digests (HeLa, Pierce) were used to simulate protein concentrations expected at single or few cell level. Further, HEK 293 cells (1 – 20 cells) were sorted and prepared with a CellenOne system (Cellenion). The UPLC systems EvosepOne in Whisper mode (Evosep) and a nanoElute (Bruker) were used and coupled to a timsTOF SCP (Bruker). Data acquisition was done in dia-PASEF mode and data were processed with Dia-NNv1.8 using a predicted human protein sequence library.

### Preliminary data (results)

Peptides from a HeLa digest were used to firstly assess sensitivity, identification rate and quantification reproducibility. Therefore, 5ng peptide loads were run on 5 different timsTOF SCP. On average 4305 protein groups, 31,000 stripped peptide sequences and 33,000 precursors were identified per instrument with an intra-instrument CV of 1 – 5 % at protein, peptide and precursor identification level. Further, a dilution series (0.125 – 20 ng) demonstrated excellent concentration responses (mean Pearson Correlation score of 0.94) and high identification rates with on average 1250 protein groups and 5600 peptides detected at 125 pg. The analysis of CellenOne sorted and prepared samples revealed several hundreds of protein groups identifiably from a single cell.

### Please explain why your abstract is innovative for mass spectrometry?

In conclusion, we show fast and in-depth proteome quantification with HeLa lysate digest dilutions at concentration range down to single cell level and applied it to real single cell samples.

#### Co-authors:

*Nagarjuna Nagaraj, Bruker Daltonik GmbH*

*Renata Blatnik, Bruker Daltonik GmbH*

*Dorte Bekker-Jensen, Evosep*

*Ole Bjeld Horning, Evosep*

*Anjali Seth, Cellenion*

*Pierre-Olivier Schmit, Bruker Daltonik GmbH*  
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*Gary Kruppa, Bruker Daltonik GmbH*

## **Session: Translational MS - Cancer and immunology, and MS**

Poster number: **LS-PA-105**

### **GENETIC ANCESTRY VARIATION IN BREAST CANCER COLLAGEN STROMA FROM BLACK WOMEN**

Abstract ID: **692**

**Presenting author: Peggi M Angel, Medical University of South Carolina, Medical University of South Carolina**

#### **Introduction**

Black women have similar breast cancer rates to white women, yet bear the highest mortality burden compared to any other race/ethnicity. Aggressiveness is a key feature in breast cancer from black women (BW). BW women are diagnosed at younger ages with higher stages and grades of breast cancer, and two times the rate of lethal triple negative breast cancer. Microscopy and transcriptomics studies report that stromal collagen predicts breast cancer progression, recurrence and survival, yet collagen translational and post-translational modifications in breast cancer disparities remains unexplored. In this work, we report on translational and post-translational regulation in breast cancer collagen stroma comparing by genetic ancestry. Data is presented on breast cancer collagen variation in American mainland and diasporic African populations.

#### **Methods**

Formalin-fixed, paraffin-embedded tissue sections and microarrays (TMAs) from the Cancer Genome Atlas, Susan G Komen Tissue Bank, Indiana University Simon Cancer Center, and Washington University School of Medicine were tested with Institutional Review Board approval. Samples were analyzed by published collagen targeting approaches using imaging mass spectrometry (IMS) and sequencing proteomics. Imaging MALDI FT-ICR or MALDI-QTOF (7 Tesla Solarix; timsTOF fleX, Bruker) were operated in positive ion mode over  $m/z$  of 700 to 3000. Data were imported to SCiLS Lab software (Bruker), normalized to total ion current and peak intensities natural log-normalized prior to statistical testing.

#### **Preliminary data (results)**

Reasons for higher breast cancer mortality in black women are not understood and are recognized to include undefined molecular causes that are beyond socioeconomic effects. Since collagen stroma regulation is highly linked to all stages of breast cancer progression and survival, we hypothesized that translational and post-translational collagen stroma variations may be altered in breast cancer from BW compared to white women (WW). The study focuses on collagen hydroxylation of proline (HYP), which controls cell attachment, cell recruitment, proliferation, and migration within the tissue microenvironment. The study includes stromal variations in normal breast tissue, breast tumor, normal adjacent tissue (NAT), normal adjacent lymph, and metastatic lymph tissue. In triple negative breast cancer, small cohorts showed significant change in levels of specific HYP sequences based on genetic ancestry. HYP peptides segmented out to reveal unique spatial localization within the tumor microenvironment. A second cohort of showed increases in collagen prolyl hydroxylase enzymes by transcriptomics. Specific sites of HYP variation within peptide sequences were identified with marked changes by genetic ancestry and by spatial location of the HYP peptides. TMA analysis combined with microscopy demonstrated changes in collagen fibers that had low but significant correlation with certain HYP peptides. In South Carolina BW, lymph metastatic tissue was dramatically altered compared to normal lymph tissue. West African homozygous status of the Atypical chemokine receptor (ACKR1/DARC) further demonstrated specific changes in collagen HYP sequences. The collective data may help understand collagen-mediated mechanisms of aggressive breast cancer towards decreasing mortality in black women.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Collagen targeted proteomics to understand mechanisms of breast cancer disparities

**Co-authors:**

*Savanna Berkhiser, Medical University of South Carolina*  
*Ashlyn Ivey, College of Charleston, Medical University of South Carolina*  
*Sean Brown, Medical University of South Carolina*  
*Anand S Mehta, Medical University of South Carolina*  
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*Michael Ostrowski, Medical University of South Carolina*  
*Graham Colditz, Washington University School of Medicine*  
*Jeffrey Marks, Duke University*  
*Harikrishna Nakshatri, Indiana University Simon Cancer Center*  
*Marvella Ford, Medical University of South Carolina*  
*Richard R Drake, Medical University of South Carolina*

Poster number: **LS-PA-106**

## **A NOVEL CHARACTERISATION OF PROTEOLYSIS TARGETING CHIMERAS USING TANDEM MASS SPECTROMETRY AND TWO-DIMENSIONAL MASS SPECTROMETRY**

Abstract ID: **731**

**Presenting author: Mohammed Rahman, University of Warwick**

### **Introduction**

Proteolysis targeting chimeras (PROTACs) are a new form of drug modalities that promote degradation of a targeted protein. These bimodal molecules contain two ligands that are connected by a chemical linker, forming a ternary complex. This directly leads to targeted degradation of a protein via ubiquitination and is different to small molecule inhibitors that require an active site. PROTACs can successfully degrade a protein target if there are sufficient binding. Synthesis of PROTACs can be a multistep batch process and there have been no characterisation studies of such molecules. Multiple fragmentation methods can be used to analyse the structure of such compounds. Recent methods in data-independent analysis (DIA) via 2DMS, can reduce the spectral complexity.

### **Methods**

Samples analysed were dissolved in 50/50 ACN and water, which were analysed using a multitude of fragmentation techniques; collision induced dissociation (CID), infrared multiphoton dissociation (IRMPD), and ultraviolet photodissociation (UVPD) on a 12 T FT-ICR MS. PROTACs were analysed in positive mode: protonated and metal adducts (lithium, sodium, potassium, and silver), and the deprotonated form in negative mode. Afterwards, compounds were analysed using a DIA technique such as 2DMS, where fragments are correlated to their precursors. 3D-peak picking algorithm can be used to extract structural information and internal calibration can achieve sub-ppm errors.

### **Preliminary data (results)**

The results obtained from this study illustrates that CID and IRMPD have similar fragmentation patterns for PROTACs, given in both activation occurs via vibrational modes. However, due to the large number of carbonyls and aromatic rings, IRMPD can provide more structural information than CID from IR activation of PROTAC moieties. Furthermore, UVPD demonstrated rich fragmentation spectra, which suggests that these molecules favour higher energy fragmentation techniques. PROTACs may also be ionised in negative mode as the deprotonated form. In this case, the negative mode fragmentation spectra supersede the one obtained using positive mode as additional moieties can be characterised. The data in this study suggests that negative mode would be ideal in characterising PROTACs. Here, IRMPD was shown to give extensive characterisation of PROTACs i.e. both ligands and the chemical linker. However, in cases where the deprotonated species, is not observable, further characterisation can be achieved using metal adducts such as lithium, sodium, potassium, and silver. In all cases with a metal adduct, UVPD was shown to provide the most structural information out of the fragmentation techniques. Metal adducts allow for new characterisation that were not seen in the protonated form. Finally, 2DMS allows the characterisation of multiple PROTACs and their adducts can be achieved in a single experiment without the need for prior chromatography or precursor isolation, which has been reported to achieve

additional structural information. Common fragments between different PROTACs can be extracted from the vertical line, which allows for identification of structural similarities.

**Please explain why your abstract is innovative for mass spectrometry?**

Characterisation of PROTACs and their adducts using CID, IRMPD, and UVPD, in both polarities and 2DMS of PROTACs.

Poster number: **LS-PA-107**

## **IDENTIFICATION OF THE CANCER EXTRACELLULAR VESICLE SURFACE PROTEOME AND ITS APPLICATION TO DETECT CANCER IN URINE**

Abstract ID: **805**

**Presenting author: Irene Bijnsdorp, Department of Urology, Amsterdam UMC, Location VUMC, Amsterdam Netherlands**

### **Introduction**

Prostate cancer (PCa) cells secrete extracellular vesicles (EVs), that can be detected in urine. Determination of the number and/or cargo of cancer-associated EVs in urine might provide a non-invasive diagnosis method for PCa. To directly capture and detect cancer specific EVs, an understanding of the EV surface protein constituents is needed. The aim of the present study was to investigate the cancer and PCa-specific-EV surface proteome *in vitro* using LC-MS/MS-based proteomics.

### **Methods**

Ultracentrifuge-isolated EVs from a panel of 17 cell lines consisting of 5 cancer types (including prostate, bladder, lung, cervical and colorectal cancer) were exposed to trypsin in duplicates, after which the surface peptides were separately quantified from the EV-luminal and total EV-fraction using label-free LC-MS/MS proteomics. EV-integrity was evaluated using electron microscopy and FACS. To explore clinical relevance, urinary EV protein profiles were measured of 76 cancer patients, which were compared to healthy controls.

### **Preliminary data (results)**

The EVs integrity, size or number was not affected by trypsinization. We identified >1500 proteins consistently at the outer surface of cancer-EVs, of which 30% were related to membrane-proteins. The core surface proteome revealed high enrichment of heat shock proteins at the outer EV membrane. In addition to vesicle-related and metabolic proteins, intracellular DNA/RNA-binding protein complexes such as nuclear and ribosomal proteins were also detected at the EV surface. Several proteins were uniquely expressed on the surface of prostate-derived EVs, and some of these proteins were also highly enriched in the urinary EVs of PCa patients. Interestingly, surface-proteins that are involved in DNA/RNA-binding were significantly enriched in urinary EVs from patients with PCa. The identified prostate cancer specific EV surface proteome thus provides novel insights that might ultimately translate to a non-invasive detection method for PCa.

**Please explain why your abstract is innovative for mass spectrometry?**

MS-based proteomics is a powerful approach to uncover the EV surface proteome at high sensitivity.

### **Co-authors:**

*Leyla A Erozensi, Department of Urology, Amsterdam UMC, Location VUMC, Amsterdam Netherlands*

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**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Amsterdam UMC, Location VUMC, Amsterdam Netherlands*

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*Guido Jenster , Department of Urology, Erasmus MC, Rotterdam, The Netherlands*

*Connie R Jimenez, Department of Medical Oncology, OncoProteomics Laboratory, Cancer Center Amsterdam (CCA),  
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Poster number: **LS-PA-108**

## **FROM TUMOUR HETEROGENEITY IN GLIOBLASTOMA TO PRECISION THERAPY**

Abstract ID: **823**

**Presenting author: Michel Salzet, PRISM U1192 Inser, ULille, CHU Lille, CLCC Oscar Lambret**

### **Introduction**

GBM represents the main malignant primary brain tumor. The prognosis is poor with a median survival estimated at 16 months in clinical studies and around 12 months in contemporary population-based studies. Approximately 5% of patients survive more than 5 years. GBMs are characterized by significant molecular heterogeneity which makes patient stratification difficult and results in large differences in mean patient survival. Morphological criteria for the diagnosis of GBM according to the World Health Organization (WHO) central nervous system tumor classification of 2021 include mitotic activity, anaplastic nuclear features, microvascular proliferation and necrosis. Efforts to further subclassify GBM have been restricted to the genomic, transcriptomic and epigenetic levels. Despite these efforts, these approaches have found limited clinical application.

### **Methods**

Molecular heterogeneity is a key feature of GBM pathology impeding patient's stratification and leading to high discrepancies between patients' mean survivals. To address the need for stratification, a spatial proteomics analysis was undertaken on a total cohort of 100 GBM patients (50 prospective and 50 retrospective) with survival ranging from a few months to more than 4 years. The prospective cohort was analyzed by spatial proteomics guided by MALDI-MS imaging and validated by IHC with the retrospective cohort. The integration of molecular data with clinical information will then be performed. Therapeutic strategy based on CAR-Macrophages will be then undertaken.

### **Preliminary data (results)**

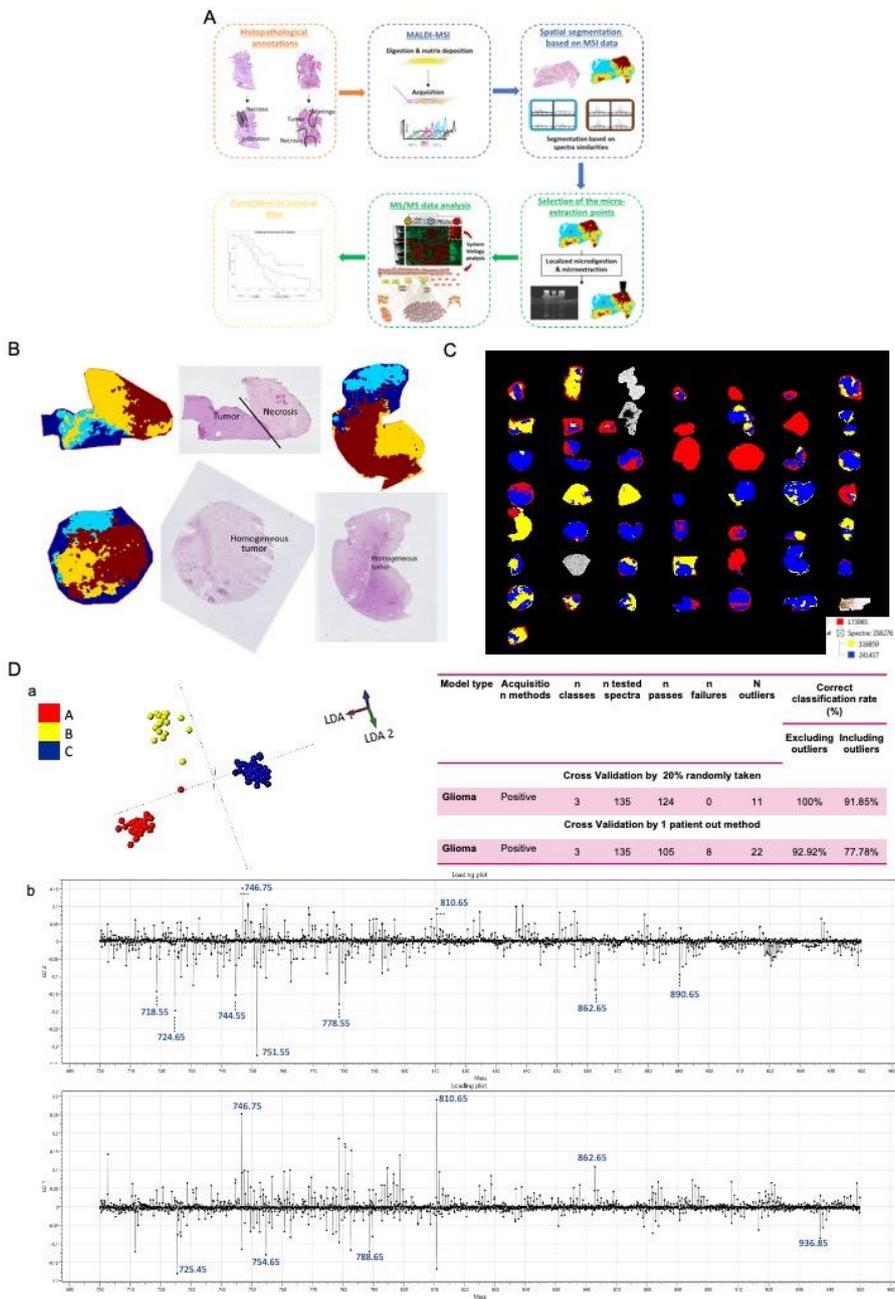
Forty-six tumors were analyzed by spatially-resolved high resolution mass spectrometry proteomics. Integrative analysis of protein expression and clinical information allowed us to identify three molecular regions associated with immune, neurogenesis and tumorigenesis signatures which were further validated based on metabolomic using Spidermass technology. Several of these molecular signatures can be enriched within the same tumor sample leading to high intra-tumoral heterogeneity. Nevertheless, a set of proteins was found statistically significant based on patient's survival times, 10 of which stem from alternative AltORF or non-coding RNA. Among these 26 proteins, which are survival markers, 10 were then validated by immunofluorescence. Thus, the classification of patients on the basis of the expression of these 10 proteins leads to a clear difference in survival between the groups. The expression of these 10 proteins and their correlation with survival data were confirmed in a retrospective cohort of 50 patients. The groups involving immune response and macrophages infiltration lead a bad prognosis. In these conditions, we developed a therapeutic strategy based on CAR-Macrophages, relying in part on the identification of specific biomarkers on the surface of tumours and more specifically on alternative membrane proteins (AltProts). Indeed, macrophages, unlike T cells, have the advantage of being very present in the tumour environment where they present antigens, and they also have a cytotoxic activity towards cancer cells. CAR-macrophages are a promising alternative therapy for GBM as we demonstrated here. This work opens the way to guide the prognosis of GBM and improve patient management.

### **Please explain why your abstract is innovative for mass spectrometry?**

GBM are highly heterogeneous tumors and a spatially-resolved proteomics approach guided by MALDI MSI, validated by metabolomic using Spidermass technology brings new insights in GBM biology to improve their stratification.

POSTER SESSION A

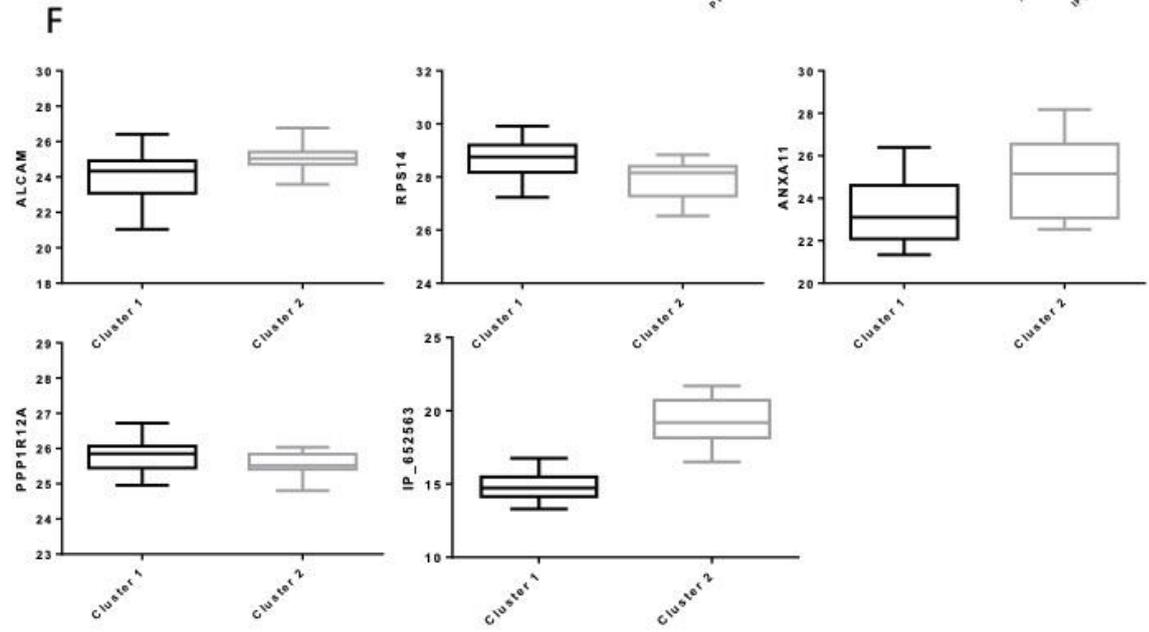
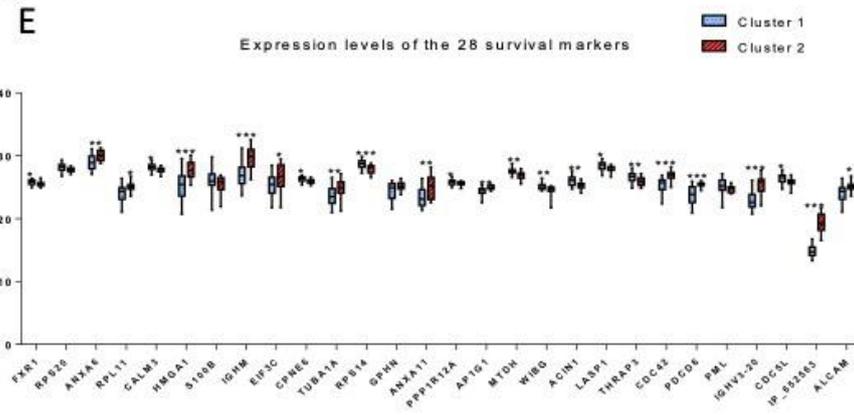
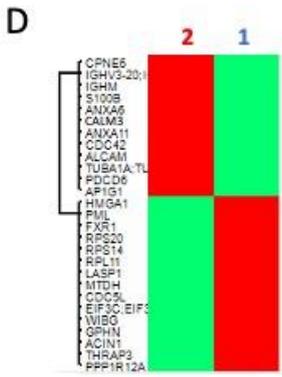
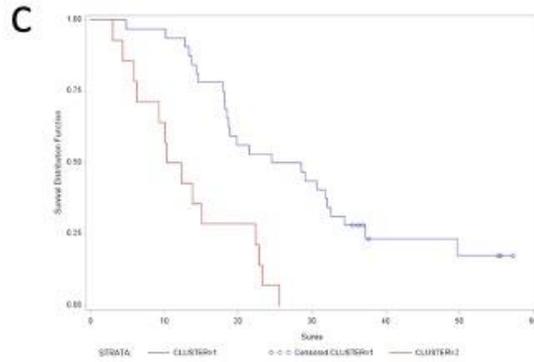
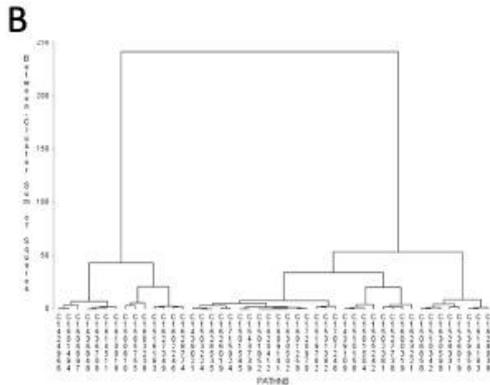
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Histological, MALDI MSI and SpiderMass data

**A**

Analysis of Maximum Likelihood Estimates								
Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
ALCAM	1	0,692	0,188	13,552	0,000	1,997	1,382	2,886
RPS14	1	1,476	0,536	7,583	0,006	4,377	1,530	12,520
ANXA11	1	0,463	0,157	8,714	0,003	1,589	1,168	2,162
PPP1R12A	1	-2,056	0,675	9,286	0,002	0,128	0,034	0,480
IP_652563	1	0,268	0,080	11,262	0,001	1,308	1,118	1,529



Proteomic and survival analysis

Poster number: **LS-PA-109**

## **SURVEYING THE CELL-CELL INTERACTION BETWEEN TUMOR AND NATURAL KILLER (NK) CELLS USING PROXIMITY LABELING (PL) AND SHOTGUN PROTEOMICS.**

Abstract ID: 155

**Presenting author: Maria Cristina Trueba Sanchez, Utrecht University**

### **Introduction**

Natural Killer (NK) cells are innate lymphocytes in charge of the immune control of virally infected and early-stage cancer cells. Lately, there has been increasing evidence that NK cell infiltration in cancer patients results in better clinical outcome across several cancer types, and therefore NK cells are currently in the spotlight of biomedical research<sup>1-3</sup>. Several studies have demonstrated the contribution of NK cells to successful immune checkpoint blockade (ICB) therapies<sup>4-5</sup> and they have been proposed as an alternative to typical T-cell adoptive cell therapy (ACT)<sup>6</sup>. Nonetheless, the knowledge about how the NK cells interact with cancer cells at the molecular level is insufficient. Here, we aim to unravel the proteome dynamics of the NK-Tumor cell interaction and the downstream signaling events that are involved.

### **Methods**

Proximity labeling (PL) methodology is used to specifically tag the proteins involved in the cell-cell interaction. Each domain of a promiscuous labeling enzyme (Split-TurboID)<sup>7-8</sup> is stably expressed on tumor and NK cells, respectively. Split-TurboID is non-specifically attached to the plasma membrane thanks to a glycosylphosphatidylinositol (GPI) anchor protein domain<sup>9-10</sup>. Upon cell-cell interaction, the Split-TurboID protein will reconstitute and promiscuously biotinylate all proteins involved in about 10 nm range<sup>11</sup> of the immunological synapse. These proteins will then be affinity purified via streptavidin enrichment and analyzed via shotgun proteomics. Four different cell lines will be used for the study.

### **Preliminary data (results)**

Our preliminary results point towards a successful stable cell line creation with TurboID promiscuous biotin ligase uniformly attached to their plasmatic membrane via the GPI-anchored domain. Cell viability in the long-term and the effect of endogenous biotinylation is being evaluated. We estimate a detailed outline of receptors and binding ligands involved in the NK driven immune response against both solid and blood tumour cells. Further experiments using PL TurboID strategy will be used in combination with phosphoproteomics to decipher the intracellular downstream signaling pathway involved in massive tumour cell death by NK cytotoxicity. Further, we will study the adaptive features of NK cells in the tumour cell-cell interaction context. Altogether our results will allow us to unravel the dynamic proteome involved in the NK immune control of different types of tumours and this will be expectantly applied to upcoming cancer cell therapies.

### **Please explain why your abstract is innovative for mass spectrometry?**

Highlight the application of a more efficient biotin ligase derived from BioID in the extracellular environment to, for the first time, study NK-tumor cell contacts using sensitive MS approaches.

### **Co-authors:**

*Maarten Altelaar, Utrecht University*

Poster number: **LS-PA-110**

## **COMPARATIVE ANALYSIS OF PRIMARY CELL LINES WITH RESPECTIVE PARENTAL TUMOUR TISSUES BY RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY: CAN WE REPRODUCE THE COMPLEXITY OF THE ORIGINAL TUMOUR FROM THE CELL LINE CULTURED FROM IT?**

Abstract ID: **159**

**Presenting author: Adrienn Molnár, ELTE Eötvös Loránd University, Waters Research Center**

### **Introduction**

For molecular diagnosis-based stratified medicine, accurate diagnosis of the disease is essential, and the best candidate for personalized treatment must be carefully selected to successfully treat patients. Chemical imaging is an emerging field that allows detailed analysis of sections taken from removed tumours, thus enabling precise diagnosis. This study aims to define the potentials of chemical imaging and rapid fingerprinting of immortalized cell lines cultured from the tumours using ambient Laser-Assisted Rapid Evaporative Ionization Mass Spectrometry (LA-REIMS). (*Figure 1.*) We aim to assess whether primary cell lines provide a faithful representation of the parental tumours from which they are derived from, to look for individual patterns and examine the comparability of *in vitro* cells to *in vivo* tumours.

### **Methods**

Immortalized cell lines were established from snap frozen, spontaneous tumours taken from veterinary surgeries and cultured in different media. Tumours were sectioned and characterized using OPO Laser-Assisted REIMS coupled to an imaging platform. Cell lines were examined with an Automated Well-Plate Reader (AWR) using REIMS™ technology fitted with a Xevo™ G2-XS TOF MS (Waters Corporation). The data acquired in negative ion mode, mass to charge range 50-1200, was processed with our in-house built software AMX (Waters) using multivariate statistics including Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). Ions were identified using MS/MS and exact mass measurements.

### **Preliminary data (results)**

Spontaneous tumours were examined during and after veterinary surgeries of dogs and cats, the removed tumours were sectioned and measured using LA-REIMS imaging with 70 µm resolution. 9 immortalized cell lines were established from the frozen tumour samples and monitored using the AWR by the measurement of cell samples frozen during the cultivation procedure. (*Figure 2.*) As expected, there is a significant difference between the homogenous cell culture and the heterogenous tissue (with a distance in the PCA space > 2.5), at least one and a half times the changes observed between passages. As the metabolic fingerprint of the cultured cell line and parental tissue was compared some characteristic peaks including phosphatidic acids, diacylglycerols, phosphatidylinositols were identified despite the tremendous observable differences. Specific groups of cells (50-100 cells per pixel) selected from the results obtained in the imaging measurements were compared to the passages of the cell lines. Therefore, examining the extent to which the metabolic fingerprint can be used to determine how far the cell line distanced from the tissue. The results show that there are already a number of variances between the cells grown in different media including their phosphatidylethanolamines and phosphatidylcholines. For the precise identification of the molecules, cyclic ion-mobility mass spectrometry will be used. In addition to this, our future plans include the understanding of all the similarities and differences between immortalized cell lines and the original tumour tissue, besides the precise characterization of the immortalized cell lines.

### **Please explain why your abstract is innovative for mass spectrometry?**

Comparison of immortalized cell lines with cancerous tissues using Rapid Evaporative Ionization Mass Spectrometry

### **Co-authors:**

*Nóra Kucsma, ELKH Research Center for Natural Sciences*

*Gabriel Horkovics-Kovats, ELTE Eötvös Loránd University, Waters Research Center*

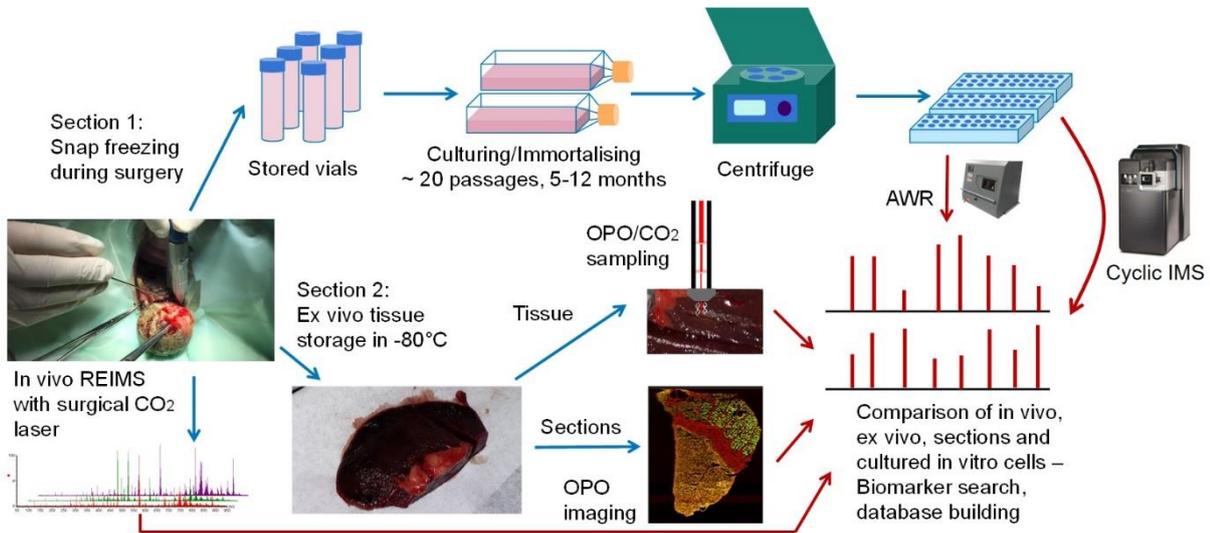
*Richard Schäffer, Waters Research Center*

*Gitta Schlosser, ELTE Eötvös Loránd University*

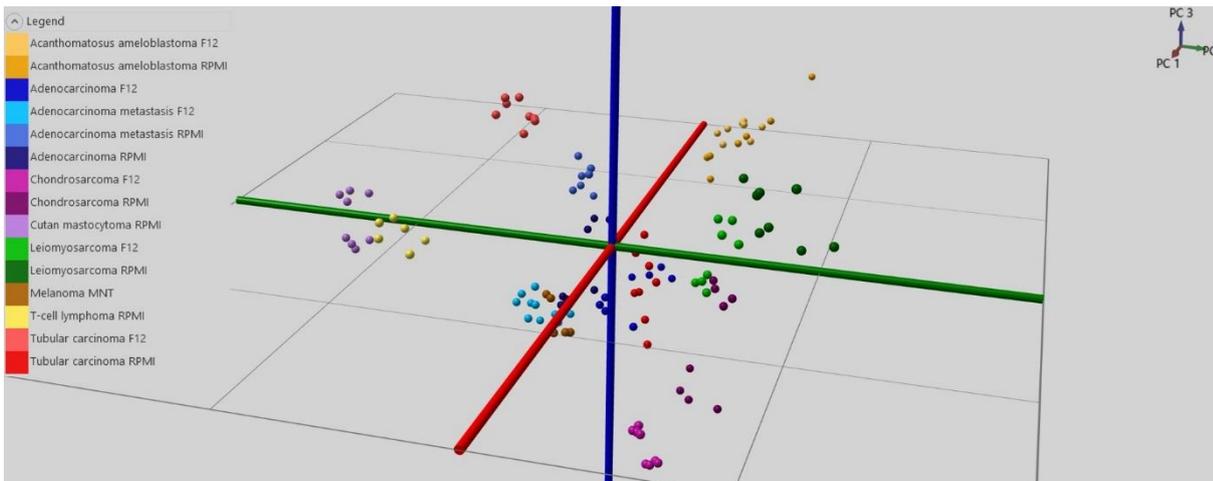
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Tuesday 30 August 2022 from 14:00 to 15:30 hours

Gergely Szakács, ELKH Research Center for Natural Sciences, Center for Cancer Research, Medical University of Vienna  
Júlia Balog, Waters Research Center



Combined workflow for the characterization of tumours and cell lines



PCA/LDA analysis of the immortalized cell lines (passage 20-21)

Poster number: **LS-PA-111**

## **ADVANTAGES OF A DYNAMIC POLYGON FOR MHC CLASS I AND II IMMUNOPEPTIDES**

Abstract ID: **214**

**Presenting author: Laura Heikaus, Bruker Daltonik GmbH**

### **Introduction**

MHC-associated peptides powerfully modulate T cell immunity and play a critical role in generating effective anti-tumor immune responses. Characterization of these peptides helps to generate therapeutic treatments and gain information on T cell mediated biomarkers. These peptides are challenging to characterize due to similar length, sequence conservation and lacking defined termini when compared to peptides generated upon enzymatic digestion. To overcome these challenges, use of PASEF (Parallel Accumulation and Serial Fragmentation) enables to generate high quality peptide spectra and resolve coeluting and isobaric peptides. Moreover, the capability to easily tailor the mobility space enables preferential detection of groups and sub-groups of relevant peptides

### **Methods**

MHC class I peptides were separated on a 100 minutes gradient by nanoElute UPLC (Bruker Daltonics) on a 25 cm pulled emitter column (IonOpticks) and analyzed on a TIMS enabled QTOF instrument using the PASEF method (timsTOF Pro, Bruker Daltonics). An estimated 200 ng of peptides were injected on column. For MHC class II peptides separation was performed on an EvoSep system (a 60 samples per day method – 21 min gradient) and analyzed on the same instrument as previously described. An estimated 25 ng of peptides was injected on the system. Data analysis was performed with PMI Byonic.

### **Preliminary data (results)**

More than 16,000 peptides and close to 4,000 protein groups (PG) were detected on average for each one of the 3 replicates for the MHC I sample (200 ng on column). Moreover, several isobaric and isomeric peptides, yet with completely different sequences, but overlapping retention time are distinctively separated by IMS (Ion-Mobility spectrometry) and subsequently fragmented without generating chimeric spectra. Two different polygons were utilized for scouting purposes. A broad polygon that included 1<sup>+</sup> ions generated approximately 20% more peptide IDs and 5% more PG. For MHC II sample, using the EvoSep platform and injecting 25 ng a standard and 1<sup>+</sup> inclusive polygon that included 1<sup>+</sup> ions mobility space are utilized. The 1<sup>+</sup> inclusive polygon enabled a detection of over 2000 peptides and more than 500 PG, which represents approximately a 20% increase of both peptides and PG over the standard polygon. Distribution of N-mer for MHC class I shows peptides with 9 amino acid residues, by far as the most abundant analytes. For MHC class II peptides, N-mer varying from 14 to 16 amino acidic residues are detected as being the most abundant ones.

### **Please explain why your abstract is innovative for mass spectrometry?**

Simultaneous analysis of 1<sup>+</sup> and higher charge state MHC class I and II

### **Co-authors:**

*Francesco Pingitore, Bruker Daltonics*

*Michael Krawitzky, Bruker Daltonics*

*Josh Elias, Chan Zuckerberg Biohub*

*Chris Adams, Bruker Daltonics*

Poster number: LS-PA-112

## TARGETED MASS SPECTROMETRY METHOD TO STUDY THE INHIBITORY EFFECT OF THE SELECTED APTAMERS ON CD28-B7 CO-STIMULATORY PATHWAY

Abstract ID: 312

**Presenting author: Abeer Malkawi, Department of Chemistry and Biochemistry, Université Du Québec à Montréal**

### Introduction

CD28-B7 is an essential co-stimulatory pathway for T-cell activation. Inhibiting T-cell activation is one of the main therapeutic strategies in autoimmune diseases, as in rheumatoid arthritis. The infusion protein CTLA4-Ig (Abatacept) competitively binds to CD80/CD86 and interrupts their interaction with CD28. Unlike Abatacept, aptamer-based medicines can be synthesized chemically in a reproducible approach that reduces the cost and the structural variations. Several aptamers have been developed to modulate the immune response by blocking or activating immune receptors, such as anti-CTLA-4 aptamer, which has an anti-tumor effect. The development of CD80/CD86 specific aptamers has a tremendous potential therapeutic advancement for RA by interrupting the binding of CD28-B7. This potential treatment will have several advantages over Abatacept.

### Methods

Our group has selected multiple aptamers specific to CD80 and CD86 proteins. The inhibitory effect of these aptamers is evaluated based on these proteins binding to CD28 using liquid chromatography-mass spectrometry (LC-MS/MS).

A multiple reactions monitoring (MRM) LC-MS/MS method was developed using signature peptides selected for CD80, CD86, CTLA-4, and CD28 proteins. The method is validated for biological matrices' accuracy, precision, and linearity. The binding between the CD28 and CD80/CD86 is established using on-beads trypsin digestion. Abatacept is used as a reference inhibitor and then the target aptamers.

### Preliminary data (results)

Specific aptamers to the CD80 and CD86 are selected using the SELEX protocol established in our lab. The signature tryptic peptides were selected based on bottom-up high-resolution MS experimental data collected for the standard proteins (TripleTOF 5600, Sciex, Concord, ON, Canada). For example, the CD80's on-bead tryptic peptides were identified, where the protein's amino acid sequence coverage reached up to 70% (in-solution digestion). <sup>95</sup>EHLAEVTLVK<sup>105</sup> (m/z 409.23, +3), and <sup>106</sup>ADFPTPSISDFEIPSNIR<sup>124</sup> (m/z 703.02, +3) are the highly abundant peptides selected for evaluating the bead-protein coupling efficiency. Unlabeled and labeled signature peptides were synthesized as standard reference materials to optimize the MRM transitions, i.e., precursor ion, production, collision energy, and cone voltage. The coupled beads with the His-tagged CD28 protein will be incubated with a particular CD80 or CD86. After eliminating the non-specific binding, the selected aptamers' ability to inhibit the CD28-CD80/CD86 binding will be determined by measuring the ratio of CD80/CD86 to CD28 on beads using the on-beads trypsin digestion and MRM LC-MS/MS method as in **Figure 1**

### Please explain why your abstract is innovative for mass spectrometry?

This targeted MS method has a significant role in studying specifically the inhibitory effect of the selected aptamers for drug development in autoimmune diseases.

### Co-authors:

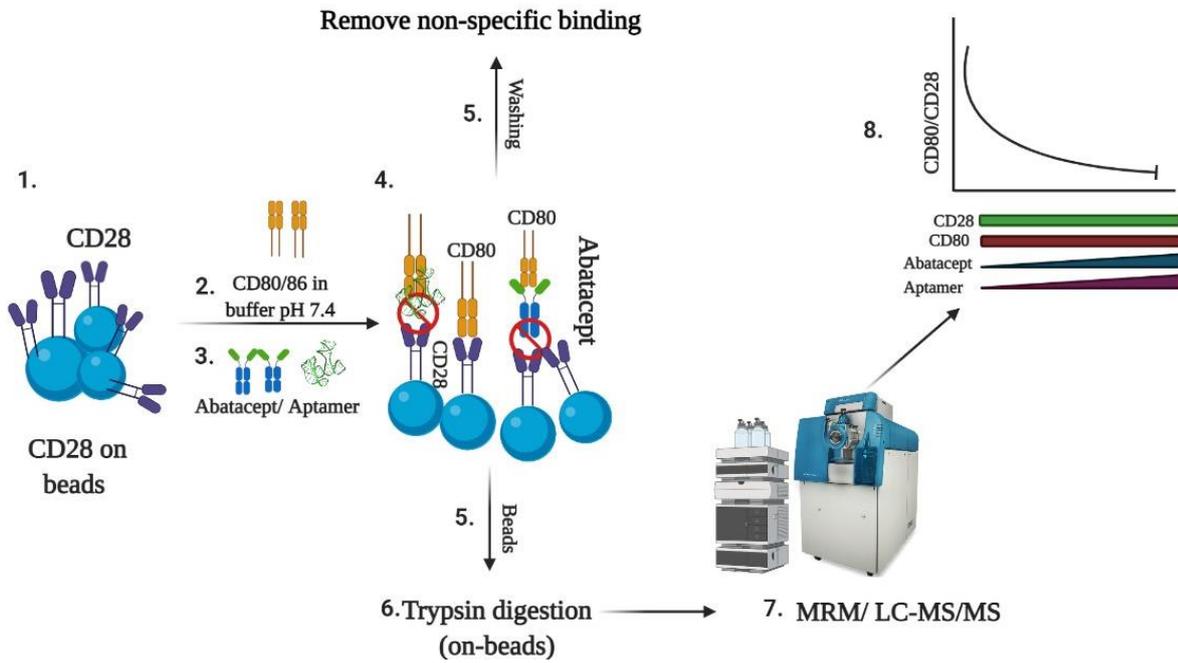
*Lekha Sleno, Department of Chemistry and Biochemistry, Université Du Québec à Montréal*

*Anas Abdelrahman, Metabolomics Section, Department of Clinical Genomics, Center for Genome Medicine, King Faisal Specialist Hospital and Research Center*

*Mohamed Siaj, Department of Chemistry and Biochemistry, Université Du Québec à Montréal*

**POSTER SESSION A**

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Poster number: **LS-PA-113**

## **MOVING TRANSLATIONAL MASS SPECTROMETRY IMAGING TOWARDS TRANSPARENT AND REPRODUCIBLE DATA ANALYSES: A CASE STUDY OF AN UROTHELIAL CANCER COHORT ANALYZED IN THE GALAXY FRAMEWORK**

Abstract ID: **401**

**Presenting author: Melanie Föll, Institute for Surgical Pathology, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany, Khoury College of Computer Sciences, Northeastern University, Boston, USA**

### **Introduction**

Mass spectrometry imaging (MSI) derives spatial molecular distribution maps directly from clinical tissue specimens. This allows for spatial characterization of molecular compositions of different tissue types and tumor subtypes, which bears great potential for assisting pathologists with diagnostic decisions or personalized treatments. Unfortunately, progress in translational MSI is often hindered by insufficient quality control and lack of reproducible data analysis. Raw data and analysis scripts are rarely publicly shared. Here, we demonstrate the application of the Galaxy MSI tool set for the reproducible analysis of an urothelial carcinoma dataset.

### **Methods**

Tryptic peptides were imaged in a cohort of 39 formalin-fixed, paraffin-embedded human urothelial cancer tissue cores with a MALDI-TOF/TOF device. The complete data analysis was performed in a fully transparent and reproducible manner on the European Galaxy Server. Annotations of tumor and stroma were performed by a pathologist and transferred to the MSI data to allow for supervised classifications of tumor vs. stroma tissue areas as well as for muscle-infiltrating and non-muscle invasive urothelial carcinomas. For putative peptide identifications, m/z features were matched to the MSiMass list.

### **Preliminary data (results)**

Rigorous quality control in combination with careful pre-processing enabled reduction of m/z shifts and intensity batch effects. High classification accuracy was found for both, tumor vs. stroma and muscle-infiltrating vs. non-muscle invasive urothelial tumors. Some of the most discriminative m/z features for each condition could be assigned a putative identity: Stromal tissue was characterized by collagen peptides and tumor tissue by histone peptides. Immunohistochemistry confirmed an increased histone H2A abundance in the tumor compared to the stroma. The muscle-infiltration status was classified via MSI by peptides from intermediate filaments such as cytokeratin 7 in non-muscle infiltrating carcinomas and vimentin in muscle-infiltrating urothelial carcinomas, which was confirmed by immunohistochemistry. To make the study fully reproducible and to advocate the criteria of FAIR (findability, accessibility, interoperability, and reusability) research data, we share the raw data, spectra annotations as well as all Galaxy histories and workflows. Data are available via ProteomeXchange with identifier PXD026459 and Galaxy results via [https://github.com/foellmelanie/Bladder\\_MSI\\_Manuscript\\_Galaxy\\_links](https://github.com/foellmelanie/Bladder_MSI_Manuscript_Galaxy_links).

### **Please explain why your abstract is innovative for mass spectrometry?**

We present one of the very first translational MSI studies that is fully transparent and reproducible. This was enabled by using the Galaxy framework for data analysis and sharing.

### **Co-authors:**

*Veronika Volkmann, Institute for Surgical Pathology, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany*

*Kathrin Enderle-Ammour, Institute for Surgical Pathology, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany*

*Dan Guo, Khoury College of Computer Sciences, Northeastern University, Boston, USA*

*Olga Vitek, Khoury College of Computer Sciences, Northeastern University, Boston, USA*

*Konrad Wilhelm, Center for Surgery, Medical Center, Department of Urology, Faculty of Medicine, University of Freiburg*

*Sylvia Timme, Institute for Surgical Pathology, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg,*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

*Germany, Core Facility for Histopathology and Digital Pathology, Medical Center-University of Freiburg and Faculty of Medicine, University of Freiburg*

*Peter Bronsert, Institute for Surgical Pathology, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany, German Cancer Consortium (DKTK) and Cancer Research Center (DKFZ), Freiburg, Germany, Tumorbank Comprehensive Cancer Center Freiburg, Freiburg, Germany*

*Oliver Schilling, Institute for Surgical Pathology, Medical Center - University of Freiburg, Faculty of Medicine, Freiburg, Germany, German Cancer Consortium (DKTK) and Cancer Research Center (DKFZ), Freiburg, Germany*

Poster number: **LS-PA-114**

## **CANCER CELL SURFACE PROTEINS STUDY FOR HIGHLIGHTED NEW POTENTIAL TARGETS FOR IMMUNOTHERAPY**

Abstract ID: **426**

**Presenting author: Melanie Rose, PRISM Inserm U1192**

### **Introduction**

All immunotherapeutic options currently available for treating cancer suffer from various limitations. One of the major problems is the high toxicity of the different treatments due to the absence of specific tumor antigens. This lack of specific therapeutic targets is caused by the heterogeneity of tumors, the low antigen expression at the surface of the cancer cells and to the workflow used to identify them. Due to their subcellular localization, cell surface proteins (surfaceome) are a rich source of immunotherapeutic target. Cancer cells often dysregulate their surfaceome, leading to the alteration of their expression pattern compared to the healthy proteome, as well as, the expression of protein variants.

### **Methods**

In this context, we investigated the glioblastoma-associated surfaceome by comparing it to astrocytes cell line surfaceome to identify new specific targets for glioblastoma treatment. For this purpose, biotinylation of cell surface proteins has been carried out in GBM and astrocytes cell lines with a membrane impermeable biotin to label primary amines on extracellular domains of cell-surface proteins. After cell lysis, biotinylated proteins will be enriched by streptavidin-affinity purification. Enriched proteins will be then digested and analyzed by shotgun mass spectrometry. Cell surface proteins were identified with Cell Surface Proteins Atlas (CSPA) and Gene Ontology enrichment.

### **Preliminary data (results)**

We found 57 surface proteins thanks to CSPA and *in silico* databases and 14 additional proteins being described as membrane proteins for a total of 71 surface proteins with differential expression between the three cell lines. Biological pathways analyses highlighted several proteins only expressed by NCH82 or U87 cell line linked to cell invasion or cell spreading, two key processes for GBM development and drug resistance. Moreover, most of the cell surface proteins are related to cell contact and cell adhesion pathway, another main process for tumor growth. Biological pathways analyses also established that several common proteins for glioblastoma cell lines and proteins only expressed by U87 are involved in immune regulation processes. Among all the surface proteins identified in the different cell lines, we have confirmed the expression of 66 of these in patients' glioblastoma using spatial proteomic guided by MALDI-mass spectrometry. Moreover, 87 surface proteins overexpressed or exclusive in GBM cell lines have been identified. Among these, we found 11 specific potential targets for GBM including 5 mutated proteins such as RELL1, CYBA, EGFR, and MHC I proteins. Matching with drugs and clinical trials databases revealed that 7 proteins were druggable and under evaluation, 3 proteins have no known drug interaction yet and none of them are the mutated form of the identified proteins.

### **Please explain why your abstract is innovative for mass spectrometry?**

Taken together, we discovered potential targets for immune therapy strategies in glioblastoma.

### **Co-authors:**

*Isabelle Fournier, PRISM Inserm U1192*

*Michel Salzet, PRISM Inserm U1192*

Poster number: **LS-PA-115**

## **THE NECROSOME COMPLEX AS A POTENTIAL THERAPEUTIC TARGET; FINDING NEW REGULATORS OF NECROPTOSIS THROUGH IP-MS BASED ANALYSIS**

Abstract ID: **430**

**Presenting author: Anastasia Piskpou, Utrecht University**

### **Introduction**

The development of Immune checkpoint blockade therapy (ICB), has enriched tremendously cancer treatment. However, its efficacy depends on the sufficient immune cell infiltration into the tumor microenvironment (TME). Consequently, in cases of cold tumors with low TME, resistance to ICB therapy is frequently observed. To this end, we need to define the regulatory cell signaling events that can stimulate tumor cell death and increase immune system activation in the TME. Necroptosis, is a form of immunogenic cell death that can be promoted upon TNF activation leading to efficient immunogenic response. To improve ICB outcomes, we need to find new regulators of necroptosis. Here, we show that such a study can be possible through colP-MS based analysis of the necrosome complex.

### **Methods**

Combinatory treatments with TNF $\alpha$ , Birinapant, and z-VAD-FMK were used to induce necroptosis in tumor cell lines. Cell viability was tested and analyzed through Incucyte® ZOOM imaging system while necrosome formation was investigated with Western Blot. To purify the necrosome complex, the Pierce™ Crosslink IP Kit was used, where the IP antibody is captured to Protein A/G Agarose resin. For column preparation, Rabbit RIPK3 antibody was used, targeting the necrosome complex. Antibody coupling to Protein A/G Plus Agarose and crosslinking success were tested by SDS-PAGE. All samples were analyzed by nano-LC-MS/MS on an Exploris Orbitrap MS.

### **Preliminary data (results)**

Preliminary results indicate that only MDA-MB231 cell line can undergo necroptosis, while both MCF7 and A549 are not affected by necroptotic treatment. Western Blot (WB) Assay validated the above results by showing formation of the necrosome complex in MDA-MB231 treated cells but not in MCF7. Phosphorylated forms of RIPK3 and MLKL were used as positive markers for necrosome formation. Interestingly, in case of A549, cells showed formation of the activated necrosome complex by WB, but no corresponding necroptotic cell death. This suggests that the necrosome undergoes additional regulation to prevent cell death initiation. Co-Immunoprecipitation results for MDA-MB231 cell line indicate the successful purification of the necrosome, as RIPK1, RIPK3 and MLKL were present in co-IP elutions. However, detailed examination of MS data revealed a very low abundance of RIPK3 in all samples, which highlights a high challenge in purification of the necrosome complex. Consequently, additional experiments with co-immunoprecipitation and MS analysis are required not only to determine which cocktail treatment leads to an active state of the complex but most importantly optimize its purification. A better understanding of the necrosome by mapping its regulators through IP-MS will reveal potential therapeutic targets to increase immune system activation in the TME, and finally tackle patient resistance to ICB therapy. IP-MS based analysis of the necrosome will shed new light into regulators of this important complex, and will expose PTM modifications responsible for modulation outcomes.

### **Please explain why your abstract is innovative for mass spectrometry?**

Through optimizing our IP-MS based analysis of the necrosome complex, we aim to contribute to the improvement of MS detection of low abundant protein complexes and their PTM regulations.

### **Co-authors:**

*Kelly Stecker, Utrecht University*  
*Elisavet Kalaitidou, Utrecht University*

Poster number: LS-PA-116

## A MASS SPECTROMETRY-BASED METHOD FOR THE DETERMINATION OF IN VIVO BIODISTRIBUTION OF SMALL MOLECULE-METAL CONJUGATE

Abstract ID: 509

Presenting author: Ettore Gilardoni, Philochem

### Introduction

Nuclear medicine plays a key role in modern oncology. The identification of primary and secondary tumor lesions is vital for a correct treatment of patients. Radionuclides as  $^{177}\text{Lu}$ ,  $^{68}\text{Ga}$ , and  $^{18}\text{F}$  are among the ones daily used in clinical practise. The need of novel molecules that selectively target tumors rather than healthy tissues is currently a hot topic where researchers and industries put lot of effort in. During the drug discovery process, the hit compounds identified must be tested in preclinical settings. However, radioactivity experiments are not easy to be carried out in terms of security and availability of materials and infrastructures.

### Methods

A LC-HRMS method was developed for the quantification of small molecule-metal conjugates.  $^{13}\text{C}$  and  $^{15}\text{N}$  stable isotopes were used as internal standard for quantification measurements of our analytes of interest in each organ. Sample preparation was optimized to reduce sample loss and increase sensitivity at the detector. Quantification data of several small molecule-metal conjugates from Philochem pipeline were carried out successfully. The methodology was further validated by comparison of LC-MS results with radiobiodistribution with gamma counter.

### Preliminary data (results)

Philochem radio-conjugates pipeline includes small molecule-radio conjugates targeting the fibroblast activation protein (i.e., OncoFAP radio-conjugates). In this study, we successfully developed a LC-MS method that can be used for the detection and quantification of OncoFAP-metal conjugates. For this purpose, several conjugated with stable isotopes (i.e.,  $^{nat}\text{Lu}$ ,  $^{nat}\text{Ga}$ ,  $^{nat}\text{F}$ ) were successfully tested. The reliability of LC-MS analysis was validated by direct comparison of MS data obtained with OncoFAP-DOTAGA- $^{nat}\text{Lu}$  and radiobiodistribution data obtained with OncoFAP-DOTAGA- $^{177}\text{Lu}$  (Figure 1). Results show that MS biodistribution of stable isotope metal conjugates is an optimal orthogonal tool for the preclinical characterization of different classes of radiotheranostics. It can be successfully applied for preliminary screenings of hit compounds and structure-activity relationship studies. Furthermore, MS-biodistribution avoids the handling of radio compounds, reducing safety issues, and opening-up new MS-based areas of research in drug development. In this perspective, radio-experiments can be performed on lead compounds and for confirmation purpose only. LC-MS also allowed to identify critical attributes in metal-chelator pairs, determining efficacy of labelling and stability issues. With our technology we were able to characterize the inefficient labelling of NODAGA chelator with  $\text{AlF}_3$  unraveling the chemical implication in the superiority of NOTA chelator in the same process.

### Please explain why your abstract is innovative for mass spectrometry?

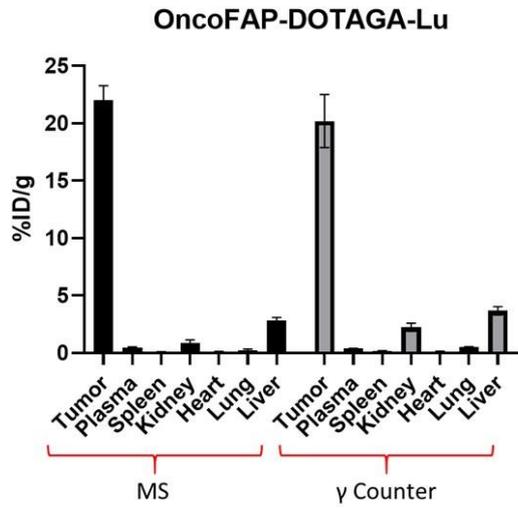
LC-MS biodistribution of small molecule-metal conjugates is a valid tool for the preclinical development of radiotheranostics.

### Co-authors:

*Andrea Galbiati, Philochem**Aurealino Zana, Philochem**Theo Sturm, Philochem**Samuele Cazzamalli, Philochem**Dario Neri, Philogen, ETH**Riccardo Stucchi, Philochem*

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours



HPLC-MS biodistribution of OncoFAP-DOTAGA-<sup>Nat</sup>Lu and radiobiodistribution of OncoFAP-DOTAGA-<sup>177</sup>Lu

Poster number: LS-PA-117

## LIPID PROFILE OF PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA

Abstract ID: 650

**Presenting author: Tialfi Bergamin De Castro, Maastricht MultiModal Molecular Imaging Institute, M4i, Maastricht University, Department of Molecular Biology, School of Medicine of São José do Rio Preto**

### Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common types of neoplasms and its main etiological factors are smoking, alcohol consumption and human papillomavirus (HPV) infection. The OSCC metabolic profile is heterogeneous and related to mutations in several tumor suppressor genes and oncogenes. Patterns have become apparent, such as Warburg effect, increased conversion of glutamine in glutamate, increased level of intermediates of citric acid/TCA cycle to reinforce energy production, increased choline metabolism, which indicates active proliferation. The phosphatidylcholine (PC), for example, is a key component of membranes and source of signaling molecules. The project aimed to analyze the glycerophospholipid profile in patients with OSCC comparing with control samples using mass spectrometry and gene expression techniques to analyze blood, tissue and clinical-pathological characteristics.

### Methods

Biocrates Life Sciences AG (Innsbruck, Austria) performed the blood analysis by LC-MS/MS using the AbsoluteIDQ® p180 Kit in 121 samples (61 OSCC and 60 controls without disease). MALDI-MSI was performed using a RapifleX Tissuetyper (Bruker Daltonik GmbH, Bremen, Germany) on 54 non-microdissected samples of OSCC from untreated patients with spatial resolution of 30 µm. The MS data was confirmed by DDA analysis using an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany) coupled to a MALDI source (Spectrograph). The gene expression analysis was performed with a 7500 Fast Real-Time PCR System (Applied Biosystems).

### Preliminary data (results)

Our preliminary results showed an increased expression of *PCYT1B* gene and decreased expression of *CHPT1* gene in cancer tissue compared to normal tissue. Both genes are related with step one and two, respectively, of the conversion of phosphocholine to PC. Comparing cancer and control groups the ROC curve showed an area under the curve (AUC) of 0.896, and a clear discrimination between the groups (figure 1). We found high levels of unsaturated PC and sphingomyelins (SM) in patients with oral carcinoma compared to controls matched by sex and age. This result may indicate an altered synthesis and secretion or plasma clearance of lipids. Neoplastic progression also showed increased biosynthesis of unsaturated lipids, which may represent *de novo* lipogenesis to meet the requirements of cell proliferation in the tumor tissue. Differently, the comparison of cases with lymph node clinically positive for metastases (cN+) and controls had an AUC of 0.912 (95% CI: 0.832-0.983), which indicates that certain metabolites may be important for clinical decisions regarding management of patients. The MSI approach showed alterations in lipid profiles between tumor and health tissue. The OSCC patients analyzed in the present study exhibited a distinct plasma metabolic profile suggestive of abnormal ketogenesis, lipogenesis and energy metabolism, which is more evident in advanced stages of the disease and may contribute to proliferation and inflammation. Such signature, if used in monitoring tests, may contribute to prognosis and treatment of OSCC.

### Please explain why your abstract is innovative for mass spectrometry?

Our previous data showed elevated levels of phosphatidylcholine and sphingomyelins in plasma of OSCC patients. Consequently, this project is using MSI in depth characterization of multiple lipid classes in OSCC

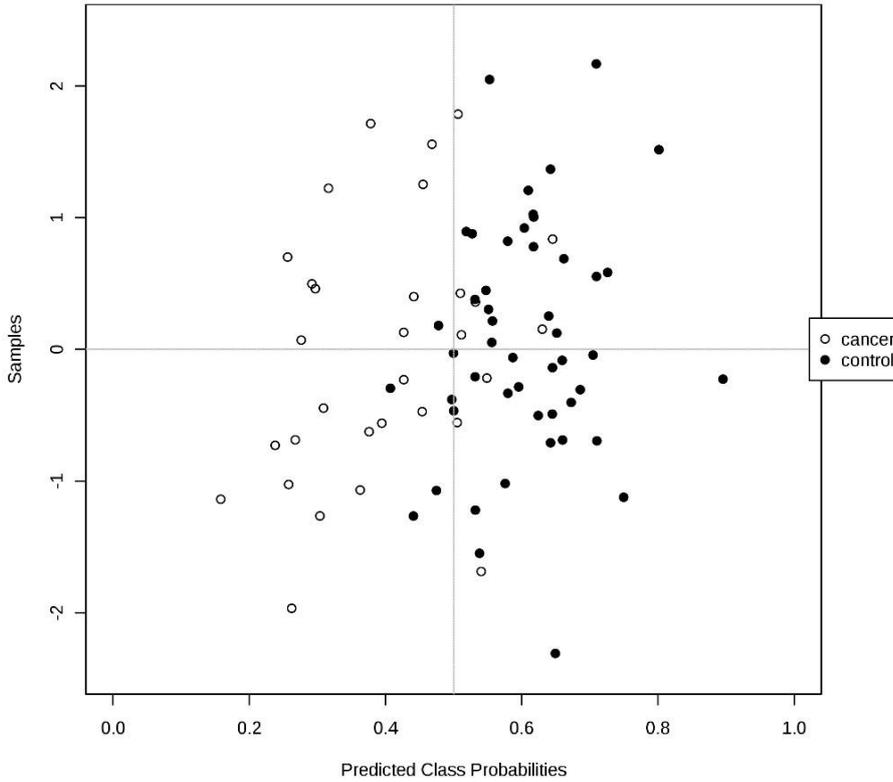
### Co-authors:

*Giovana Mussi Polachini, Department of Molecular Biology, School of Medicine of São José do Rio Preto*  
*Luis Fabiano Soares Smarra, Department of Molecular Biology, School of Medicine of São José do Rio Preto,*  
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*Rossana Veronica Mendonça López, Brazilian Biosciences National Laboratory, LNBio*  
*Ana Carolina de Mattos Zeri, Oncology Translational Centre, Cancer Institute of São Paulo*  
*Ismael Dale Cotrim Guerreiro da Silva, Department of Gynecology, Federal University of São Paulo*  
*Michiel Vandenbosch, Maastricht MultiModal Molecular Imaging Institute, M4i, Maastricht University*

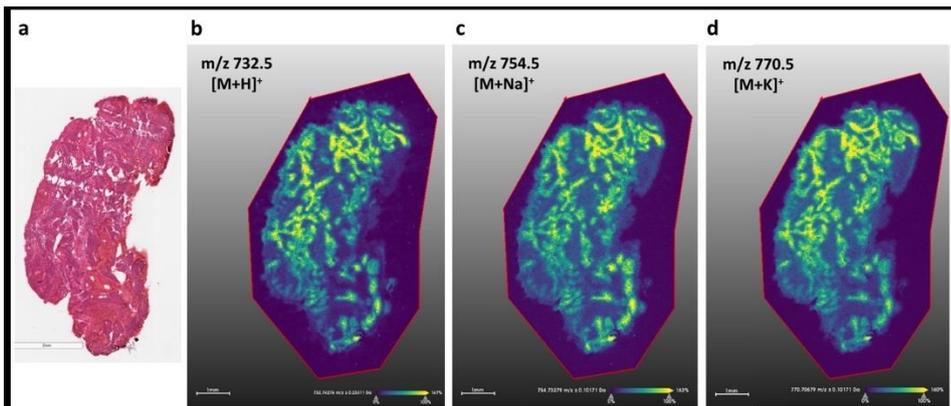
POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Ron M.A. Heeren, Maastricht MultiModal Molecular Imaging Institute, M4i, Maastricht University  
Eloiza Helena Tajara da Silva, Department of Molecular Biology, School of Medicine of São José do Rio Preto,  
Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo



Predicted probabilities shows a discrimination between cancer and control groups.



H&E staining and MS images of PC 32:1.

Poster number: **LS-PA-118**

## **CHEMORESISTANT CANCER CELL LINES ARE CHARACTERIZED BY MIGRATORY, AMINO ACID METABOLISM, PROTEIN CATABOLISM AND IFN1 SIGNALLING PERTURBATIONS**

Abstract ID: **870**

**Presenting author: Manuela Klingler-Hoffmann, University of South Australia**

### **Introduction**

Ovarian cancer (OC) is the deadliest gynaecological malignancy. 70% of ovarian cancers are diagnosed at a late stage, because of its often-asymptomatic progression, when the 5-year survival rate for patients is reduced to approximately 35%. This outcome has only slightly improved over the last 30 years, despite substantial progress being made in other cancers. Inherent or acquired chemoresistance and cancer dissemination within the peritoneal cavity remain the major barriers to curative ovarian cancer treatment. Describing the molecular changes observed in the chemo resistant phenotype of ovarian cancer cell lines and their associated biological functions might provide important insight into the pathogenesis of ovarian cancer and might help to inform future therapies.

### **Methods**

We have developed carboplatin-resistant cell line models using two ovarian cancer cell lines OVCAR5 and CaOV3 with the aim of identifying chemoresistance-specific molecular features. Chemotaxis and chick embryo chorioallantoic membrane (CAM) invasion assays revealed enhanced migratory and invasive potential in OVCAR5-resistant cells when compared to parental cell line. To provide a comprehensive molecular characterisation of chemo resistant ovarian cancer cell lines, we performed mass spectrometry-based metabolomic and proteomic analyses. Biological process analysis was performed using the DAVID database and the KEGG global metabolomic network.

### **Preliminary data (results)**

Our data revealed signalling and metabolic perturbations in the chemo resistant cell lines. A comparison with the proteome of patient-derived primary ovarian cancer cells grown in culture showed a shared dysregulation of cytokine and type 1 interferon signalling, potentially revealing a common molecular feature of chemoresistance. In addition, some of the proteins identified as differentially regulated are associated with patient survival using the Kaplan Meier plotter. A preliminary investigation of patient-derived cells highlights the need to perform broad biological and molecular analyses, and comprehensive *in vitro* and *in vivo* studies using a larger patient cohort.

### **Please explain why your abstract is innovative for mass spectrometry?**

We compare metabolomics and proteomics analysis for their ability to correctly group chemosensitive, chemoresistant cell lines and primary cells.

### **Co-authors:**

*Mitchell Acland, The University of Adelaide*  
*Noor A Lokman, The University of Adelaide*  
*Clifford Young, University of South Australia*  
*Dovile Anderson, Monash University*  
*Mark Condina, University of South Australia*  
*Chris Desire, University of South Australia*  
*Tannith M Noye, The University of Adelaide*  
*Wanqi Wang, The University of Adelaide*  
*Carmela Ricciardelli, The University of Adelaide*  
*Darren J. Creek, Monash University*  
*Martin K. Oehler, University of South Australia, The Adelaide University, Royal Adelaide Hospital*  
*Peter Hoffmann, University of South Australia*

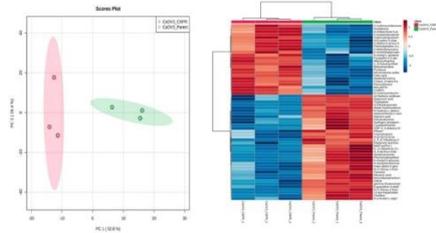
**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

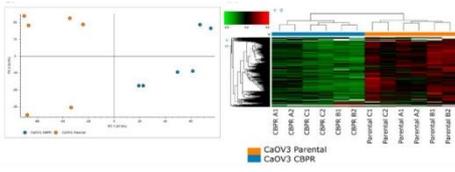
Parental Carboplatin-resistant



Metabolomics



Proteomics



Proteomic and metabolomic analysis of ovarian cancer cell lines

Poster number: **LS-PA-119**

## **PROTEOMICS CHARACTERIZATION OF FFPE LUNG CANCER SAMPLES**

Abstract ID: **913**

**Presenting author: Lilla Turiák, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network**

### **Introduction**

Lung cancer is among the most diverse and lethal types of cancer, therefore identifying alterations in proteins participating in events leading to this disease is crucial to improve current treatment options. We aimed to perform a detailed proteomic characterization of formalin-fixed paraffin-embedded (FFPE) tissue sections from patients with small cell (n=9) or non-small cell lung cancer (adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, n=10, 9, 10, respectively).

### **Methods**

FFPE tissues were dewaxed, antigen retrieval was performed followed by on surface tryptic digestion on cancerous and tumor-adjacent normal regions. Peptides were desalted then analyzed using nanoLC-MS(MS). Label free quantitation was carried out using MaxQuant. Statistical analysis was performed in RStudio, altered biological processes were identified using gene set enrichment analysis (GSEA).

### **Preliminary data (results)**

Principal component analysis (PCA) revealed considerable differences in molecular characteristics between the tumorous and the tumor-adjacent regions. Furthermore, hierarchical clustering displayed lung cancer type-specific molecular alterations. In order to identify the dysregulated biological processes shared between or specific to a specific lung cancer type GSEA was used. We have identified several biological processes disrupted in all investigated cancer types such as the degradation of the extracellular matrix and suppression of the complement and coagulation cascade as well as the activation of the MTORC1 signaling pathway. The dysregulated pathways revealed could contribute to a more precise classification of lung cancer types. Proteins with altered expression unique to a specific lung cancer type were also identified and these could be the targets of future studies and can also bear potential diagnostic and prognostic value.

### **Please explain why your abstract is innovative for mass spectrometry?**

Pilot study comparing four types of lung cancer in the same cohort using on-surface tryptic digestion of FFPE tissues.

### **Co-authors:**

*Simon Sugár, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network*

*Fanni Bugyi, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network*

*Gábor Tóth, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network*

*Ilona Kovalszky, Semmelweis University, Department of Pathology and Experimental Cancer Research*

*Tamás Tornóczki, Department of Pathology, Medical School and Clinical Center, University of Pécs*

*László Drahos, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network*

Poster number: **LS-PA-120**

## **CHOLESTEROL PROFILE OF BREAST CANCER, USING TOF-SIMS**

Abstract ID: **930**

**Presenting author: Auraya Manaprasertsak, Lund University**

### **Introduction**

Globally, about ten million die from cancer each year. In most cases, cancer spreads to remote organs and develops a resistance to therapy. To reduce the deadly impact of cancer, novel targets for therapy or markers for early detection are necessary. Cancer cells are known to deregulate cholesterol synthesis and to rely on its utilization for cell proliferation. We ask if this increased cholesterol synthesis leaves a vulnerability in the cholesterol structure that can be targeted in therapy or diagnosis.

### **Methods**

Breast tissues were collected from the murine models, which are FVB-PyMT and FVB as with and without cancer, respectively. The breast tissues were sectioned using cryostat microtome and freeze dried prior to the ToF-SIMS analysis. The ToF-SIMS spectra were recorded in positive mode, using  $\text{Bi}_3^+$  as the primary ion source. The peak areas from cholesterol were calculated in ratio to compare the breast tissues with and without cancer.

### **Preliminary data (results)**

The present study provides evidence to distinguish breast tissue with and without cancer from cholesterol distribution on tissue. The ratio of intact cholesterol peak ( $m/z$  385:  $\text{C}_{27}\text{H}_{45}\text{O}^+$ ) and fragment cholesterol peak ( $m/z$  369:  $\text{C}_{27}\text{H}_{45}^+$ ) from mouse breast tissue with and without cancer were 22% and 29%, respectively. The breast tissue without cancer exhibited more ratio of the intact cholesterol ( $m/z$  385) distribution due to the cholesterol deregulation in the cancer cell. We hypothesize that this reflects either a weakness in the molecular structure and decay or that the synthesis is incomplete. Further studies of cholesterol profiles could advance our understanding of its synthesis and utilization in cancer. Since cholesterol is a key component in cancer cell metabolism, insights to its synthesis can identify novel targets for therapy or diagnosis.

### **Please explain why your abstract is innovative for mass spectrometry?**

We utilized ToF-SIMS to investigate cancer to the chemical profile of cholesterol induced by cancer cell metabolism. We investigated breast tissue sections of mice with and without cancer.

### **Co-authors:**

*Emma Hammarlund, Lund University*  
*Per Malmberg, Chalmers University of Technology*

Poster number: **LS-PA-121**

## **AUTOMATED AND GENERIC WORKFLOW FOR PERSONALIZED MINIMAL RESIDUAL DISEASE MONITORING USING REAL-TIME DATA INDEPENDENT ACQUISITION – NEURAL NETWORK PROCESSING (DIA-NN) ON PASER**

Abstract ID: **962**

**Presenting author: Charissa Wijnands, Radboud University Medical Center**

### **Introduction**

Multiple Myeloma (MM) is the second most common hematologic malignancy characterized by clonally expanding plasma cells that produce monoclonal immunoglobulins (M-protein). The M-protein provides a patient-specific biomarker, however current M-protein detecting methods are not specific nor sensitive. As such, our lab has developed an LC-MS/MS method, targeting the variable region of the M-protein (MS-MRD), providing a personalized test-solution 1000-fold more sensitive.

Our current manual MS-MRD workflow relies on patient-specific PASEF-PRM methods, generated from PASEF-DDA runs, and manual data analysis using Skyline software. Although effective, the required manual investment per patient is time-consuming and error-prone making the real-life implementation in patient care challenging. We aim to generate an automated and generic workflow for which we evaluated the PASEF-DIA technology as a valid alternative for PASEF-PRM.

### **Methods**

The PaSER (Parallel Search Engine in Real-time; Bruker Daltonics) platform enables real-time data processing of DDA and DIA spectral data that are streamed directly from the timsTOF Pro instrument (Bruker Daltonics) to the PaSER box upon acquisition. Here we set out to develop an automated workflow that: (i) identifies M-protein clonotypic peptides from MM patient peripheral blood serum intake samples using the ProLuCID search engine and personalized protein sequence database to (ii) generate a patient-specific library and DIA-NN method for (iii) subsequent quantitation and reporting of M-protein concentrations in samples from MM-patients undergoing treatment.

### **Preliminary data (results)**

The personalized MS-MRD workflow starts with identification of clonotypic peptides that, after analytical validation, can be used for M-protein monitoring. To explore the detectability of clonotypic peptides using database assisted peptide identification in PaSER, serum samples from 10 newly diagnosed MM patients were analyzed using PASEF-DDA in combination with ProLuCID real-time database searching. Sequence coverage was on average 81% for the heavy chain and 88% for the light chain of the M-protein and 59/60 previously manually selected peptides were identified in duplicate injections.

To evaluate if PASEF-DIA can be used as generic solution to replace personalized PASEF-PRM methods we measured a dilution series from a pool of 5 MM patients with confirmed M-protein concentrations of >10g/L. All samples were analyzed by PASEF-PRM and PASEF-DIA and data processed using Skyline or DIA-NN, respectively. With both methods a linear decreasing signal intensity is observed as the dilution factor increases. The results further indicate that the LLOD on peptide level is more sensitive using DIA-NN compared to PRM-Skyline. In addition, DIA-NN provides a time-saving and more efficient method. In the same time that it takes to analyze one sample using PRM-Skyline, approximately 36 samples could be analyzed using DIA-NN. In conclusion our data suggests that automated and generic workflow for MS-MRD with PaSER and DIA-NN seems feasible without loss of sensitivity and DIA-NN provides a method that is less time consuming and labor intensive.

### **Please explain why your abstract is innovative for mass spectrometry?**

Proof-of-concept for the feasibility of implementing automated generic workflows in personalized diagnostic tests using real-time data processing and data independent acquisition for protein expression monitoring.

Poster number: LS-PA-122

## UNTARGETED METABOLITE PROFILING OF GINKGO EXTRACT EGB 761 WITH LC-HRMS

Abstract ID: 926

**Presenting author: Markus Schmitt, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co**

### Introduction

The *Ginkgo biloba* leaves special extract EGb 761<sup>®</sup> alleviates age-associated cognitive impairment. Targeted MS methods have been utilized in the past to study *in vivo* bioavailability and pharmacokinetics of the main constituents with known pharmacological activity, i.e. terpene lactones and flavonoids (glycosides). However, the potential contribution of the remaining ~63 % of the extract's constituents to the pharmacodynamics of EGb 761<sup>®</sup> remains elusive. In addition to the parent compounds from the extract, pharmacological effects can be mediated by metabolites formed by intestinal microbiota or upon absorption by host drug metabolism. The *in vivo* metabolite profiling presents a number of challenges due to the complex chemical composition of plant extracts with often unknown metabolites in a complex matrix.

### Methods

In the present study an approach was developed to compare different metabolite extraction protocols for rat brain tissues based on untargeted LC-HRMS analysis to capture as many treatment specific metabolites as possible. By using tissues from treated rats and controls, an optimized extraction method was developed without prior knowledge of target metabolites. Number and intensities of features specific for the treatment group were compared between different protocols. To improve metabolite confirmation, parallel acquisition with high resolution MS (Orbitrap) for unknown compounds was combined with low resolution MS (linear ion trap) for known EGb 761<sup>®</sup> constituents and potential metabolites.

### Preliminary data (results)

Optimization of metabolite extraction protocols in target tissues such as brain is difficult without knowledge of the chemical properties of these metabolites. The potential loss of unknown metabolites during sample preparation would not even be recognized. Thus, conditions for protein precipitation of brain homogenate and choice of the antioxidants were optimized for untargeted analysis and known EGb 761<sup>®</sup> constituents and metabolites, respectively. This was followed by liquid-liquid extraction with ethyl acetate or direct injection of the supernatant. Our data show that liquid-liquid extraction mostly enriched compounds specific for the EGb 761<sup>®</sup> treatment whereas with direct injection, features were detected that were either up- or downregulated in the treatment group. This indicates that direct injection after protein precipitation is a suitable approach to study changes in the metabolome after EGb 761<sup>®</sup> treatment. On the other hand, specific metabolites of EGb 761<sup>®</sup> were best detected using liquid-liquid extraction to enrich the analytes and remove matrix effects. The group of compounds specific for EGb 761<sup>®</sup> included the known constituents terpene lactones and flavonol glycosides. Especially in the brain, terpene lactones were the main features specific for the treatment group. However, additional compounds were found only in the treatment group in the plasma and brain samples. Among them we tentatively identified phenolic acids and its glucuronide and sulfate conjugates as another group of dominant features detected in the treatment group.

### Please explain why your abstract is innovative for mass spectrometry?

The present study shows an approach to utilize high resolution mass spectrometry to develop untargeted metabolite extraction protocols for plant extract bioanalytics in a complex matrix such as brain tissue.

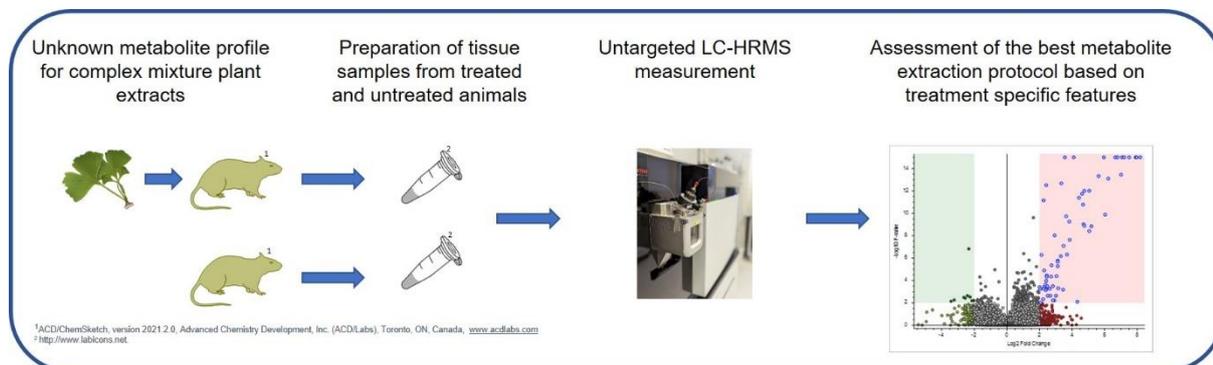
### Co-authors:

Heike Schneider, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co  
Simone Kaiser, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co  
Marianne Zeller, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co  
Gabriele Luderer, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co  
Thomas Gantert, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co

## POSTER SESSION A

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

Žarko Kulić, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co  
Martin D. Lehner, Department of Preclinical Research and Development, Dr. Willmar Schwabe GmbH & Co



Workflow to optimize extraction protocols of unknown metabolites from tissues prior to MS analysis

Approach to identify treatment specific unknown metabolites from plant extracts

**POSTER SESSION A**

Monday 29 August 2022 from 11:30 to 13:00 hours  
Monday 29 August 2022 from 14:00 to 15:30 hours  
Tuesday 30 August 2022 from 14:00 to 15:30 hours

# POSTER SESSION B

## Theme: Mass spectrometry across disciplines

### Session: Environmental MS: Geo, Water, Aerosols, VOC's and POC's

Poster number: AD-PB-001

## GADOLINIUM-BASED MRI CONTRAST AGENTS: FROM THE BODY TO THE AQUEOUS ENVIRONMENT AND BACK?

Abstract ID: 183

Presenting author: Uwe Karst, University of Münster

### Introduction

Since more than 25 years, gadolinium-based contrast agents (GBCAs) are used in magnetic resonance imaging, one of the most important modern tools for medical diagnostics, due to their strong paramagnetic properties. Initially being considered as extremely stable and therefore non-toxic, a more differentiated point of view has been established in recent years among radiologists and chemists. Additionally, a significant amount of anthropogenic gadolinium has been released into the environment, rendering GBCAs an important group of emerging contaminants. While these compounds are difficult to ionize by electrospray ionization mass spectrometry, liquid phase separations coupled to inductively coupled plasma-mass spectrometry (ICP-MS) have shown promise for the determination of GBCAs in the aqueous environment as well as in drinking waters.

### Methods

To investigate the distribution and fate of GBCAs in water samples, powerful methods of speciation analysis are required. In this work, a fully automated single platform approach for total metal analysis and syringe-driven chromatography in combination with ICP-MS was developed to identify and quantify several contrast agents in environmental and in drinking water samples. A method based on anion-exchange chromatography (IC) was set up to achieve the separation of the polar to ionic substances, whereas the hyphenation to quadrupole-based ICP-MS led to a highly sensitive element specific detection.

### Preliminary data (results)

The developed IC/ICP-MS method enables a fast separation of several commonly administered contrast agents in less than seven minutes, which is a significant improvement of analysis time in comparison to previously published methods. Limits of detection between 45 and 80 pmol/L turned out to be sufficient for the detection and quantification of GBCA in environmental samples without prior sample enrichment. To investigate the release and distribution of GBCA into the environment, the method was finally applied for speciation analysis of water samples obtained from different surface and drinking waters around the City of Münster.

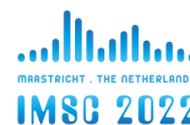
It could be shown that the vast majority of gadolinium is still present in form of the original GBCAs not only in surface waters after the wastewater treatment plant, but also in local drinking water originating from different water purification plants. As many studies have recently shown a deposition of Gd from GBCAs in the human body, including bones, skin and several areas of the brain, the question arises if an oral uptake of GBCAs is likely to result in a similar degree of deposition. To address this question, laser ablation (LA)-ICP-MS of human tissue samples was used to analyze the deposition of gadolinium from GBCAs and lanthanum from orally administered lanthanum carbonate, an emerging phosphate binder for dialysis patients.

Please explain why your abstract is innovative for mass spectrometry?

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



Gadolinium-based contrast agents are excreted, survive wastewater treatment and water purification plants and may enrich in the human body after ingestion

**Co-authors:**

*Marcel Macke, University of Münster*

*Derrick Quarles, Elemental Scientific, Inc.*

*Michael Sperling, University of Münster*

Poster number: **AD-PB-002**

## THE MYSTERY OF THE CHUKOTKA STINKY GRAY WHALES

Abstract ID: **198****Presenting author: Albert T Lebedev, Lomonosov Moscow State University**

### Introduction

Hunting on gray whales is an important element of subsistence and culture of Chukotka Native people. In 1990s, the hunters began reporting an increase in the number of hunted gray whales that exhibited a strong medicinal odor (so-called "stinky" whales). Some whales are noted as stinky from their blow at sea, but in other cases the odor only becomes obvious during cutting. Tissues from these stinky whales are deemed inedible (not palatable) and can cause stomach aches and other symptoms if consumed. Previous analyses failed to identify the chemical substance responsible for the medicinal odor. To resolve the problem tissues of normal and stinky whales (32 samples) were collected in 2021 at several sites in Chukotka.

### Methods

Samples 1-2g of whales tissues were mixed and mashed with Na<sub>2</sub>SO<sub>4</sub> using porcelain mortar, transferred to screw cap vial and agitated for 10 min at 60C and 250 rpm. Headspace of each sample was extracted with agitation by 50/30 µm divinylbenzene/Carboxen/Polydimethylsiloxane fiber for 20 min for solid-phase microextraction (SPME). Analysis was carried out with Shimadzu GCMS-QO2010 Ultra and LECO GCxGC-HRT+ instruments in splitless mode in the following program: 3 min desorption at 250C, initial oven temperature 40C (5 min), ramp 10C/min to 200C, then 20C/min to 300C (hold 1 min). Mass range - m/z 30-500.

### Preliminary data (results)

The problem of "stinky" whales was first tackled with GC-MS about 15 years ago. Conclusion was made that the crucial group of stinky compounds involves aldehydes, forming due to oxidation of the fats and aliphatic hydrocarbons, present in the analyzed samples in high concentration. However, it was mentioned that the quality of the samples was not good, as they were defrosted and refrosted many time before the analysis. The present set of samples was frosted only once and the smell of the "stinky" samples was quite pronounced. High levels of aldehydes were found in all samples of the normal whales, thus proving, that they were not responsible for the smell. The analysis revealed the presence of a wide array of small molecules with the known unpleasant odor. These were nitrogen and sulfur containing compounds including trimethylamine, dimethylsulfide, dimethyldisulfide, thiazole, indole, cadaverine, etc. The levels of these compounds were rather high, while in the normal whales they were absent or detected in the trace levels. The result obtained allows eliminating any organic pollutants, contaminating the waters of the Chukotka Sea. The source of the detected smelly compounds should involve distorted metabolism of the whales due to some disease or some bacterial contamination with releasing of the corresponding bacterial metabolic products. To answer these questions additional metabolomic/biological studies should be carried out.

### Please explain why your abstract is innovative for mass spectrometry?

GC-MS allowed establishing the smelly metabolome components in the samples of the gray whales tissues collected in the Chukotka Sea.

### Co-authors:

*Olga V Polyakova, Lomonosov Moscow State University*  
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Poster number: **AD-PB-003**

## **A CHARGE DETECTION MASS SPECTROMETER FOR CHARACTERIZING DUST GRAINS IN THE MARS ATMOSPHERE**

Abstract ID: **431****Presenting author: Daniel Austin, Brigham Young University**

### **Introduction**

We are developing a charge detection mass spectrometer (CDMS) for future Mars missions that will characterize the mass and charge of dust suspended in the Martian atmosphere. Atmospheric dust is a significant driver of the Martian climate and also represents a risk for both robotic and future human exploration of the red planet. Although much of the particle size distribution has been determined previously using optical scattering, the population of sub-micron-sized grains remains unknown, and the electrical charge on dust has not yet been measured. Accurate measurements of these properties is essential to design mechanical systems, filters, solar panels, and other dust-sensitive components. CDMS allows measurement of both mass (a surrogate for size) and charge of individual grains.

### **Methods**

The Mars CDMS includes a series of charge-sensitive and biased electrodes printed on the facing surfaces of two printed circuit boards (PCBs). During operation, Mars atmosphere is ingested into the instrument. As dust grains pass between the two PCBs, the resulting image charge provides the magnitude of the charge and the velocity of the particle. Biased electrodes alter the velocity based on  $m/z$ . Combining the measured  $m/z$  from the velocity change with the charge amplitude gives the absolute mass. The measured mass and charge for each particle is compiled into distributions.

### **Preliminary data (results)**

Preliminary experiments have used charged micron-sized particles including glass beads, polystyrene (PS) spheres, and finely-ground Mars regolith simulant (MMS-1). Electrospray charging is used for the PS and regolith simulant particles, and contact charging has been used with the glass beads. Electrosprayed suspension is introduced into the vacuum chamber and differentially pumped and desolvated before reaching the CDMS analyzer. Measured charge sensitivity is around 1000 electrons, although efforts to improve this number suggest a lower detection limit is possible. Mass accuracy is limited by both the charge accuracy and by the accuracy with which the change in velocity can be measured, which in turn limits the usable  $m/z$  range of the instrument. Because the instrument ingests Mars atmosphere, a higher throughput is desirable, but this must be balanced by the maximum pressure at which the electrostatic effect on velocity is greater than any aerodynamic effects. In the present experiments, the CDMS is operated at pressures of less than 1 Torr. The effects of collisions with background gas on dust grain trajectories through the instrument are significant above this pressure. An Amptek charge sensitive amplifier has been used for preliminary measurements, and a custom amplifier has also been developed and tested.

### **Please explain why your abstract is innovative for mass spectrometry?**

This is the first charge detection mass spectrometer designed for study of Mars atmospheric dust.

### **Co-authors:**

*Elaura Gustafson, Brigham Young University*  
*Kate Hales, Brigham Young University*  
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Poster number: AD-PB-004

## FATE OF BISPENOLS DURING CONVENTIONAL WASTEWATER TREATMENT

Abstract ID: 458

**Presenting author: Anja Vehar, Department of Environmental Sciences, Jožef Stefan Institute, Jamova cesta 39, 1000, Ljubljana, Slovenia, Jožef Stefan International Postgraduate School, Jamova cesta 39, 1000, Ljubljana, Slovenia**

### Introduction

Bisphenols (BPs) are synthetic organic compounds used in the production of epoxy resins and polycarbonate. BPA, the most common bisphenol, is a known endocrine-disrupting compound, and the concern over its safety has resulted in its gradual replacement by other BPs. Since they share a common structure of two hydroxyphenyl groups, they may also share endocrine-disrupting potential. The main sources of BPs are industrial and, on a smaller scale, municipal effluents, which are treated in wastewater treatment plants (WWTP). According to their physicochemical properties, they are only partially removed from the wastewater and thus they can be released into the environment *via* effluent discharge or sludge disposal. This study aims to determine their fate in a full-scale conventional WWTP.

### Methods

Samples of wastewater and sludge from different treatment stages, including primary treatment, secondary treatment with sequencing batch reactors, and anaerobic sludge digestion, were sampled (Figure 1). Aqueous samples were prepared following the method of Kovačič et al., 2019 and solid samples following the newly developed method to determine 16 BPs in sludge, based on solid-phase extraction and gas chromatography-mass spectrometry. Concentrations were recalculated into mass flows, used for determining the extent of adsorption to primary and secondary sludge and level of biodegradation.

### Preliminary data (results)

Ten out of 16 BPs were present in the influent, concentrations ranging from 2 ng L<sup>-1</sup> (BPP, 22BPF) to 434 ng L<sup>-1</sup> (BPS) and only 5 BPs were determined in the plant effluent (BPAP: 2 ng L<sup>-1</sup> to BPA: 79 ng L<sup>-1</sup>). The most abundant BPs in WW and sludge were BPA and BPS, and to a lesser extent 22BPF, 24BPF and 44BPF. Concentrations of BPS, BPA, 44BPF and 22BPF increased during the mechanical stage with the highest values in primary settler effluent. On average, 14% of the total BPs were adsorbed to primary and secondary sludge, 18 % remained in the effluent and 68% were biodegraded in sequencing batch reactors (Figure 2). In sludge, the highest concentrations of BPs were observed in the anaerobically stabilised sludge, compared to the primary and secondary sludge. During anaerobic digestion, BPS, 22BPF, BPE, BPAF and BPP were removed to some extent (11% BPE to 79% BPAF), but the mass flows of 24BPF, 44BPF, BPA and BPBP were higher in the anaerobically stabilised sludge than in primary and secondary sludge together. The highest daily emissions *via* effluent release (1.48 g day<sup>-1</sup>) and sludge disposal (4.63 g day<sup>-1</sup>) were for BPA. Overall, the data shows that the concentrations of BPs in sludge are not negligible, and for that reason, their emissions into the environment should be monitored and further studied.

### Please explain why your abstract is innovative for mass spectrometry?

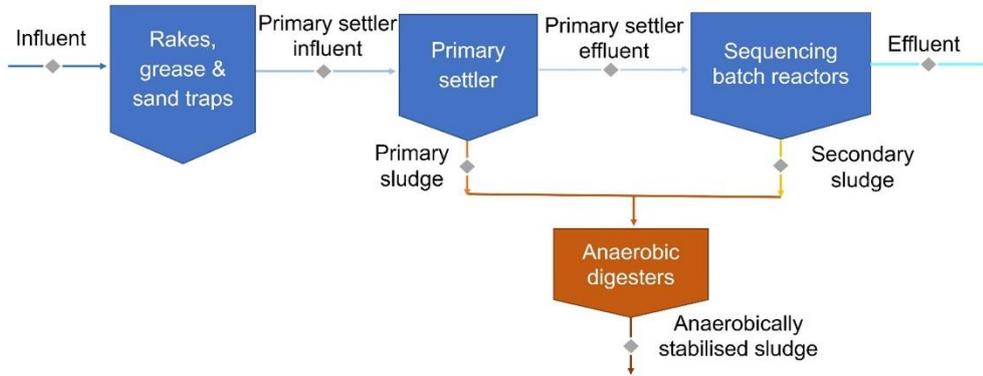
This was the first method that was developed for determining 16 BPs in sludge with GC-MS.

### Co-authors:

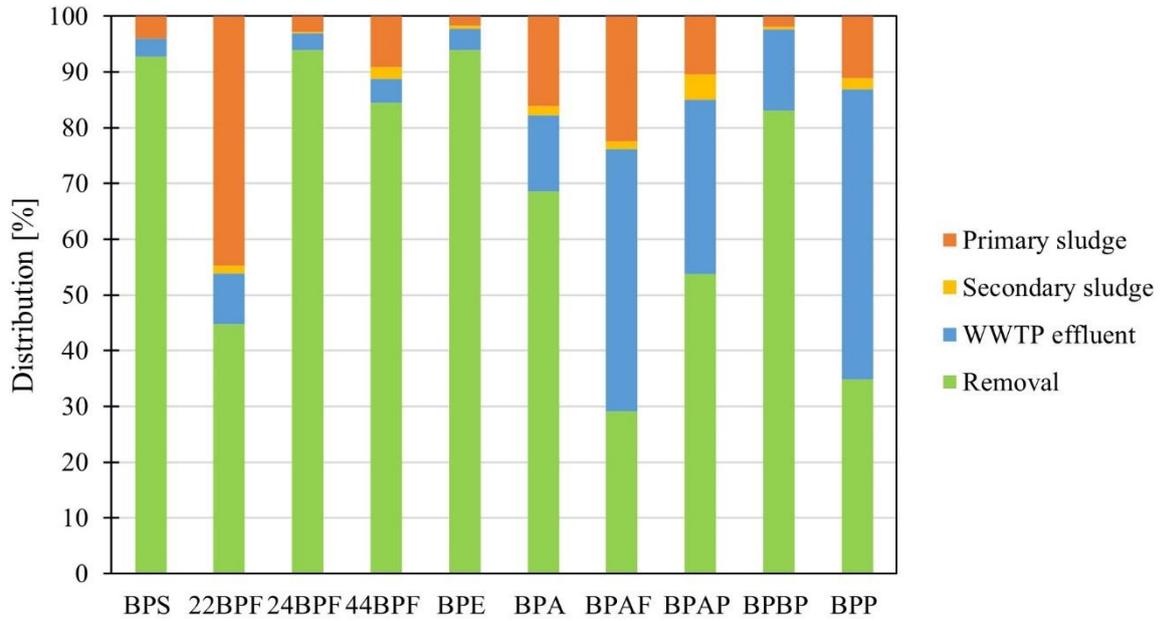
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Schematic of WWTP and sampling points.



Distribution and removal of BPs.

Poster number: **AD-PB-005**

## **SENSITIVE AND ROBUST QUANTIFICATION OF 15 COMMON UV FILTERS IN COMMERCIAL SUNSCREENS**

Abstract ID: **505**

**Presenting author: Jessica Smith, SCIEX**

### **Introduction**

Recent research has highlighted concerns related to the potential endocrine disrupting properties of some UV filters in sun care products. In light of recent developments, some states in the US are set to ban any sunscreen products that contain both octocrylene and avobenzone by 1<sup>st</sup> January 2023. In addition to this, other states in the US have already banned both oxybenzone and octinoxate due to damaging effects on the coral reefs. These developments have raised safety concerns. Therefore, it is paramount to assess the limits of a range of common UV filters that are found in commercial sunscreen products. Here a rapid and robust method has been developed for the sensitive detection of 15 common UV filters.

### **Methods**

A stock solution of 1000 µg/mL of 15 UV filter standards was prepared in methanol. A dilution series was prepared between 1-200 ng/mL to construct a calibration curve. Sunscreens (10 mg) were added to methanol (10 mL) and vortexed for 5 minutes. The resulting mixtures were then centrifuged for 5 minutes (@4500 RPM). The supernatant was then filtered through a 0.22µm PTFE syringe filter.

Chromatographic separation was performed using Phenomenex Luna Omega Polar C18 analytical column (C18 100Å, 3µm, 100 x 4.6). The MS system operated in sMRM mode using ESI in positive ion and negative ion mode.

### **Preliminary data (results)**

This analytical method provides simple sample preparation and a reproducible method for sensitive detection of common UV filters found in commercial sunscreen products. Calibration curves were generated for 15 UV filters across a concentration range of 1- 200 ng/mL. Accurate quantification was achieved across a linear range, where  $r^2 > 0.99$ , highlighting accuracy in quantification across this range. Precision and accuracy values for compounds of interest were within an acceptable criterion, with % CV <10% and accuracy within  $\pm 30\%$  of the expected value. Chromatographic evaluation shows good peak-peak separation with a total run time of 12 minutes. With regulation changes in both the US and EU, here we provide our customers with an economical, robust, and reproducible method for quantification of UV sunscreen filters.

### **Please explain why your abstract is innovative for mass spectrometry?**

UV sunscreen filters, octocrylene, oxybenzone, avobenzone, octinoxate, MRM, sensitive detection, robust, quantification, simple sample preparation, reproducible.

Poster number: **AD-PB-006**

## **USING PODCASTS FOR SCIENCE COMMUNICATION: AN EXAMPLE OF PUBLIC PERCEPTION TO MASS SPECTROMETRY APPLICATIONS**

Abstract ID: **541**

**Presenting author: Nayyer Rehman, WRG Europe Ltd, Mednarodna podiplomska šola Jožefa Stefana**

### **Introduction**

Public engagement in science is critical; it influences policies and budgeting related to scientific development. Podcasts are increasingly used as a tool for public engagement because of their personalised style and convenience. However, research is needed to assess what contributes to a podcast being an effective science communication tool. For example: What effects does presentation style and language accessibility have on audience perceptions? How does two-way exchange between presenter and audience influence trust?

Mass spectrometry, one of the most advanced and complex techniques for chemical analysis, is used in areas of significant public interest, namely pharmaceuticals, clinical diagnostics, genetics, geographic origin, chemical contaminants, and allergen management. Thus it presents an ideal topic for a podcast series to develop recommendations for optimal podcast design and delivery.

### **Methods**

Experts in the field of mass spectrometry were invited to participate in an informal conversation about their research and its benefits to people. These discussions were used as the basis for the development of a series of short-form podcasts (between 20 and 30 minutes).

Each podcast focused on the application of mass spectrometry in a different area of public interest. In addition to narrative analysis, focus groups were used to explore how the different features of the podcasts influenced knowledge development, perceptions, trust, and interest.

### **Preliminary data (results)**

Preliminary results provide evidence that podcasts are an effective means of increasing public knowledge of complex scientific topics such as mass spectrometry. An open access guidance document is being produced which can be utilised by scientists to maximise the generation of public interest in their area of research using podcasts, trust in the content being communicated and the willingness to further engage.

### **Please explain why your abstract is innovative for mass spectrometry?**

The creation of a new tool to effectively communicate developments in mass spectrometry and its applications in areas of public interest, leading to increased public engagement and feedback opportunities.

### **Co-authors:**

*Vicky Edkins, WRG Europe Ltd*  
*Nives Ogrinc, Institut "Jožef Stefan"*

Poster number: **AD-PB-007**

## NON-TARGET ANALYSIS OF DISINFECTION BYPRODUCTS IN DRINKING WATER USING ONLINE NANOLC-FT-ICR MS

Abstract ID: 551

**Presenting author: Limei Han, Department of Analytical Chemistry, Helmholtz Centre for Environmental Research (UFZ), 04318 Leipzig, Germany**

### Introduction

Chlorine disinfection of drinking water is a common process for waterborne disease control. During the process also disinfection byproducts (DBPs) form due to the unwanted reaction of chlorine with ubiquitous dissolved organic matter (DOM) in raw water.

The identification of unknown DBPs typically uses non-target analysis with high-resolution mass spectrometry (HRMS), such as Orbitrap or FT-ICR MS, etc. While the classical sample pretreatment solid-phase extraction (SPE) process can enrich the low concentrated DOC in the raw samples, it will also lose highly polar DBPs due to its weak extraction efficiency for highly polar compounds.

The objectives of this work were to develop a new online nLC-FT-ICR MS method to study the polar fraction of DBPs that were overlooked by traditional SPE pretreated measurements.

### Methods

Chlorinated drinking water samples were collected from Canitz drinking water treatment plant (Sachsen, Germany). The separation was performed with a nano-LC system (nLC), on a C18 column (75  $\mu\text{m}$  x 150mm, 2  $\mu\text{m}$ , Thermo Fisher Scientific, USA). After the separation on the column, the samples were directly introduced to the FT-ICR MS, which was coupled via the Captive spray nanoESI source for the ionization in negative mode. The nLC chromatogram from 11 to 21 min was split into 20 segments (spectra) with 0.5 min-width based on the model compounds elution profile. Formulas were assigned with the following elemental rules  $\text{C}_{1-60}^{13}\text{C}_{0-1}\text{H}_{1-122}\text{O}_{0-40}\text{N}_{0-2}\text{S}_{0-1}^{34}\text{S}_{0-11}^{35}\text{Cl}_{0-6}^{37}\text{Cl}_{0-6}$ .

### Preliminary data (results)

In total, 21095 (4874 unique) and 19856 (4474 unique) DOM molecular formulas (MFs) were detected in the direct measurement (CI-DW-nLC) and SPE processed measurement (CI-DW-SPE-nLC) of chlorinated drinking water samples, respectively. In the early four highly polar segments (11-13 min), over 8.3 times more MFs were uniquely detected in CI-DW-nLC (1650 MFs) as compared to the CI-DW-SPE-nLC (198 MFs). Most of the unique MFs detected in the CI-DW-nLC were eluted in these early segments, which shows that the direct measurement of the samples overcomes the bias from the traditional SPE pretreatment process.

In addition, the number of heteroatom-containing DOM compounds in CI-DW-nLC was also higher than those in CI-DW-SPE-nLC. In total, over 57% of the detected MFs were containing at least one nitrogen or sulfur (formula classes CHNO, CHNOS, CHOS), while only 51% of the compounds contains heteroatoms in CI-DW-SPE-nLC. Especially after 14 min (14-21 min), the percentage of heteroatom-containing compounds in all corresponding segments were higher in CI-DW-nLC as compared to CI-DW-SPE-nLC (between 3.3% and 17.3%). This confirms that SPE is also biased towards non-polar CHO compounds.

When investigating the potential DBPs, 4989 (3270 unique) MFs were detected, of which, only 33% (22%) were detected in CI-DW-SPE-nLC. Especially for the detection of potential DBPs in the high polarity segments (11-13 min), the intensity weighted oxygen to carbon (O/C) ratio was between 0.52 and 0.56, indicating that their polarity is very high, which in both chlorinated surface and groundwater were lower than 0.4.

### Please explain why your abstract is innovative for mass spectrometry?

1. Direct measurement of drinking water samples with the online nLC-FT-ICR MS method.
2. Highly polar potential DBPs were discovered.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

**Co-authors:**

*Oliver Lechtenfeld, Department of Analytical Chemistry, Helmholtz Centre for Environmental Research (UFZ), 04318 Leipzig, Germany, ProVIS – Centre for Chemical Microscopy, Helmholtz Centre for Environmental Research (UFZ), 04318 Leipzig, Germany*

*Thorsten Reemtsma, Department of Analytical Chemistry, Helmholtz Centre for Environmental Research (UFZ), 04318 Leipzig, Germany, Institute of Analytical Chemistry, University of Leipzig, 04103 Leipzig, Germany*

Poster number: **AD-PB-008**

## **DETECTION OF MYCOTOXINS IN HIGHLY MATRIX LOADED HOUSE-DUST SAMPLES BY QTOF-HRMS, IM-QTOF-HRMS, AND TQMS: ADVANTAGES AND DISADVANTAGES**

Abstract ID: **602****Presenting author: Benedikt Cramer, University of Muenster, Institute of Food Chemistry**

### **Introduction**

The analysis of mycotoxins in environmental samples represents an important tool for exposure assessment and for the evaluation of potential risks to human health. Currently, mass spectrometric detection by triple quadrupole (TQMS) systems is the established method of choice. However, screening methods using high resolution mass spectrometers (HRMS) find increasing application as they provide advantages such as enhanced selectivity and retrospective data analysis. Complex sample matrices are known to have enormous effects on mass spectrometric analyses. In the presented study, the impact of the complex sample matrix house dust on the performance of TQMS as well as quadrupole time-of-flight (QTOF)-HRMS instruments with and without ion mobility (IM) separation and a novel Bruker VIP-HESI ion source was evaluated.

### **Methods**

House dust samples were extracted with acetonitrile-water. For instrument comparison, extracts were fortified with different levels of multiple mycotoxins and analysed by ultra high performance liquid chromatographic (UHPLC) separation and mass spectrometric detection. The following instruments were tested: EVOQ Elite TQMS, Impact II QTOF, TIMS-TOF PRO IMS-QTOF (all Bruker). Both HRMS instruments were operated with electrospray ionization using the standard Apollo II source and the advanced VIP-HESI. After comparison and optimization, naturally contaminated dust samples were analysed.

### **Preliminary data (results)**

All performed experiments revealed strong signal suppression in mass spectrometric detection due to matrix components. Furthermore, TQMS remains the overall most sensitive detection method for these trace analytes (Figure 1). However, the application of the VIP-HESI ion source compared to the standard source during QTOF analysis resulted in a significant increase in sensitivity for most of the investigated mycotoxins as the LODs were reduced by a factor 2.5 on average compared to standard ESI ionisation (Figure 2). No general sensitivity gain was observed, when TIMS was applied ahead of QTOF detection. However, the application of TIMS strongly reduced noise and interferences, but also the application of HRMS alone compared to TQMS gave more selectivity. Consequently, peak integration and detection were easier, faster and better applicable for automated algorithms. Based on these data it seems likely, that in the future the use of HRMS instruments will be more widespread in many areas of (quantitative) application as QTOF-HRMS and especially IM-QTOF-HRMS instruments bring additional advantages in speed, nontarget screening capabilities, retrospective data analysis and multi-analyte detection. However, in the performed experiments the TQMS instrument enabled by far the most sensitive detection of mycotoxins in house dust (compare Figure 2). Therefore, a set of 21 naturally contaminated samples was analysed applying the developed UHPLC-TQMS approach revealing the presence of 8 mycotoxins deriving from different indoor moulds.

### **Please explain why your abstract is innovative for mass spectrometry?**

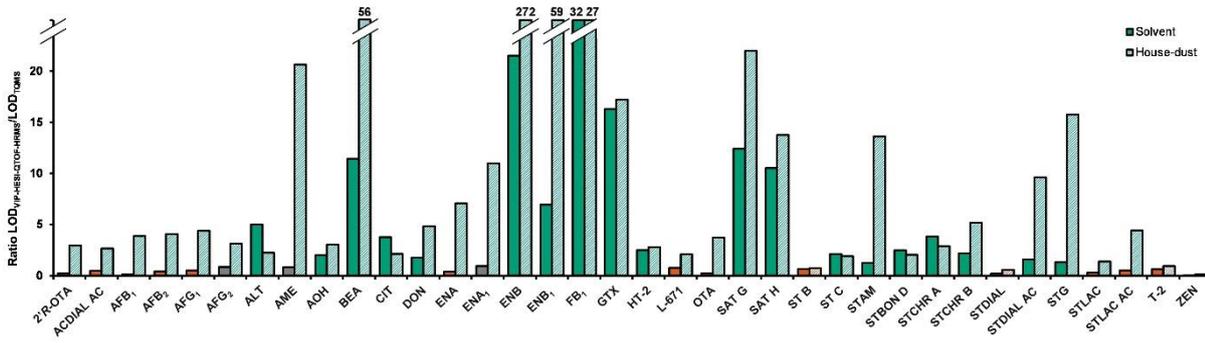
Technical improvements push HRMS instruments further into the field of trace analysis. Recent technical advances are evaluated for their suitability to overcome matrix effects caused by complex, non-purified samples.

### **Co-authors:**

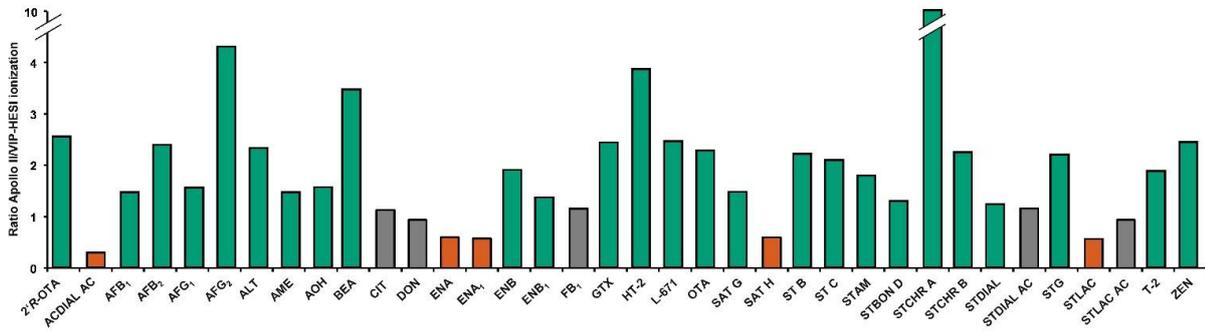
*Viktoria Lindemann, University of Muenster, Institute of Food Chemistry*  
*Jessica Schmidt, University of Muenster, Institute of Food Chemistry*  
*Hans-Ulrich Humpf, University of Muenster, Institute of Food Chemistry*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



Comparison of LODs of mycotoxins in dust and neat solvent.



Effects of ionisation sources on the sensitivity of QTOF-HRMS detection.

Poster number: **AD-PB-009**

## A METHOD FOR MONITORING ESTROGENS IN WHOLE SURFACE WATERS BY GC-MS/MS

Abstract ID: **607**Presenting author: **Ana Kovačič, Jožef Stefan Institute, Ljubljana, Slovenia**

### Introduction

Natural and synthetic estrogens are key endocrine-disrupting chemicals. Despite occurring at ultra-trace levels (below  $\text{ng L}^{-1}$ ), it is believed that they are contributing to an increase in feminized fish and other endocrine disruptive effects, and hence, their inclusion in the Watch list was not unexpected. One of the main sources of estrogens to surface waters is wastewater effluent. Once in surface waters, they can partition into different compartments, i.e., water and suspended particulate matter. For this reason, there is an urgent need for a methodology to monitor estrogen levels below the environmental quality standards (EQS) set by the Water Framework Directive requirements.

### Methods

In this study, a precise and accurate gas chromatography-mass spectrometry method (GC-MS/MS) for the analysis of estrone (E1),  $17\beta$ -estradiol ( $17\beta$ -E2),  $17\alpha$ -estradiol ( $17\alpha$ -E2), 17-alpha-ethinylestradiol (EE2), and estriol (E3) in whole water samples with  $\text{ng L}^{-1}$  limit of quantification (LOQ) was developed and validated in accordance with CEN/TS 16800:2020 guidelines.

### Preliminary data (results)

We established the equilibration conditions of native and isotopically labelled estrogens in a whole surface water sample ( $T = 4\text{ }^{\circ}\text{C}$ ,  $t = 12\text{ h}$ ). The method involved extracting 0.5 L of whole synthetic surface water containing 0.5 L natural water (Evian),  $7\text{ mg L}^{-1}$  dissolved organic carbon, and  $50\text{ mg L}^{-1}$  of suspended particulate matter, using Atlantic® ReadyDisk DVB. The optimized steps of sample extraction (conditioning with 10 mL of methanol, ethyl acetate, and ultrapure water, washing with 10 mL 20% MeOH, drying for 45 min, and eluting with 3 times 4 mL ethyl acetate) resulted in recoveries of 88% to 99%. The most efficient derivatization was achieved by adding 25  $\mu\text{L}$  of trimethylsilyl iodide and 25  $\mu\text{L}$  of pyridine and heating at  $90\text{ }^{\circ}\text{C}$  for 1 h. From an analytical perspective, it is safe to store derivatized extracts for seven days, at  $-20$  or  $4\text{ }^{\circ}\text{C}$ .

### Please explain why your abstract is innovative for mass spectrometry?

The developed traceable MS-based methods with LOQs for estrogens between 0.1 ( $< 30\%$  EQS) and  $1\text{ ng L}^{-1}$  will contribute to improved monitoring and risk assessment of aquatic systems.

### Co-authors:

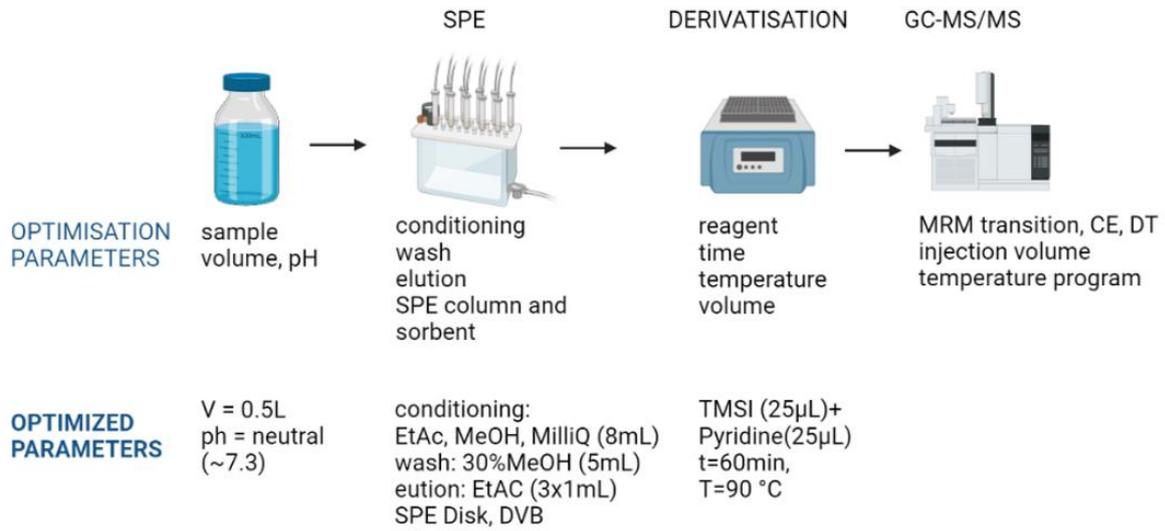
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*Lorin Steinhäuser, Bundesanstalt fuer Materialforschung und - pruefung BAM, Berlin, Germany*

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Method development for determination of estrogens using GC-MS/MS



Method development for determination of estrogens using GC-MS/MS.

Poster number: **AD-PB-010**

## **MONITORING OF ALBENDAZOLE TRANSFER FROM SHEEP DUNG TO SOIL AND FODDER PLANTS USING UHPLC-MS/MS**

Abstract ID: **615**

**Presenting author: Martina Navrátilová, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic**

### **Introduction**

Albendazole, commonly used anthelmintic drugs (ADs), is used to prevent and treat parasitic infections in agriculture and humans. Just as antibiotic residues can negatively affect biota – by contributing to an increase in the resistant population of bacteria, ADs residues can have the same effect on helminths. Efforts to increase the use of organic fertilisers over mineral fertilisers have grown in importance in recent years, which brings the risk of transferring anthelmintic residues to the fields and field products. Our study aimed to determine whether albendazole could be transferred to soil and plants after field fertilisation. Answers could help us better understand the connection between the presence of the anthelmintic drugs in the environment and the presence of the resistant helminths.

### **Methods**

We fertilised two fields (each approx. 50 m<sup>2</sup>) with fodder plants, the first with dung from albendazole (10 mg/kg b.w) treated sheep and from non-treated sheep. Samples of soil in two depths and two plant species, *Medicago sativa* and *Trifolium pratense*, all in various distances from dung pots (0 – 75 cm), were collected during 13 weeks after fertilisation.

QuEChERS (soil) and LLE (plants) extraction methods were used to determine albendazole and its transformation products, such as albendazole sulfoxide and albendazole sulfone. Then, the UHPLC-MS/MS method was used to quantify the albendazole and its transformation products.

### **Preliminary data (results)**

The UHPLC-MS/MS method was optimised and validated in terms of selectivity, sensitivity, recovery, linearity and matrix effects according to SANTE/11813/2017 guideline: Analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. We focused on the anthelmintic drug albendazole, its two main transformation products, albendazole sulfoxide and albendazole sulfone, as the most abundant anthelmintic in Central Europe. Our study successfully detected albendazole sulfoxide and albendazole sulfone in the soil-plant system. Even two months after the first contact of fodder with treated dung, albendazole sulfoxide was still present in plants and connected soil.

### **Please explain why your abstract is innovative for mass spectrometry?**

A fast and sensitive method was developed for determining emerging contaminants in the environment.

### **Co-authors:**

*Andrea Sochová, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic*

*Ivan Vokřál, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic*

*Petra Matoušková, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic*

*Lenka Skálová, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic*

Poster number: AD-PB-011

## ASSESSING CAPABILITIES OF LC-IM-TOFMS FOR RELIABLE QUANTIFICATION IN WATER ANALYSIS

Abstract ID: 629

**Presenting author: Sven Kochmann, University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Institute of Analytical Chemistry**

### Introduction

Separation by ion mobility (IM) of gas-phase ions reflects their size and shape and is a good complement to liquid chromatography time-of-flight mass spectrometry (LC-TOFMS). LC-IM-TOFMS can increase selectivity, identification confidence, and the number of detected compounds, in particular isomers, while maintaining the same conditions as LC-TOFMS. Due to these benefits, several qualitative workflows have been developed and established, but the complexity of LC-IM-TOFMS datasets has hindered development of automated quantitative workflows to this point. In this work, we assess the capabilities of LC-IM-TOFMS for reliable and robust quantification in the context of water analysis by developing a workflow using established open-source software to tackle the complex datasets (Figure 1). Our workflow is validated by comparison to an established LC-TOFMS workflow.

### Methods

LC was performed using an Agilent 1290 Infinity II UHPLC system with a Waters Atlantis HSS T3 RPLC (150 mm × 2.1 mm, 1.8 μm particles). 0.1% v/v formic acid in water and 0.1% v/v formic acid in acetonitrile were used as eluents; the elution program was optimized while maintaining a maximum retention time of less than 30 minutes. For IM separation and MS detection, an Agilent 6560 IM-QTOFMS (a low-field drift tube IM system) equipped with a Dual AJS ESI ion source was used in positive mode. Data processing was performed in MS-DIAL and Skyline.

### Preliminary data (results)

Water samples with 49 suspect compounds as well as calibration standards with 50 calibration compounds were prepared within a trial for interlaboratory comparison of (semi-)quantitative LC/HRMS non-targeted screening in the frame of the network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances (NORMAN). Water samples and calibration standards were measured using the LC-TOFMS method developed within the interlaboratory study as well as with an LC-IM-TOFMS method. The LC-TOFMS dataset was used as benchmark for quantitative results obtained in LC-IM-TOFMS.

For TOFMS, calibration compounds were identified with a high degree of confidence (Level 1 according to Schymanski *et al.*) and range from 0.85 nmol L<sup>-1</sup> to 8.9 μmol L<sup>-1</sup> concentrations. Most compounds showed a linear response using ESI-TOFMS. The remaining compounds were identified with lower degrees of confidence (Level ≤3) and not fully quantified due to insufficient sensitivity and selectivity. Most of the suspect compounds were identified with a high degree of confidence (Level 1) and quantified inside the calibration range (see above). The remaining compounds were identified with lower degrees of confidence (Level ≤3) and not quantified.

For IM-TOFMS, datasets were successfully evaluated using a workflow on MS-DIAL and Skyline. MS-DIAL was used for peak-picking, retention time and arrival time alignment, and identify confirmation. Further processing of the LC-IM-TOFMS dataset in Skyline allowed full use of the IM dimension for absolute quantification. Results were compared with LC-TOFMS data.

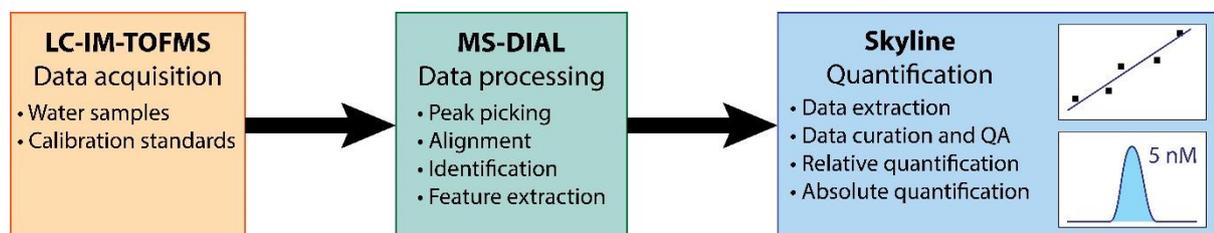
### Please explain why your abstract is innovative for mass spectrometry?

Development of a workflow for LC-IM-TOFMS that provides reliable, robust, and quantitative information about chemicals of concern in water samples.

### Co-authors:

Tim Causon, University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Institute of Analytical Chemistry

*Louise Malm, Stockholm University, Department of Materials and Environmental Chemistry*  
*Anneli Kruve, Stockholm University, Department of Materials and Environmental Chemistry*  
*Stephan Hann, University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Institute of Analytical Chemistry*  
*Teresa Steininger-Mairinger, University of Natural Resources and Life Sciences, Vienna, Department of Chemistry, Institute of Analytical Chemistry*



Proposed workflow for LC-IM-TOFMS.

Poster number: **AD-PB-012**

## **MASS SPECTROMETRIC NON-TARGET SCREENING IN PRACTICE - QUO VADIS?**

Abstract ID: **698**

**Presenting author: Thomas Letzel, AFIN-TS**

### **Introduction**

Mass spectrometric non-target screening (NTS), non-target analysis and untargeted screening are synonyms for the fact that mass spectrometric driven ion extraction, fragmentation and fragment detection is leading to new insights into very complex samples (sometimes without former molecular knowledge of the experiment-performing analyst).

### **Methods**

The analytical performances are wide-spread in using various chromatographic separation, (ion mobility) as well as (tandem)mass spectrometric detection and mostly quality-assured. The subsequent data evaluation and data interpretation steps are ongoing research topics to realize flexible but reproducible data handling as steps like the so called peak picking, componentization, alignment and others. These developments are ongoing but on a good way.

### **Preliminary data (results)**

However, new challenges in NTS come up, if one wants to answer specific questions in different disciplines which is mostly very application specific and needs adjusted but robust holistic solutions. On the other hand, there are solutions available that can be used in interdisciplinary context.

This presentation includes the similarities and differences of NTS concepts and workflows in different disciplines. They are presented for real life solutions in environmental analysis (with the identification of emerging compounds in water samples), in plant metabolomics samples (with the molecular reflection of metabolomics pathways), in food analysis (with focus on authenticity check) and in clinical analysis (with a view on disease biomarkers).

### **Please explain why your abstract is innovative for mass spectrometry?**

The state-of the art reflection gives direct consequences for the future of NTS in research and in practice; both will be discussed.

### **Co-authors:**

*Stefan Bieber, AFIN-TS*

Poster number: AD-PB-013

## SEX PHEROMONES IN DIATOMS

Abstract ID: 714

**Presenting author: Franziska Klapper, Institute for Inorganic and Analytical Chemistry, Bioorganic Analytics, Friedrich Schiller University Jena, Lessingstrasse 8, D-07743 Jena, Germany**

### Introduction

Benthic diatoms like *Seminavis robusta* are unicellular algae shaping biofilms in marine and freshwater ecosystems. They have a unique life cycle constituted of a vegetative phase dominated by cell division and a sexual phase. The latter is initiated at a specific cell size and is supported by pheromones guiding cells of different mating types.[1] Comparative metabolomics analyses of cell exudates of different mating types (+/-) and cell sizes using LC-MS data allowed identification of at least three pheromones involved in the mating process. I-Diproline was elucidated as the first sex pheromone in diatoms [2]. Structure elucidation of the two sex inducing pheromones (SIP) [3], however, is challenging since they are produced in minute amounts and of higher molecular weight compared to I-diproline.

### Methods

Structural analysis of SIP<sup>+</sup> was performed by high resolution mass spectrometry through studying the isotopic distribution and conducting MS<sup>n</sup> experiments, both, in negative and positive ionization mode. Mass inaccuracy of MS Orbitrap measurements in higher *m/z* ranges required isotopic labeling experiments with <sup>15</sup>N nitrate and <sup>13</sup>C carbonate to determine the sum formula. MS<sup>2</sup> experiments and subsequent MS<sup>3</sup> experiments using in source fragmentation allowed to match fragments resulting from different collision energies. Furthermore, reduction reactions of SIP<sup>+</sup> were monitored.

### Preliminary data (results)

Using isotopic labeling, MS<sup>2</sup> as well as NMR experiments we could identify first properties of SIP<sup>+</sup>.

1. Chepurnov, V.A., et al., *Sexual Reproduction, mating system, and protoplast dynamics of Seminavis (Bacillariophyceae)*. J. Phycol., 2002. **38**(5): p. 1004-1019.
2. Gillard, J., et al., *Metabolomics Enables the Structure Elucidation of a Diatom Sex Pheromone*. Angew. Chem. Int. Ed., 2013. **52**(3): p. 854-857.
3. Moeys, S., et al., *A sex-inducing pheromone triggers cell cycle arrest and mate attraction in the diatom Seminavis robusta*. Sci. Rep., 2016. **6**: p. 19252.

### Please explain why your abstract is innovative for mass spectrometry?

Combination of negative and positive ionization mode of ESI-MS<sup>2</sup> and MS<sup>3</sup> experiments allowed to identify first properties of SIP<sup>+</sup>.

### Co-authors:

Wim Vyverman, Laboratory of Protistology and Aquatic Ecology, Department of Biology, University Gent, Krijgslaan 281 S8, 9000 Gent, Belgium  
Georg Pohnert, Institute for Inorganic and Analytical Chemistry, Bioorganic Analytics, Friedrich Schiller University Jena, Lessingstrasse 8, D-07743 Jena, Germany, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, D-07745 Jena, Germany

Poster number: **AD-PB-014**

## **CHARACTERISATION OF BIO-OILS AT MOLECULAR LEVEL BY SPECIFIC DERIVATIZATION OF FUNCTIONAL GROUPS.**

Abstract ID: **832**

**Presenting author: Anthony Abou Dib, LCP-A2MC, FR 2843 Institut Jean Barriol de Chimie et Physique Moléculaires et Biomoléculaires, FR 3624 Réseau National de Spectrométrie de Masse FT-ICR à très haut champ, Université de Lorraine, ICPM, 1 boulevard Arago, 57078, Metz Cedex 03, France**

### **Introduction**

A wide range of high-value compounds from sustainable alternatives to petroleum, such as lignocellulosic biomass can be produced by different conversion treatments. Among them, pyrolysis at temperature up to 500°C without oxygen leads to the production of a liquid (called bio-oil) containing a wide variety of oxygenated compounds derived from cellulose, hemicellulose, and lignin. At present, the investigation of bio-oil composition includes GC, FTIR and NMR which unravels partly the molecular diversity or give overall data on structures or chemical features. FT-ICR MS petroleomic approach provides elemental composition details at the molecular level and yields a raw formula to each detected feature. To go deeper, this contribution proposes to combine an integrated fractionation-derivatization workflow to evidence the difference bio-crude oil class of compounds.

### **Methods**

To characterize the poly-functionality compounds of bio-crude oil, derivatization and fractionation steps have been used prior analyses by ESI and APPI FT-ICR MS. Fractionation was done by solid-phase extraction using an anionic exchange phase. The investigated bio-oil was produced by fast pyrolysis of Oak.

After basification, the bio-oil is loaded on the SPE cartridge. The effluent 1 (water/methanol washing) contained the not-retained bio-oils components. A second wash step with a 1 M pentafluoro-pyridine n-hexane solution led to the effluent 2. The final elution was done with formic acid (effluent 3). 3-chloroanilin was added to each effluent before analysis.

### **Preliminary data (results)**

By alkaline treatment of bio-oils, nearly all the alcohols, phenols and acidic compounds were converted into alcoholates, phenolates and carboxylates, respectively and, were adsorbed. Consequently, oxygenated compounds which did not contain these chemical functions were not retained by the SPE phase. The use of PFP ensured the specific elution by derivatization of the adsorbed phenolates. In contrast, elution with formic acid converted carboxylates into carboxylic acids (respectively, alcoholates into alcohols) which were no longer retained. The use of 3-chloroanilin ensured the derivatization of carbonyls. As a result, each of the three effluents contained specific oxygenated class compounds:

- Effluent 1 contained neither the phenolic nor the acidic compounds. Among them, bio-oil components with at least one carbonyl group were derivatized by 3-chloroaniline and formed Schiff base.
- Effluent 2 gathered the phenolic compounds, which have been derivatized by PFP and contained a tetrafluoropyridyl moiety. The phenolic compounds with carbonyl group were also derivatized by 3-chloroaniline.
- Effluent 3 included alcoholic and acidic bio-oil components which could be also derivatized by PFP and/or 3-chloroaniline if they also contained additionally phenolic and/or carbonyl groups, respectively.

After optimization, the time required to perform fractionation/derivatization is not longer than two hours. Hundreds or even thousands of signals are observed on the mass spectra. Data processing is facilitated by the presence of stable isotope (Cl or F) in the derivatization agents. The elemental formula of the original  $C_xH_yO_z$  compound is obtained by subtracting the derivatization reagent and adding the neutral loss.

### **Please explain why your abstract is innovative for mass spectrometry?**

The use of fractionation and derivatization by agents labelled with stable isotopes combined to petroleomic analysis ensures the specific analysis and distinction of the different oxygenated classes of bio-oil components.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

**Co-authors:**

*Vincent Carre, LCP-A2MC, FR 2843 Institut Jean Barriol de Chimie et Physique Moléculaires et Biomoléculaires, FR 3624 Réseau National de Spectrométrie de Masse FT-ICR à très haut champ, Université de Lorraine, ICPM, 1 boulevard Arago, 57078, Metz Cedex 03, France*

*Frédéric Aubriet, LCP-A2MC, FR 2843 Institut Jean Barriol de Chimie et Physique Moléculaires et Biomoléculaires, FR 3624 Réseau National de Spectrométrie de Masse FT-ICR à très haut champ, Université de Lorraine, ICPM, 1 boulevard Arago, 57078, Metz Cedex 03, France*

Poster number: AD-PB-015

## LOCALISING ORGANIC CONTAMINANTS AND THEIR BIOTRANSFORMATION PRODUCTS IN WHOLE BODY CROSS-SECTIONS OF AQUATIC INVERTEBRATES USING TWO MS-IMAGING TECHNIQUES

Abstract ID: 854

**Presenting author: Johannes Raths, Swiss Federal Institute of Aquatic Science and Technology, Eawag, Dübendorf, Switzerland, Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerland**

### Introduction

Internal concentrations at the toxicological target sites are the parameter determining an adverse effect towards an organism. Currently applied sample workup for aquatic invertebrates, such as *Gammarus pulex*, includes sample homogenisation and solvent extraction. This way, all spatial information is lost. The application of imaging approaches represents a promising alternative. However, methods for invertebrates are so far limited. The application of mass spectrometry imaging would allow the localisation and discrimination between parent compounds and their BTPs, as well as to analyse multiple compounds within the same sample.

The present study aims to assess the spatial distribution of organic pollutants and their BTPs in the tissue *G. pulex* by applying different MS-imaging and dissection methods followed by online SPE-LC-HRMS/MS in order to evaluate their feasibility.

### Methods

Specimens of *G. pulex* were exposed via the aquatic phase to a mix of pesticides (cyprodinil, fluopyram, terbutryn) and pharmaceuticals (carbamazepine, citalopram, diclofenac). Several adjacent whole body cryosections (16 µm thickness) were created, supported by the application of cryotape. The produced cross-sections were either stained (haematoxylin and eosin (HE staining)), analysed by matrix-assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) or by desorption electrospray ionization (DESI-) MSI. Additionally, dissected and non-dissected gammarids were analysed after liquid extraction by online SPE-LC-ESI-HRMS/MS to confirm the internal concentrations. All mass scans were performed on a QExactive orbitrap mass spectrometer.

### Preliminary data (results)

The whole body internal concentrations determined by LC-HRMS/MS ranged from 2.5 µg/g to 14 µg/g. Five of the six compounds (except diclofenac) could be detected using DESI-MSI whereas only three (citalopram, cyprodinil, terbutryn) could be detected properly using MALDI-MSI. The parent compounds generally followed a rather uniform distribution in the amphipod tissue, indicating a passive diffusion driven bioaccumulation. Higher intensities were observed in the regions of the gastrointestinal-system and in case of citalopram the nervous system. Several BTPs of terbutryn and cyprodinil could be detected in the gastrointestinal-system of the cross-sections analysed by MALDI-MSI (Figure 1). The location of the hepatopancreas was confirmed by the use of specific sulfate-based lipid biomarkers ( $m/z$  666.40 and 680.41).

The LC-HRMS/MS analysis of dissected gammarid tissue confirmed the higher parent concentrations in the gastrointestinal-system, and its highest contribution of the total BTP amount (Figure 2). The hepatopancreas of invertebrates is known to provide similar functions than both liver and pancreas of vertebrates, including enzymes responsible for the transformation of xenobiotics, such as the CYP-450 family. The observed BTPs are most likely CYP-facilitated (i.e. hydroxylation). The observations indicate that the gastrointestinal-system plays a major role in biotransformation in amphipods.

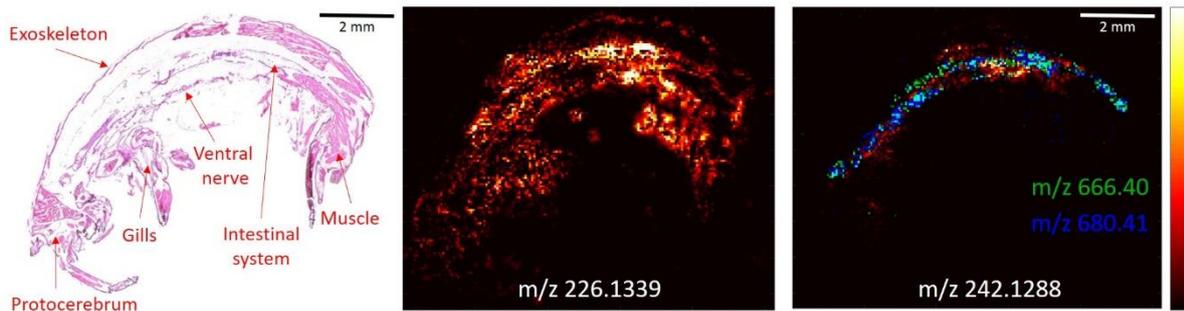
The present study demonstrates the suitability of MSI for investigations on the spatial distribution of organic contaminants and biotransformation sites in *G. pulex*. However, both applied methods required relatively high tissue concentrations and DESI-MSI appeared to be the more sensitive method. Further improvements would be needed to address environmental concentrations.

**Please explain why your abstract is innovative for mass spectrometry?**

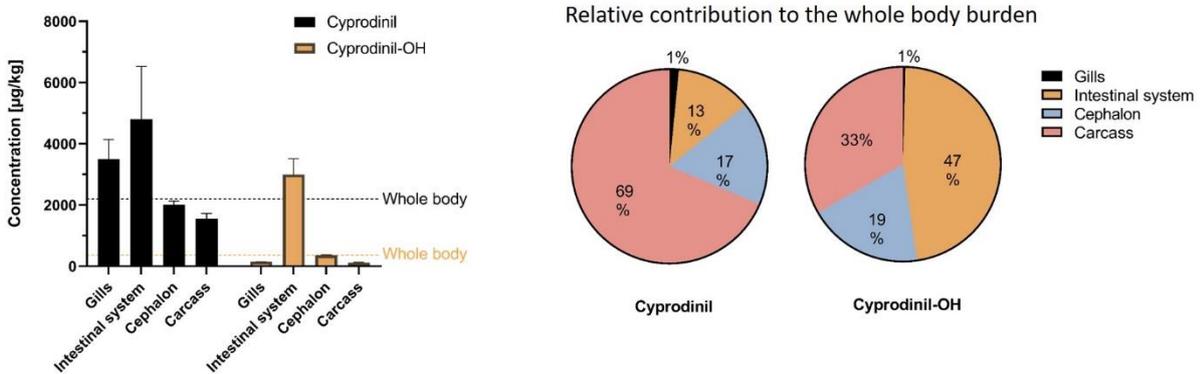
The comparison of two different imaging MS techniques to determine the spatial distribution of parent compounds and formed biotransformation products in aquatic invertebrates.

**Co-authors:**

*Fernanda Endringer Pinto, Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark*  
*Chirstian Janfelt, Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark*  
*Juliane Hollender, Swiss Federal Institute of Aquatic Science and Technology, Eawag, Dübendorf, Switzerland, Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerland*



HE-Stain , MSI of cyprodinil (226) & cyprodinil-OH (242) & biomarkers (green, blue).



Concentrations in the dissected compartments (left) and relative contribution (right).

Poster number: **AD-PB-016**

## ULTRA-SHORT TRANSIENT CHROMATOGRAPHY AND DIRECT ANALYSIS OF HIGHLY INTERFERED AND CHALLENGING ELEMENT WITH THE FIRST QUADRUPLE QUADRUPOLE ICPMSMS

Abstract ID: **888****Presenting author: Soumya Gupta, IPREM-UMR5254, E2S UPPA, CNRS, 2 avenue Angot, 64053 Pau cedex, FRANCE**

### Introduction

An increase in the presence of pollutants in water sources poses a problem for both human health and the balance of ecosystems. It is essential to regulate and remove toxic contaminants from water sources efficiently. HPLC with ICPMS has been widely used for the past few years; however, the advantages over SP/SC & LC-ICPMultiQuadMS/MS have not been studied thoroughly for the highly interfered elements which suffer from polyatomic/ isobaric interferences. The new generation and the innovative geometry of ICPMS/MS can provide a much higher selectivity by employing the synergy of an active reaction/collision cell, the high-speed rate detector and eradicating most of the interferences which are improving the limit of detection significantly over ICPMS in MultiElemental Analysis in Transient or Ultra-Short Signal.

### Methods

Sulfur, Silicon, Phosphorus, Selenium, or Gadolinium-containing molecules or complexes were purchased from Res, Agilent Technologies. UHPLC 1D or 2D separations were done in an Acquity 2D from Waters using PFP & AR ACE column 75mm x 2.1mm x 1,7mm. MS/MS data were acquired on PerkinElmer (NexION 5000) instrument in multi-mode (Mass Shift, daughter loss, parent scan, broadband, narrowband, SP/SC).

### Preliminary data (results)

Heteroelements containing congeners (sub ppb level) were separated and accurately quantified in less than 10 minutes of chromatographic run. Cleaner chromatogram, sup-ppb level, and more specific mass spectra with fewer interferences were obtained by optimizing the method in MSMS separation. Details of optimization method conditions can be found in the dialogue box in figure 1. Figure 1 also shows the signal-to-noise ratio (s/n) for phosphorus and Sulphur to recognize the best method for the acquisition of both elements. Figure 2 displays the calibration curves of different organic compounds containing phosphorus and Sulphur by method 2. The higher the organic solvent was introduced in the plasma, the more compatible it with the targeted compounds, which results in lower peak intensity. For example, Vamidotion 13.3%, Azamethiphos 38.5%, Dichlorvos 68.3%. This innovative analytical approach can also be compared in parallel analysis of water by Non-Target Screening with the help of a high-resolution Tim stop mass spectrometer.

### Conclusion

MSMS could be a filter of choice to improve the monitoring of halogenated, sulfur, or Rare earth-containing molecules in our environment or (nano)material. This method presents a similar performance to UPLC-HRMS, but it is faster, cheaper, provides accurate and element-specific quantification, and is less sensitive to matrix interferences.

### Please explain why your abstract is innovative for mass spectrometry?

ICPMSMS with chromatography will benefit Non-Target Screening Trace Elements containing Emerging Pollutants detailed quantification by adding this additional stage of specificity and at the sub-ppb level in a single run.

### Co-authors:

*Fan Yang, IPREM-UMR5254, E2S UPPA, CNRS, 2 avenue Angot, 64053 Pau cedex, FRANCE*  
*Pierre Luc Dupont, PerkinElmer SAS, 16 av. du Quebec - Bat Lys 9140 Villebon S/Yvette, FRANCE*  
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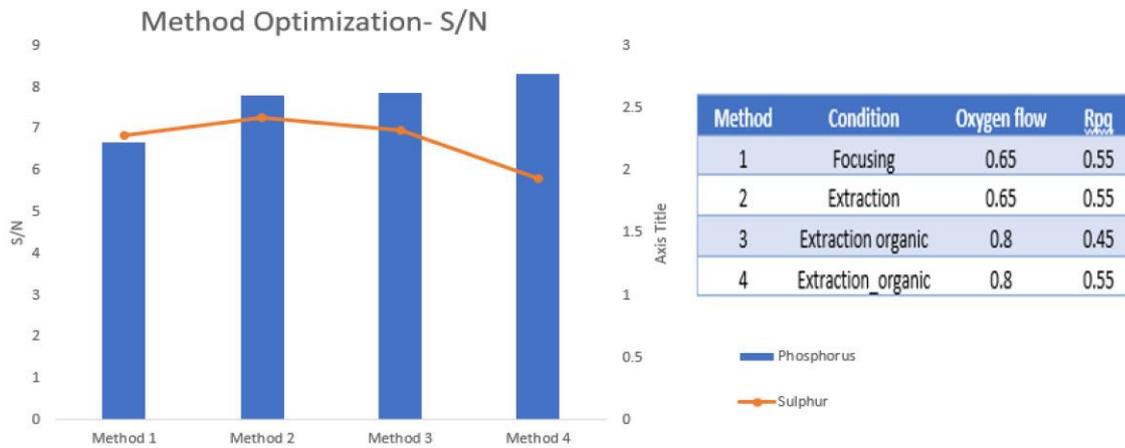


Figure 1- Signal/noise data for different acquisition methods.

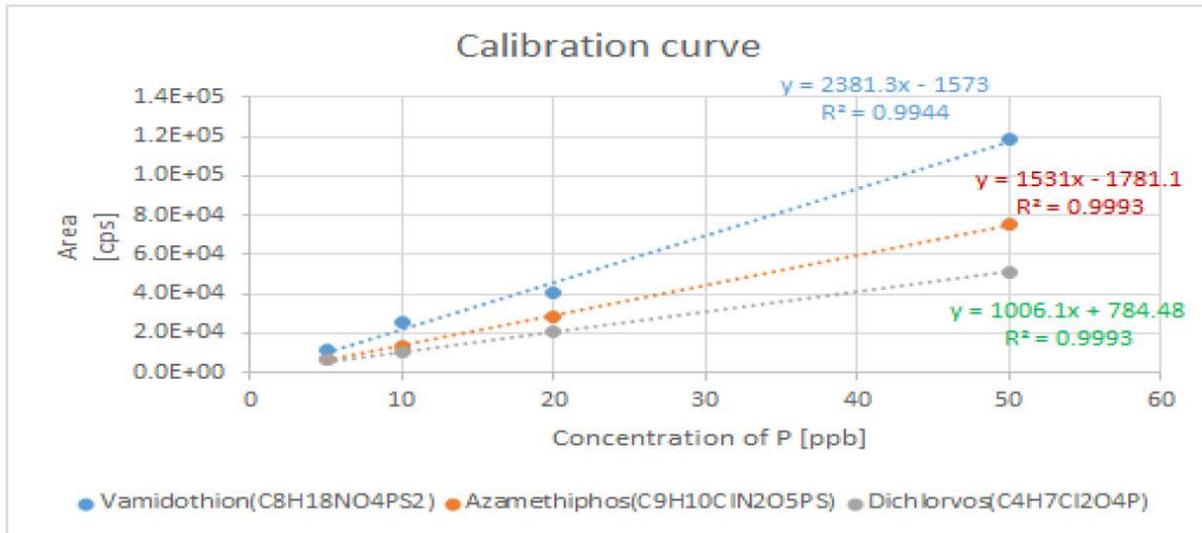


Figure 2- Calibration curves of three compounds taken from chromatograph

Poster number: AD-PB-017

## TRANSCRIPTOMICS, NEUROPEPTIDOMICS AND MASS SPECTROMETRY IMAGING OF THE MINIATURE BRAIN OF THE CATAGLYPHIS NODUS ANT

Abstract ID: 950

Presenting author: Susanne Neupert, University of Kassel

### Introduction

Cataglyphis ants exhibit an age-related polyethism with very distinct behavioral-stages. Due to their role in underlying physiological and developmental processes, signaling molecules like neuropeptides play a crucial role in behavioral transition regulations. Here, we combined transcriptomic and peptidomic analysis to obtain a comprehensive peptidomic data set of the Cataglyphis brain. We further applied imaging mass spectrometry to unmask the spatial distribution of neuropeptides and peptides encoded on different peptide genes on 14  $\mu\text{m}$  thin consecutive brain sections by mass spectrometry imaging (MSI), and applied immunocytochemistry as control of the IMS results.

### Methods

As the neuropeptidome of *C. nodus* was unknown, we collected a comprehensive peptidomic data set obtained by transcriptome analysis of the ants' central nervous system combined with brain extract analysis by Q-Exactive Orbitrap mass spectrometry (MS) and direct tissue profiling by MALDI-TOF MS. Next we characterized the spatial distribution of a subset of peptides encoded on 16 precursor proteins with high resolution by MALDI MS imaging (MALDI MSI) on 14  $\mu\text{m}$  brain sections. The accuracy of our MSI data was confirmed by matching the immunostaining patterns for tachykinins with MSI ion images from consecutive brain sections.

### Preliminary data (results)

The transcriptome of *C. nodus* brain tissues was assembled, followed by BLAST searches using known neuropeptide precursors from different insects. In total, a set of 49 *C. nodus* prepropeptide sequences subdivided into 30 complete neuropeptide-, 7 complete neuropeptide-like-, 11 complete and 1 non-complete protein hormone genes were yielded that originate from 42 different genes. For chemical identification of transcriptome-predicted peptides, we used the *C. nodus* precursor sequences as database and analyzed brain extracts by ESI-Q Exactive Orbitrap MS followed by PEAKS 10.5 software package. In total, we identified 71 peptides with likely bioactive function, encoded on 49 neuropeptide-, neuropeptide-like and protein hormone prepropeptide genes, including a novel neuropeptide-like gene (fliktin). As a first step to uncover potential functions of neuropeptides, we optimized available MSI protocols for neuropeptide detection in the *C. nodus* brain to gain comprehensive information of the spatial distribution of these signaling molecules. We evaluated different experimental steps including sample preparation, tissue embedding material, tissue thickness, tissue rinsing procedure, tissue storage, matrix composition and matrix application tools for *C. nodus* samples. Among the characterized *C. nodus* brain neuropeptides, we were able to visualize the spatial distribution of 35 peptides encoded on 16 precursor genes on 14  $\mu\text{m}$  brain sections. To evaluate the precision of our MALDI-MSI neuropeptide data set, we performed immunostainings against TK combined with MALDI-MSI in consecutive brain sections. To visualize neuroanatomical landmarks in the brain, we co-stained the brain sections with fluorophore-conjugated phalloidin to delineate neuropil regions and fiber connections in brain regions.

### Please explain why your abstract is innovative for mass spectrometry?

This work represents the initial step to address the functional peptidomic changes underlying age, stage- or/and task-specific behavioral transitions

### Co-authors:

Jens Habenstein, University of Würzburg

Franziska Schmitt, University of Würzburg

Sander Liessem, University of Cologne

Alice Ly, SCiLS, Zweigniederlassung Bremen der Bruker Daltonik GmbH

Dennis Trede, SCiLS, Zweigniederlassung Bremen der Bruker Daltonik GmbH

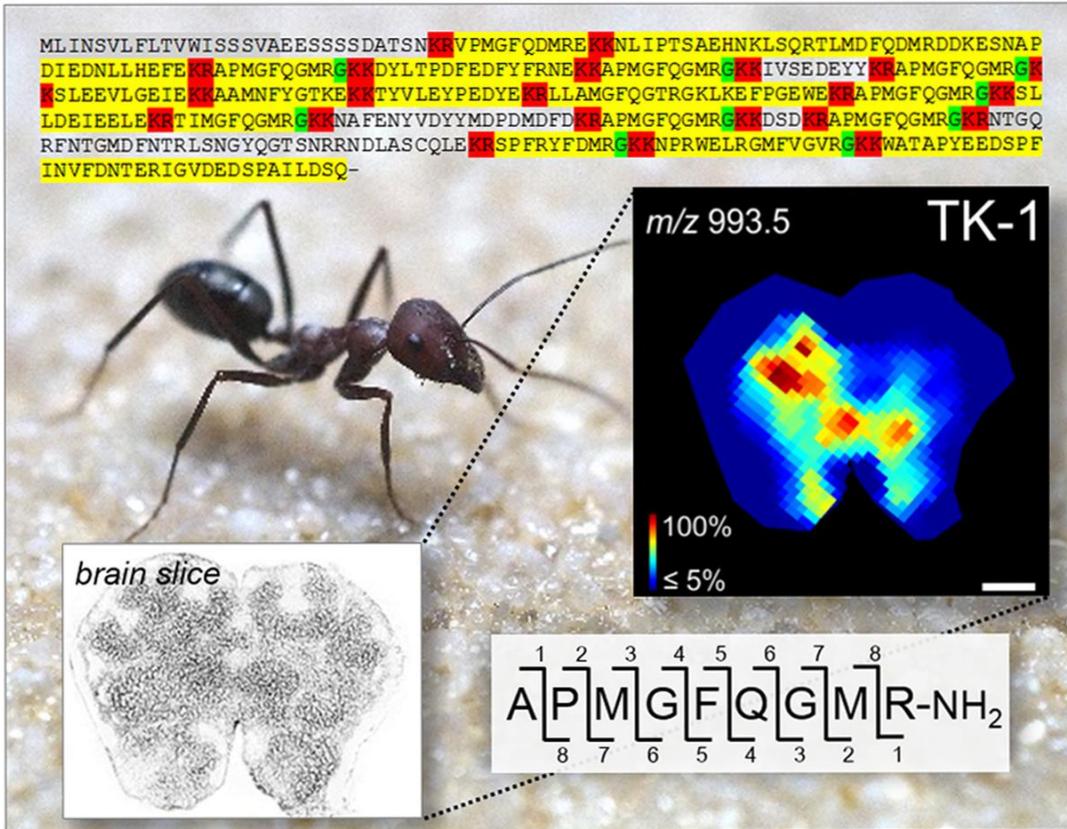
Christian Wegener, University of Würzburg

Reinhard Predel, University of Cologne

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

Wolfgang Rössler, University of Würzburg  
Susanne Neupert, University of Kassel



Poster number: AD-PB-018

## STRATEGIES FOR ANNOTATIONS OF PHENOLIC COMPOUNDS BY MS-BASED MOLECULAR NETWORKING AND MANUAL MS/MS DATA CURATION

Abstract ID: 965

**Presenting author:** Luís Guilherme Pereira Feitosa, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP) - Universidade de São Paulo (USP)

### Introduction

Molecular Networking (MN) is a cheminformatic tool that allow the grouping of similar MS/MS spectra of related structures, and compound annotations based on the comparisons of experimental MS/MS data with spectral libraries. GNPS (Global Natural Products Social Molecular Networking) is an online and open-access platform to perform the MN. However, the accuracy of GNPS annotations is limited due to equipment variations and isomerism. Furthermore, uses of MS<sup>3</sup> data and orthogonal parameters are crucial to distinguish isomers in homologous series of Natural Products. Thus, manual data curation is required to accurate annotations. In this sense, we purpose the structures of flavonoids and of hydroxycinnamic acids derivatives in plants from Compositae family based on MN, fragmentation patterns of congeners, and gas phase reactions with ESI.

### Methods

We performed the metabolomics of plants from genus *Mikania* (Compositae) obtained from regions of Atlantic Forest and Cerrado biomes in Brazilian territory. The leaves of 19 *Mikania* species were milling to obtain powder samples. Then, samples were extracted with methanol: water and analyzed by LC-ESI-MS/MS (Ion Trap analyzer) in negative mode ionization, using a C18 column and elution with the gradient of water: acetonitrile, both with formic acid 0.1%. The MS/MS data were preprocessed in MZmine software and then processed in Feature-Based Molecular Network (FBMN) workflow of GNPS platform (<https://gnps.ucsd.edu/>). The molecular network was visualized in Cytoscape software.

### Preliminary data (results)

The MN of *Mikania* exhibits clusters with similar structures of flavonoid-*O*-glycosides, and hydroxycinnamic esters of quinic and octulopyranosonic acids (Figure 1). After the grouping of spectra in molecular network, we performed the manual data curation of spectrometric data. Fragmentation patterns of flavonoids are well known and widely described in literature. Flavonoid annotations were based in neutral losses of *O*-glucosyl and sulfate groups, as well characteristic losses of -CH<sub>3</sub> and CH<sub>4</sub> in methoxylated aglycones, and product ions of RDA reactions. Moreover, annotation of flavonoids considered chemotaxonomic data from a *in house* database. FBMN allowed the visualization of consensus spectra of caffeoylquinic acids (CQA and Di-CQA), feruloylquinic acid (FQA) and feruloyl, caffeoylquinic acid (FCQA) isomers in distinct nodes of cluster. These isomers were annotated from comparisons of experimental MS<sup>2</sup> data from consensus spectra and MS<sup>3</sup> spectra from raw data with profile fragmentations of reference standards available in literature. The order of elution in C18 was an orthogonal parameter for the annotations of CQA, Di-CQA and FQA isomers (Figure 1). Hydroxycinnamic esters of 2,7-dehydro-octulopyranosonic acid (OA) and 2,7-dehydro-3-deoxy-octulopyranosonic acid (DOA) were described in Compositae species, including MS<sup>2</sup> and MS<sup>3</sup> data. However, fragmentations with ESI of these compounds were not completely elucidated yet. Thus, we purpose the mechanisms of main fragmentations of OA and DOA derivatives found in *Mikania*, including pathways related to losses of phenolic groups and RDA reaction to suggests their positions (Figure 2). These fragmentations contribute with annotations of congeners of hydroxycinnamic esters of OA and DOA.

### Please explain why your abstract is innovative for mass spectrometry?

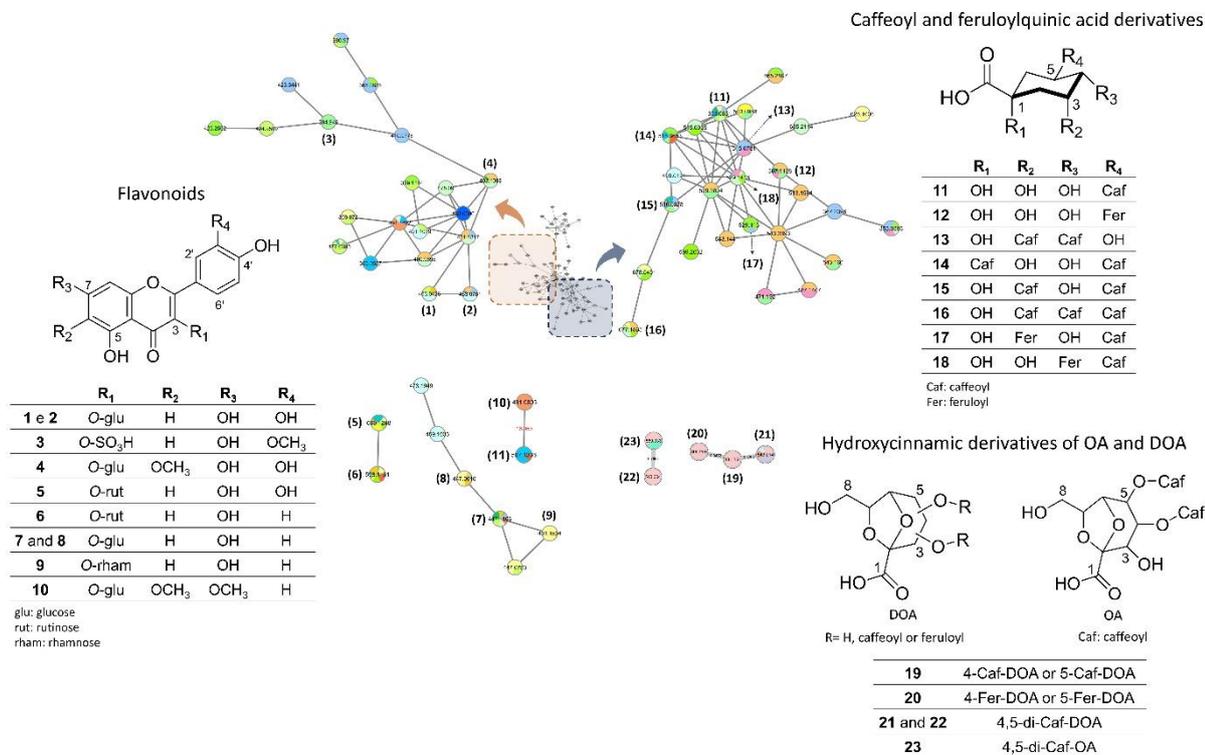
Different strategies in mass spectrometry may be used for manual annotations of phenolics from the organization of MS/MS spectra in molecular networks, aiming improve the accuracy of structural suggestions.

### Co-authors:

Marcelo Monge Egea, Universidade Federal de Uberlândia (UFU)

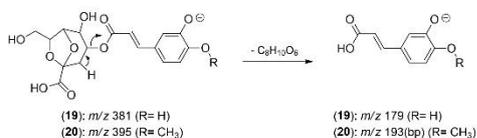
Norberto Peoporine Lopes, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP) - Universidade de São Paulo (USP)

Dioneia Camilo Rodrigues de Oliveira, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP) - Universidade de São Paulo (USP)

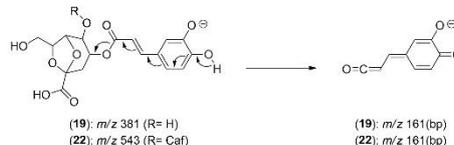


Clusters with phenolic compounds annotated in molecular network of *Mikania*.

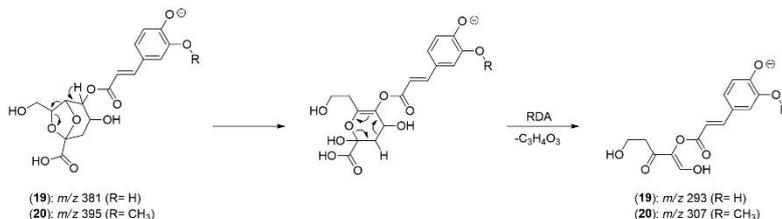
**Pathway A: hydrogen rearrangement (CRF)**



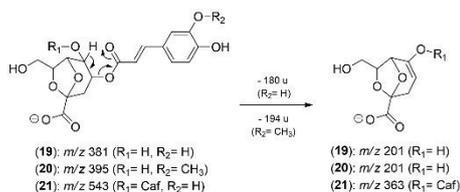
**Pathway B: remote hydrogen rearrangement (CRF)**



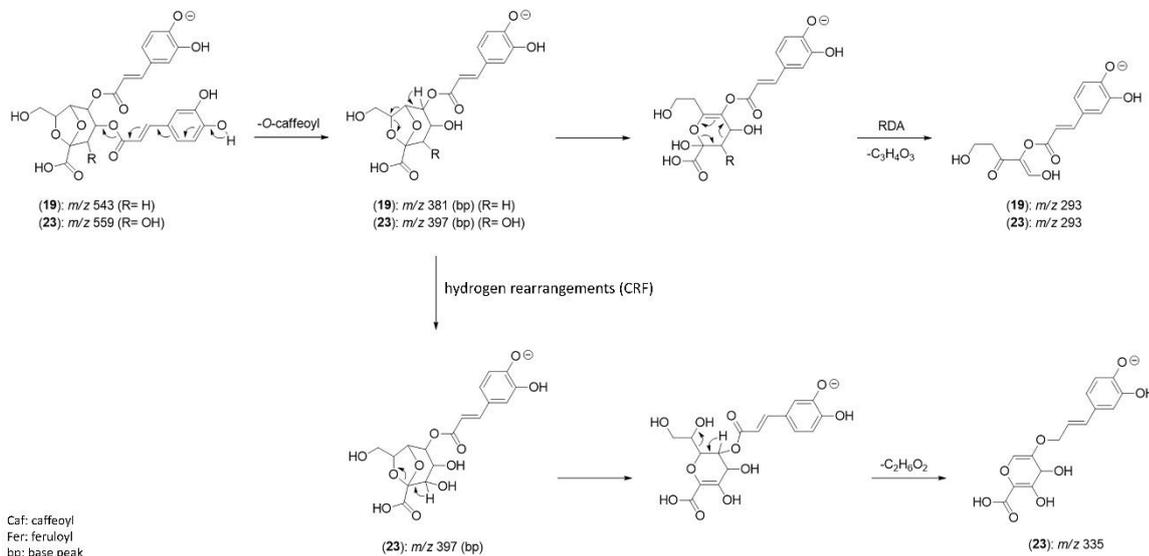
**Pathway C: hydrogen rearrangement and RDA reaction (CRF)**



**Pathway D: McLafferty-type rearrangement (CRF)**



**Pathway E: remote hydrogen rearrangement and RDA reaction (CRF)**



Proposed fragmentation mechanisms for hydroxycinnamic esters of OA and DOA.

Poster number: AD-PB-019

## THE ACCURATE QUANTIFICATION OF ETHYLENE OXIDE IN THE PRESENCE OF ACETALDEHYDE: THE SEPARATION OF ISOBARIC COMPOUNDS USING SIFT-MS

Abstract ID: 981

Presenting author: Stefan J Swift, J. Heyrovsky Institute of Physical Chemistry

### Introduction

A re-call of over 105 products made from sesame foodstuffs (originating from India) occurred in Europe in 2020 due to the detection of up to 3500 times the recommended maximum residue limit per kilogram of the highly toxic and carcinogenic compound Ethylene Oxide (EtO), which was used as a pesticide in the manufacturing process. EtO is ingested or inhaled into the body, is metabolized and spread throughout the bloodstream. Its acute toxicity originates from its ability to form hydroxyethyl adduct species with DNA and hemoglobin, giving rise to its carcinogenic nature. EtO was also previously measured as an air pollutant and is on the EPA list of 187 toxic species. Therefore, this species' accurate characterization and quantification within the air is vital.

### Methods

The challenge which arises in using Mass Spectrometry to accurately quantify EtO is that it is isobaric with acetaldehyde, a species abundantly found in many foods. Selected Ion Flow Tube – Mass Spectrometry (SIFT-MS), however, has the advantage of being able to change its reagent ion used ( $\text{H}_3\text{O}^+$ ,  $\text{NO}^+$ ,  $\text{O}_2^+$ ,  $\text{O}^-$ ,  $\text{OH}^-$ ,  $\text{O}_2^-$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ ), which allows for the separation of isobaric species. Once the reagent ion enters the flow tube and collides with the analyte, the kinetics and the branching ratios are investigated and characterized. This in-turn allows for real-time analysis of headspace and air samples.

### Preliminary data (results)

Our preliminary results indicate that the ion-molecule reactions between EtO and the reagent ions are highly variable both in the kinetic rate constants and the nature of the product ions formed. EtO and acetaldehyde can be individually and accurately quantified using the different reactions with specific reagent ions. Results are also presented and compared for both He and  $\text{N}_2$  carrier gases. The energetics of the reactions were calculated using the DFT method B3LYP with the 6-311 ++G(d,p) basis set. These results confirm our understanding of the ion-molecule reactions of EtO and acetaldehyde with the  $\text{H}_3\text{O}^+$  and  $\text{NO}^+$  reagent ions.

### Please explain why your abstract is innovative for mass spectrometry?

Exploiting additional reagent ions in SIFT-MS allows for more isobaric substances to be investigated, including ethanol overlapping with formic acid or acetic acid overlapping with the  $^{18}\text{O}$  isotopologue of acetone.

### Co-authors:

*Ann-Sophie Lehnert, Syft Technologies GmbH*

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*Leslie P Silva, Syft Technologies Inc.*

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*Kseniya Dryahina, J. Heyrovsky Institute of Physical Chemistry*

*Patrik Španěl, J. Heyrovsky Institute of Physical Chemistry*

## **Session: Homeland security, explosives and environmental monitoring**

Poster number: AD-PB-020

### **INVESTIGATION OF WASTEWATER SAMPLES FROM A MOSCOW SCHOOL IN ORDER TO DETERMINE THE PRESENCE OF SUBSTANCES SUBJECT TO CONTROL IN THE RUSSIAN FEDERATION USING ORBITRAP**

Abstract ID: 556

**Presenting author: Artem Zharikov, Skolkovo Institute of Science and Technology**

#### **Introduction**

The number of adolescents who use drugs and psychotropic substances in the world compared to 2010 has increased by 22%. The projection based on demographic change suggests that by 2030 the number of people who use drugs worldwide will increase by 11%, for example in Africa by 40% due to its rapidly growing and young population. In the observed dynamics of the increase in the incidence of drug addiction in recent years, the problem of drug addiction is becoming more and more topical every year. In connection with the above, it is very important to monitor wastewater in educational institutions for the presence of substances subject to control in the Russian Federation in adolescents.

#### **Methods**

The essence of the scientific study was sampling, sample preparation, qualitative analysis using HRMS, and data analysis. Sampling was carried out using pneumatic and mechanical means of sampling in plastic containers. Sample preparation consisted of solid-phase purification on a C18 sorbent to remove interfering impurities and concentration of the extract, followed by qualitative analysis using HRMS. The mass spectrum was recorded in the full scan mode in an orbital ion trap (Q-Orbitrap).

#### **Preliminary data (results)**

Data processing was carried out using the Xcalibur and Compound Discoverer programs when searching for compounds in the mzCloud database and in the end, we managed to identify 4 substances banned for circulation in the Russian Federation - these are Methadone, Benzoyllecgonine, 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and Cocaine at the intensity of  $5.12 \times 10^8$ ;  $9.87 \times 10^7$ ;  $2.88 \times 10^9$ ;  $4.27 \times 10^8$ , provided that the sample was concentrated 250 times during sample preparation and match in database mzCloud 90,6; 94,3; 95,2; 95,9 respectively.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Further development of new databases is needed to make the HR-MS approach a tool to control the qualitative identification of drugs and psychotropic substances in wastewater.

#### **Co-authors:**

*Yury Kostyukevich, Skolkovo Institute of Science and Technology*

Poster number: AD-PB-021

## ULTRA-SHORT TRANSIENT CHROMATOGRAPHY AND DIRECT ANALYSIS OF HIGHLY INTERFERED AND CHALLENGING ELEMENT WITH THE FIRST QUADRUPLE QUADRUPOLE ICPMSMS

Abstract ID: 617

**Presenting author: Soumya Gupta, IPREM-UMR5254, E2S UPPA, CNRS, 2 avenue Angot, 64053 Pau cedex, FRANCE**

### Introduction

An increase in the presence of organic and inorganic pollutants in water sources poses a problem for both human health and the balance of ecosystems. It is essential to regulate and remove toxic contaminants from water sources efficiently. HPLC with ICPMS has been widely used for the past few years. However, the advantages over SP/SC & LC-ICPMultiQuadMS/MS have not been studied thoroughly for the highly interfered elements which suffer from polyatomic/isobaric interferences. The new and innovative geometry of ICPMS/MS can provide a much higher selectivity by employing the synergy of an active reaction/collision cell, the high-speed rate detector and eradicating most of the interferences which are improving the limit of detection significantly over ICPMS in MultiElemental Analysis in Transient Signals.

### Methods

Sulfur, Silicium, Phosphorus, Selenium, or Gadolinium containing molecules or complexes were purchased from Res, Agilent Technologies. UHPLC 1D or 2D separations were done in an Acquity 2D from Waters using Acclaim RSLC 120 C18 2.2 $\mu$ m x 2.1mm x 100 mm. MS/MS data were acquired on PerkinElmer (NexION 5000) instrument in multi-mode (Mass Shift, daughter loss, parent scan, broadband, narrowband, SP/SC).

### Preliminary data (results)

Heteroelements containing congeners (sub ppb level) were separated and accurately quantified in less than 15 minutes of chromatographic run. Cleaner chromatogram, sup-ppb level and more specific mass spectra with fewer interferences were obtained by optimising the method in MSMS separation. Details of optimisation method conditions can be found in the dialogue box in figure 1. Figure 1 also shows the signal to noise ratio (s/n) for phosphorus and Sulphur to recognise the best method for the acquisition of both elements. Figure 2 displays the optimised method's calibration curves of different organic compounds containing phosphorus.

This innovative analytical approach can also be combined with water analysis by Non-Target Screening (NTS) by a high-resolution timsTOF mass spectrometer. In NTS, some emerging contaminants or transformation products do not have the available standards. It is challenging to find an analogue compound to do quantification or semi-quantification. Still, in ICP-MS/MS, the quantification is based on elements, providing more accurate quantification results in NTS.

Both elements were analysed with oxygen gas, and in the mass-shift mode of the ICPMSMS., the calibration range was from 5ppb to 50 ppb, with the lowest calibration point, we have an s/n at 10.  $r^2 = 0.99$ . LOD (limit of detection) is 1.5ppb, LOQ (limit of quantification) is 5ppb

### Please explain why your abstract is innovative for mass spectrometry?

ICPMSMS hyphenated with chromatography or direct analysis will benefit NTS Trace Elements detailed global quantification at the sub-ppb level in a single run, only in a few minutes.

### Co-authors:

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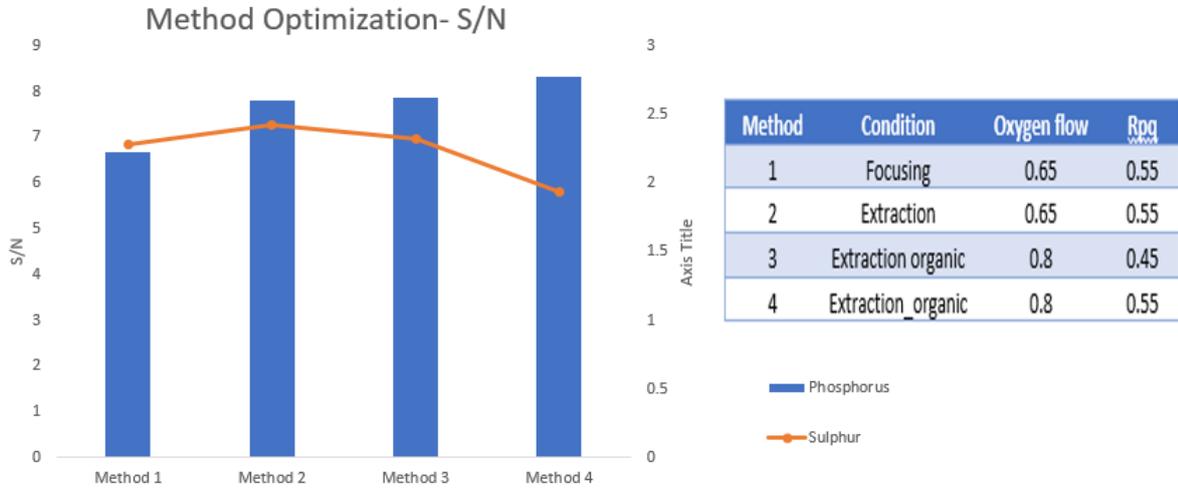
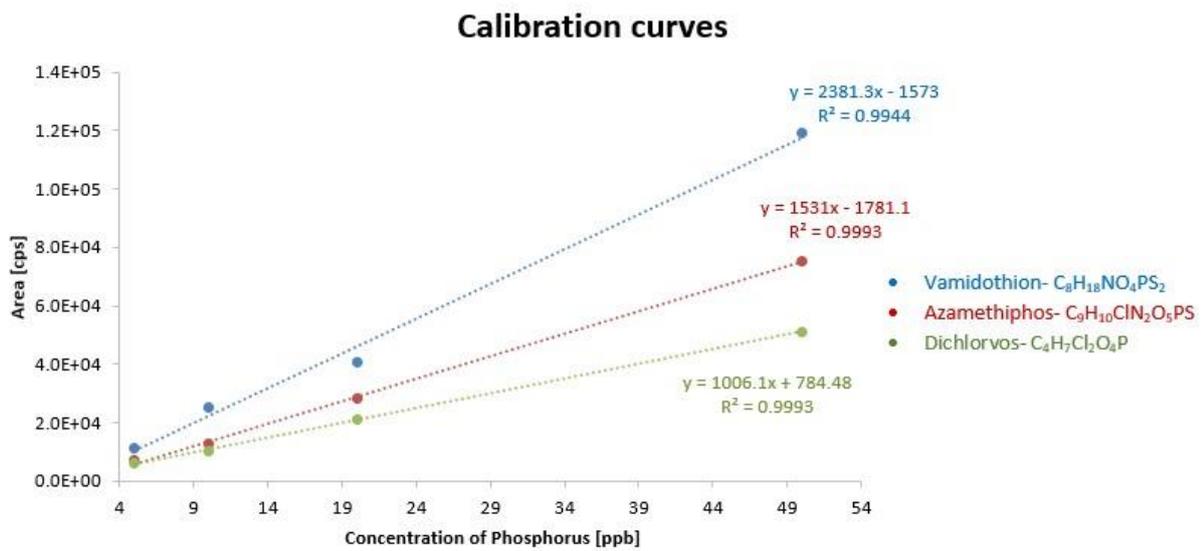


Figure 1- Signal/noise data for different acquisition methods.



Calibration curves of three different compounds containing Phosphorus

Poster number: **AD-PB-022**

## **IDENTIFICATION OF BIOMARKERS OF POPULATION SIZE IN WASTEWATER USING TOTAL CORRELATION MASS SPECTROMETRY (TOC-MS)**

Abstract ID: **625**

**Presenting author: Jack Rice, Verdel Instruments**

### **Introduction**

The need for accurate, real time monitoring of public health has been thrown into sharp relief since 2020. Wastewater based epidemiology (WBE) is an established and increasingly popular technique for assessing public health through the analysis of wastewater. A key part of WBE is population normalisation, which relies on accurate estimates of population size. Unfortunately, the sources of this information are often static, such as census data, or estimates based on house size.

A previous study in Australia, performed on census day 2011, used Bayesian statistics to identify a panel of pharmaceuticals and lifestyle chemicals that accurately estimated population size. This work assessed the feasibility of using ToC-MS to identify similar, suitable biomarkers for assessing population size in UK wastewater.

### **Methods**

A panel of previously identified population size biomarkers were analysed via direct infusion using ToC-MS. ToC-MS is a new MS/MS technique, based on 2D-MS, for data independent analysis without chromatographic separation or quadrupolar isolation. The ToC-MS instrument combines a Q-ToF and stored waveform ion modulation with ultraviolet photodissociation (UVPD) to simultaneously fragment all analytes whilst intrinsically linking fragments to parent molecules.

UVPD fragmentation patterns were identified for each biomarker, which were then spiked into influent wastewater and concentrated using solid phase extraction (SPE) with Oasis HLB cartridges. After identification, unspiked influent wastewater was analysed to identify other potential biomarkers.

### **Preliminary data (results)**

The biomarkers selected for this study were acetaminophen, atenolol, caffeine, carbamazepine, codeine, furosemide, hydrochlorothiazide, ibuprofen, naproxen, and salicylic acid. All analysis was performed by direct infusion at a rate of 0.25  $\mu\text{L}/\text{min}$  and initial analysis was focused on identifying previously unknown UVPD fragmentation patterns, using positive and negative ESI and ToC-MS. This allowed for the simultaneous identification of several fragments for each biomarker, which were then confirmed structurally. Biomarkers and deuterated analogues were then spiked into 5 mL of wastewater that had been eluted from SPE cartridge with methanol. Two equal aliquots were taken and modified with either 0.1 % (v/v) formic acid or ammonium hydroxide, for analysis with positive and negative ESI, respectively. A majority of the previously identified fragments were detected, including at least one fragment per biomarker.

Influent wastewater, spiked with deuterated analogues, was then analysed to identify selected biomarkers as well as a range of other potential population size biomarkers *in-situ*. As before, influent wastewater was eluted from cartridge using 2 mL of methanol, with equal aliquots taken and spiked with either 0.1 % (v/v) formic acid or ammonium hydroxide. Across two separate analytical runs, one with positive and one with negative ESI, ToC-MS analysis was able to identify between one and three previously identified fragments of each biomarker, except carbamazepine, which was not detected in the sample. Further data analysis and preliminary quantification is currently ongoing but has identified several similar analytes previously detected in UK wastewater, and their associated fragments.

### **Please explain why your abstract is innovative for mass spectrometry?**

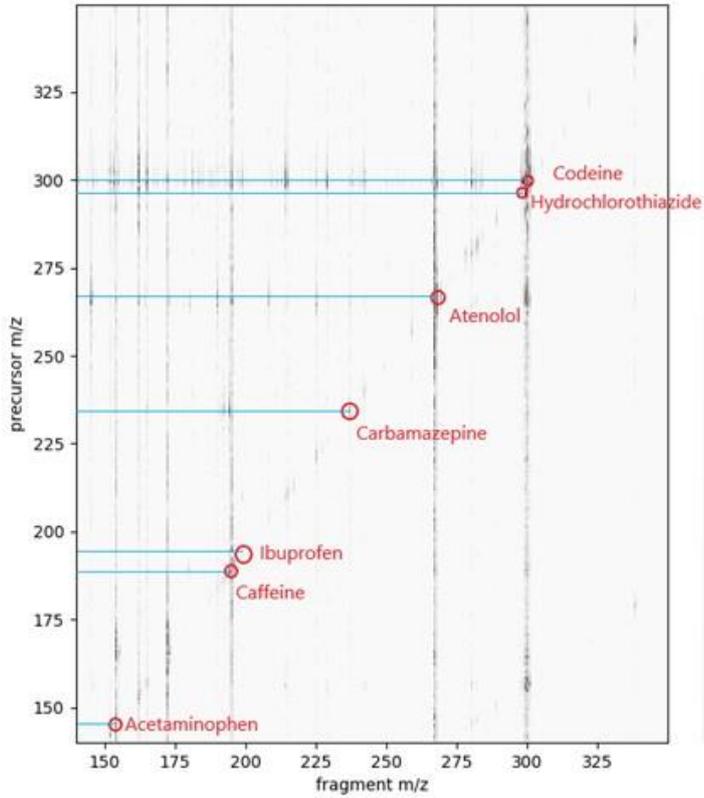
Data independent analysis of wastewater via ToC-MS

Use of UVPD for tandem mass spectrometry analysis of biomarkers in wastewater

UVPD fragmentation of pharmaceuticals

**Co-authors:**

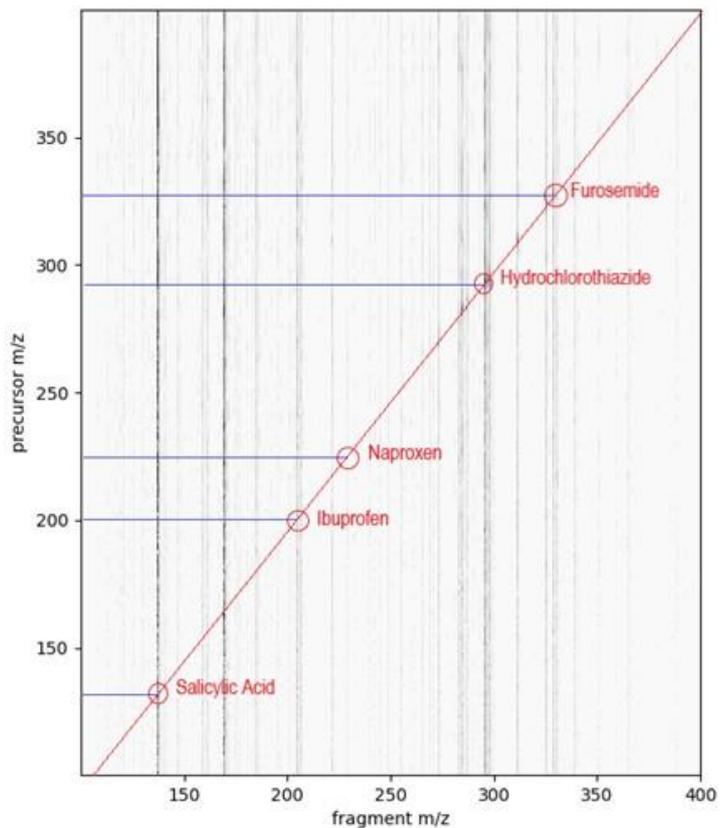
*Nathan Cassidy, Verdel Instruments*  
*Cath Whitaker, Verdel Instruments*  
*Robert Burch, Verdel Instruments*  
*Peter B O'Connor, Verdel Instruments, University of Warwick*



Positive ESI, ToC-MS spectra showing biomarkers and their associated fragments

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Negative ESI, ToC-MS spectra showing biomarkers and their associated fragments

Poster number: **AD-PB-023**

## FLUORINATED POLYMERS DART-MS STUDY

Abstract ID: **693****Presenting author: Frederic Progent, CEA,DAM,DIF,F-91297 Arpajon**

### Introduction

Polymers are ubiquitous in industry and in our environment, their identification is therefore a major analytical challenge for characterizing raw materials (structure, impurities, etc.) or for identifying polymers. For example, mass spectrometry can be used to monitor the aging of compounds or trace polymers in environmental samples. This study is focused on fluoropolymers which are used in a variety of industries including electronics, medical equipment, chemicals and industrial manufacturing, alternative energy or military applications.

### Methods

In order to identify the structure and ultimately to be able to detect trace of polymers, analyses are carried out on bulk samples and on concentrated solutions of fluorinated polymers. Our investigations were focused on a polyvinylidene fluoride (PVDF) and copolymers such as Viton A<sup>®</sup> and Tecnoflon<sup>®</sup> (dipolymers constituted of hexafluoropropylene and vinylidene fluoride). The analyses were performed on an Orbitrap mass spectrometer, equipped with DART (Direct Analysis in Real Time) or ASAP ([Atmospheric Samples Analysis Probe](#)) sources allowing a direct introduction of samples in the ionization region. The contribution of DART-FT-ICR-MS analyses is also discussed.

### Preliminary data (results)

Optimal DART settings were obtained through a parametric study. An experimental design was performed to determine the key factors in our context. Mass spectra were exploited through Van Krevelen diagrams, Kendrick maps, and various processing softwares (ex: Polymerix<sup>®</sup>, Statistica). The study of the mass spectra allowed the research of end groups and the identification of polymers. The possibilities of evaluating the characteristic data of a polymer (average mass, polydispersity, etc.) are discussed.

The contribution of FTICRMS (Fourier Transform Ion Cyclotron Mass Spectrometry) mass spectrometry, combined with a DART ion source, is compared with results obtained by Orbitrap.

Polymers studied were aged in an enclosure, under UV irradiation to search possible markers of their transformation.

Further research will focus on the search for impurities present in polymers as well as the analysis of trace polymers.

### Please explain why your abstract is innovative for mass spectrometry?

The study combines atmospheric pressure ion sources (DART, ASAP) and high resolution Mass Spectrometry (Orbitrap, FT-ICR-MS) for structural elucidation of fluoropolymer and for search of trace polymers.

### Co-authors:

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*FREDERIC AUBRIET, Laboratoire de Chimie et Physique-Approche Multi-échelles des Milieux Complexes (LCP-A2MC), Université de Lorraine 1 Boulevard Arago, Metz Technopole Cedex 03 F-57078*

Poster number: **AD-PB-024**

## **STICKING IT TO COLLECTION AND ANALYSIS: UTILIZING PRESSURE-SENSITIVE ADHESIVE PAPER COMBINED WITH PORTABLE MASS SPECTROMETRY FOR DETECTION OF THREATS**

Abstract ID: **964****Presenting author: Dan Carmany, Excet Inc, U.S. Army**

### **Introduction**

One of the challenges facing field-focused methods involves the detection of trace amounts from within complex matrices. The use of pressure-sensitive adhesive (PSA) coated paper has been shown to be effective for easy collection of trace amounts of analytes from porous and non-porous surfaces. These methods are being transitioned to a portable mass spectrometry instrument, the Bayspec Continuity. Collection requires an individual to press the substrate onto a surface and lift it back up. Drugs and explosives were identified using this methodology. Detection limits from contaminated surfaces were determined to be in the low nanogram (ng) range. Common interferents did not inhibit analysis and the substrates could be stored, extracted, and reanalyzed on benchtop instrumentation.

### **Methods**

Analytes were collected and analyzed on PSA substrates using the Bayspec Continuity. Analyte standards were spotted onto various surface types and collected by pressing the PSA substrates onto the dried spots. Limits of detection (LOD) were determined by spotting various analyte concentrations onto a surface and normalizing with an internal standard. For the reanalysis studies, substrates were extracted after PS-MS analysis with methanol and analyzed on a benchtop instrument. Various MS front ends were designed and developed to transition the method from precut substrates to direct analysis from a yellow sticky note.

### **Preliminary data (results)**

Previous studies demonstrated the effectiveness of PSA substrates to collect, stabilize, and directly identify threat agents without the need for sample preparation. This approach is being transitioned for on-site/field forward threat identification. All examined threat agents were identified by PS-MS on the portable instrument. A MATLAB/Python script was written for quantitation. The LODs for analytes spotted directly onto the substrates was <50ng using the Bayspec. The drugs were sampled off cloth, concrete, and aluminum with LODs <5 $\mu$ g. The explosives were sampled off cloth and aluminum with LODs <15 $\mu$ g.

Sufficient analyte remained on the PSA substrate following PS-MS to allow for confirmation using benchtop instrumentation. Substrates spotted with analytes were analyzed by PS-MS and extracted immediately or stored at various temperatures for up to 4 weeks prior to reanalysis. The extractions were analyzed on a QqQ. More than 40% of the original analyte amount was found to be extracted from the substrates. The stability study showed that the explosives could be extracted when stored for at least 4 weeks. However, TNT required cold storage to remain stable.

Chalk dust, and spackling powder were used as interferents for the explosives and a mixture of inositol, lactose, and mannitol for the drugs. Identification of the explosives was not affected by the interferents. Each of the drugs could also be identified with the interferents.

Direct analysis of a yellow sticky note instead of a precut substrate was possible using a swab attachment produced by Bayspec or by internally developed PS-MS substrate casings.

### **Please explain why your abstract is innovative for mass spectrometry?**

Transitioned collection and analysis for small molecule analytes from benchtop instrumentation to a portable mini-MS.

### **Co-authors:**

*Elizabeth Dhummakupt, U.S. Army*  
*Nicholas Manicke, Indiana University–Purdue University Indianapolis*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Chau Nguyen, Indiana University–Purdue University Indianapolis*  
*Paul Demond, Excet Inc*

## Session: Space exploration & Astro chemistry

Poster number: AD-PB-025

### MOLECULAR GROWTH IN LASER VAPORIZED $\text{Si}_n^{+0}$ + $\text{C}_2\text{H}_2$ CHEMISTRY FOR AN ASTROPHYSICAL CONTEXT

Abstract ID: 754

Presenting author: Sandra Wiersma, IRAP, Université de Toulouse III - Paul Sabatier, CNRS, CNES

#### Introduction

A rich, dusty chemistry is present in the outflows of dying, carbon-rich, red giant stars, in which silicon is of major importance. The materials formed in these so-called circumstellar shells are recycled in the formation of new solar systems, giving a larger chemical library to the next generation. Our view of the molecular species involved in hydrocarbon growth and dust formation – in particular organosilicon dust – is still very limited. We study the chemistry of  $\text{Si}_n^{+0}$  with  $\text{C}_2\text{H}_2$  to gain insight in the dust nucleation and molecular growth using complementary mass spectrometric techniques.

#### Methods

Using the PIRENEA 2 setup, a rotating silicon rod is irradiated using a 532 nm Nd:YAG laser, after which the plasma plume is cooled and transported by a gas pulse from a solenoid valve with a 2 bar backing pressure. This gas is either helium, or helium seeded with a small fraction of  $\text{C}_2\text{H}_2$ . The resulting beam is then analyzed using a quadrupole mass spectrometer with a  $m/\Delta m \approx 40$ . The formed species can also be deposited on a gold-plated target, and then analyzed using the laser desorption/ionization and high-resolution time-of-flight setup AROMA with a  $m/\Delta m \approx 10,000$ .

#### Preliminary data (results)

In pure He,  $\text{Si}_n$  clusters sized  $n=3-12$  are readily detected using PIRENEA 2. The addition of a mere 0.004% of  $\text{C}_2\text{H}_2$  strongly suppresses the formation of pure  $\text{Si}_n$  clusters, and shows a marked rise of  $\text{SiC}_{2n}\text{H}_{2n+m}$  species, starting at  $\text{SiC}_4\text{H}_4$ . At a  $\text{C}_2\text{H}_2$  content of 0.02%, the formation of  $\text{Si}_n$  clusters is completely suppressed. At the highest  $\text{C}_2\text{H}_2$  percentage, 1%, the distribution of  $\text{SiC}_{2n}\text{H}_{2n+m}$  species has grown to at least  $\text{SiC}_{16}\text{H}_{16\pm 2}$ . Deposition samples prepared in the presence of  $\text{C}_2\text{H}_2$  were analyzed ex-situ with AROMA. The analysis reveals relatively low amounts of  $\text{Si}_n$  species, but does show the presence of many complex hydrocarbons. Typical polycyclic aromatic hydrocarbons (PAHs) are observed such as  $\text{C}_{14}\text{H}_{10}$ ,  $\text{C}_{16}\text{H}_{10}$  and  $\text{C}_{20}\text{H}_{12}$ , which appear preferentially at increasing  $\text{C}_2\text{H}_2$  concentrations. Conversely,  $\text{SiC}_{2n}\text{H}_{2n+m}$  species are only found in trace amounts.

Differences between the two mass spectroscopic characterizations need to be understood from two perspectives. Firstly, deposited material can be processed through surface reactions. During deposition for 1-2 hours, heterogeneous reactions likely occur. Secondly, laser desorption will favor molecular species that are more loosely bound to the surface, so this method does not probe the composition of the entire sample. These preliminary results show that the cationic Si/C/H species that are formed in the gas-phase do not show a stoichiometric Si/C ratio form when they are exposed to acetylene, but instead adsorb an increasing amount of acetylene units to a single Si. Ultimately, this results in the (preferential) formation of PAHs.

#### Please explain why your abstract is innovative for mass spectrometry?

We combine two complementary mass spectrometric characterizations to probe the molecular growth in laser vaporized  $\text{Si}_n^{+0}$  reacting with  $\text{C}_2\text{H}_2$ .

#### Co-authors:

Dianailys Nuñez-Reyes, IRAP, Université de Toulouse III - Paul Sabatier, CNRS, CNES  
Hassan Sabbah, IRAP, Université de Toulouse III - Paul Sabatier, CNRS, CNES  
Sébastien Zamith, LCAR, Université de Toulouse III - Paul Sabatier, CNRS

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Loïc Noguès, IRAP, Université de Toulouse III - Paul Sabatier, CNRS, CNES*  
*Anthony Bonnamy, IRAP, Université de Toulouse III - Paul Sabatier, CNRS, CNES*  
*Christine Joblin, IRAP, Université de Toulouse III - Paul Sabatier, CNRS, CNES*

## Theme: Food and (bio)pharma

### Session: Biopharmaceuticals & Vaccines

Poster number: FP-PB-001

## SEQUENCE VERIFICATION AND SIDE PRODUCT IDENTIFICATION OF SYNTHETIC RNA OLIGONUCLEOTIDES BY LC-ESI-PASEF AND OLIGOQUEST SOFTWARE

Abstract ID: 115

**Presenting author: Detlev Suckau, Bruker Daltonics GmbH & Co. KG**

### Introduction

Oligonucleotide characterization by mass spectrometry has gained significant interest recently with the increased use of DNA and RNA as research reagents as well as pharmaceutical drug molecules. Typical DNA primer molecules are in the range of 20mers while single stranded guide RNA (sgRNA) easily involves the analysis of 100mers at 33 kDa molecular weight.

We developed a workflow for the characterization of RNA oligonucleotides in the range 10-100mers using RP-UPLC-ESI-PASEF (Parallel Accumulation Serial Fragmentation) and the automatic assignment of the MS/MS fragment ions using a prototypic software tool "OligoQuest".

### Methods

Oligonucleotides were methylated at each position; they were UPLC separated and PASEF analyzed on a timsTOF Pro 2 (Bruker). In the workflow, multiple PASEF spectra are accumulated per charge state and the monoisotopic MS/MS peaklist is calculated using the SNAP algorithm. OligoQuest matches monoisotopic fragment ion list against the theoretical fragment ions calculated from the RNA or DNA sequence including multiple modifications. OligoQuest calculates the 5'-, 3'- and internal fragment ions and it provides a visual overview of the match (Fig. 1).

### Preliminary data (results)

The analysis of an RNA 24mer with methylated nucleotides yielded an MS spectrum with an accurate match of the theoretical mass (7969.408 Da), a sequence coverage (SC) of 100% and an intensity coverage of 47%. In addition, 3 early-Rt side products (5-0.5% intensity) were observed with intact masses that indicated a loss of mU (-320 Da), mG (-359 Da) and mC (-319 Da). The simultaneous loss of mG+mU and mC+mG and the addition of mG and mC+mG was also observed in spurious MS peaks. All side products were separated from the target 24mer at 5.8 min (Fig. 2).

The major side product 24mer-mU yielded a PASEF spectrum, which confirmed the assumption (100% sequence coverage) that one of the 4 mU residues 11-14 was not properly added to the sequence (see Fig. 1). The 24mer-mG form matched best to lack of mG-9 with a SC 92%. For the 24mer-mC form 2 positions for lack of mC are possible: mC10 and mC16, none of them could be ruled out at SCs of 90-91%.

The described workflow yielded extensive coverage of the synthetic target RNA 24mer fragment ion spectrum and was well suited to localize sites of missing residues in various side products.

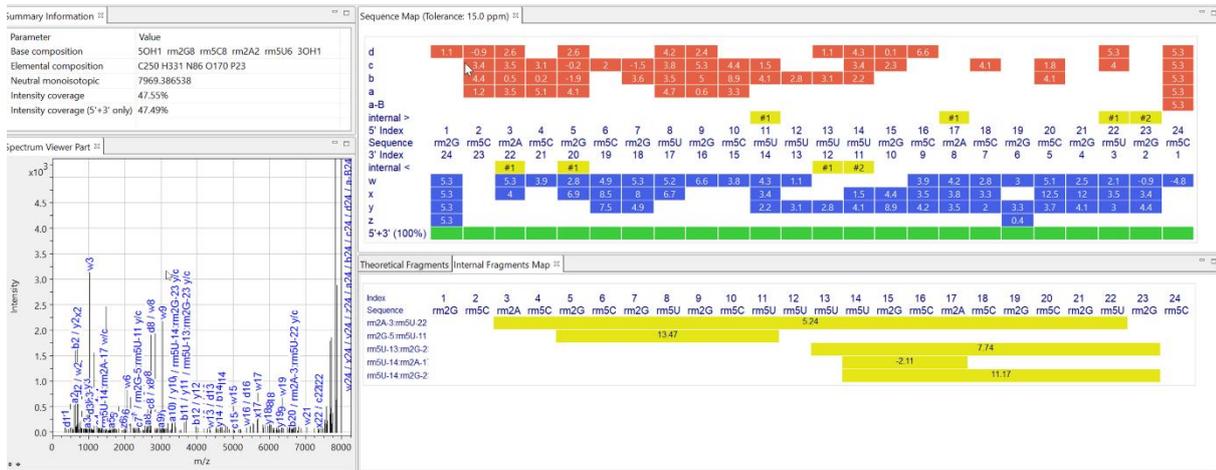
The combination of intact mass analysis with the annotated PASEF spectra in OligoQuest reduce analysis times for target oligonucleotide sequence verification and side product characterization dramatically as the need for manual investigation of MS/MS (PASEF) spectra is greatly diminished.

**Please explain why your abstract is innovative for mass spectrometry?**

Automated analysis of modified RNA molecules and their impurities

Co-authors:

Julia Schneider, Axolabs GmbH  
 Eckhard Belau, Bruker Daltonics GmbH & Co. KG  
 Christian Albers, Bruker Daltonics GmbH & Co. KG  
 Alexander Bunkowski, Bruker Daltonics GmbH & Co. KG  
 Stuart Pengelley, Bruker Daltonics GmbH & Co. KG  
 Dan Fabris, State University New York  
 Peter Sander, Bruker Daltonics GmbH & Co. KG  
 Stephan Seiffert, Axolabs GmbH  
 Ingo Röhl, Axolabs GmbH



Oligonucleotide MS/MS data representation in OligoQuest

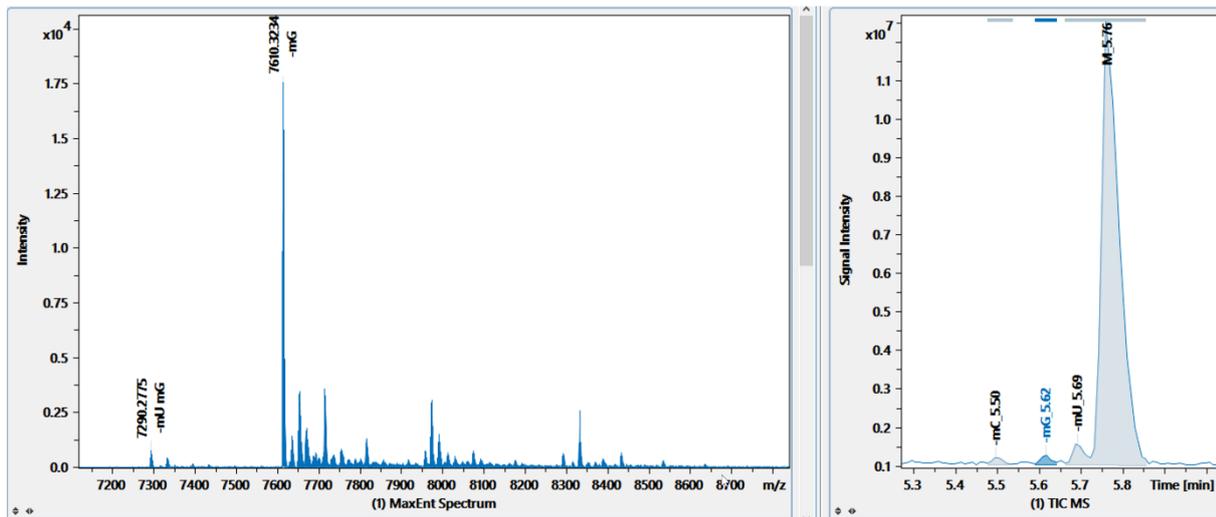


Fig. 2 24mer RNA side product analysis. The chromatogram with the 24mer and the 3 major side product peaks is shown. The peak at 5.52 min is selected and the respective charge deconvoluted spectrum with the 24mer-mG peak displayed

Chromatogram of synthetic oligonucleotide with side products

Poster number: **FP-PB-002**

## **CHARACTERIZATION OF PROTEIN BASED BIOTHERAPEUTICS WITH NEXT-GENERATION MALDI TOP-DOWN SEQUENCING**

Abstract ID: 132

**Presenting author: Arndt Asperger, Bruker Daltonics GmbH & Co KG**

### **Introduction**

Biotherapeutics represent an important class of drug molecules with steadily increasing impact in the pharma market. Due to their molecular size and heterogeneity, characterization of protein-based biologics is a challenging task.

In recent years, MALDI top-down sequencing (MALDI-TDS) has become a widely used method for the characterization of protein biotherapeutics delivering instant confirmation of primary sequences and protein terminal status, such as N-terminal pyroglutamylation or C-terminal lysine loss. It has also been successfully applied to localize glycosylation or PEGylation sites and has provided high sequence coverage to facilitate curation of erroneous reference sequences.

In this study we demonstrate unique capabilities for Next-Generation MALDI-TDS at highest isotopic fidelity and previously unseen mass accuracy (<2 ppm) and further combined with Trapped Ion Mobility Spectrometry (TIMS) technology.

### **Methods**

SARS-CoV-2 RBDs (with a C-terminal His<sub>6</sub>-tag added) were expressed in CHO and HEK293 cells (InVivo Biotech Services). N-linked glycans were released with PNGase F (Promega). Further enzymes, SialEXO and OglyZOR (Genovis), were applied for removal of O-glycans and sialic acids, respectively.

Next Generation MALDI Top-Down Sequencing spectra were acquired from approx. 20-40 pmol RBD using sDHB as a MALDI matrix on a Bruker timsTOF flex instrument equipped with ESI/MALDI dual ion source. Data were analyzed in DataAnalysis, BioPharma Compass 2021 and Biotoools software (all Bruker).

### **Preliminary data (results)**

Next-Generation MALDI-TDS provides in one spectrum accurate intact mass information and highly confident C- and N-terminal sequence readout. High resolving power in the low m/z range allows for acquisition of meaningful MALDI-TDS data in the critical mass range below m/z 1000. Short length MALDI-ISD fragments appear well resolved from complex chemical background and enable sequence verification down to the very terminal amino acid residues. TIMS has the potential to further enhance MALDI-TDS as it allows to dissect C- and N-terminal TDS spectra and, therefore, is of great benefit to simplify data analysis when elucidating unexpected sequence errors or unknown protein sequences. Furthermore, TIMS enhances T3-Sequencing by efficient removal of isobaric interferences yielding cleaner MS/MS spectra.

To highlight the performance of the instrument (timsTOF flex, Bruker Daltonics) in Next-Gen MALDI-TDS, we present here data obtained from bovine carbonic anhydrase II. Reference data achieved an intact mass accuracy of 0.5 ppm and a sequence validation percentage (SVP) of 86.9% with an average fragment mass deviation of <2 ppm.

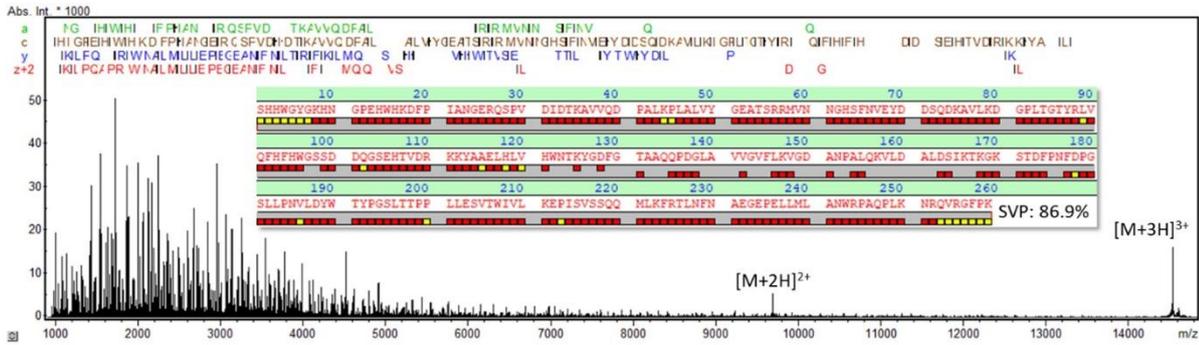
Furthermore, we describe pharma application examples illustrating the potential of Next-Gen MALDI-TDS for efficient characterization of protein based biotherapeutics: For adalimumab subunits, pre-fractionated by LC, intact mass accuracy of 0.5 ppm and SVPs between 70 and 83% were achieved. Next-Gen MALDI-TDS of a recombinantly expressed SARS-CoV-2-S-glycoprotein RBD allowed for unambiguous assignment of O-glycosylation type present in the antigen product as well as highly confident identification of the active site of O-glycosylation in the RBD's N-terminal region.

### **Please explain why your abstract is innovative for mass spectrometry?**

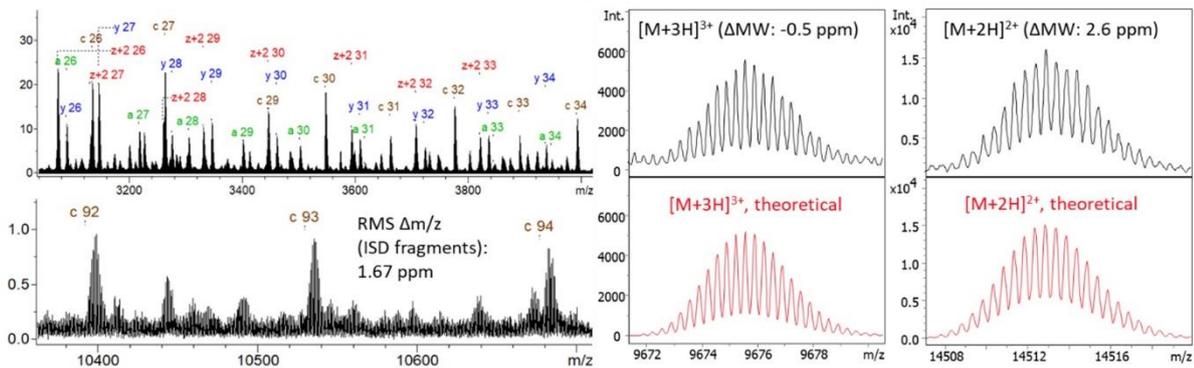
Top-down approach utilizing MALDI-ISD in combination with Trapped Ion Mobility and UHR-TOF for N- and O-glycosylation analysis of SARS-CoV-2 RBD

Co-authors:

Detlev Suckau, Bruker Daltonics GmbH & Co KG  
 Anja Resemann, Bruker Daltonics GmbH & Co KG  
 Waltraud Evers, Bruker Daltonics GmbH & Co KG



Total view on sequence annotated spectrum. Sequence view inset displays sequence validation percentage (SVP) achieved (86.9%). Upper brick row refers to assignments based on N-terminal ISD fragments; lower brick row refers to assignments based on C-terminal ISD fragments

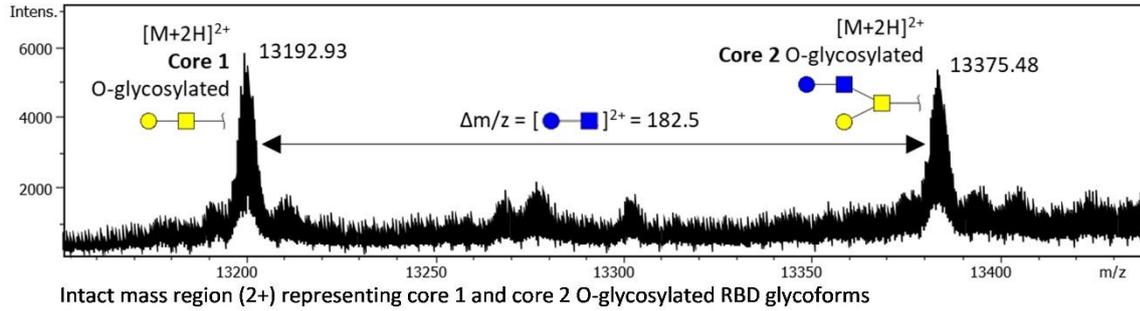


Right: View on 2+ and 3+ intact mass ion signals detected in the MALDI-TDS spectrum. High match quality with theoretical isotope patterns documents the outstanding data quality. Left: View on ISD fragment m/z regions illustrating the high quality of sequence annotation.

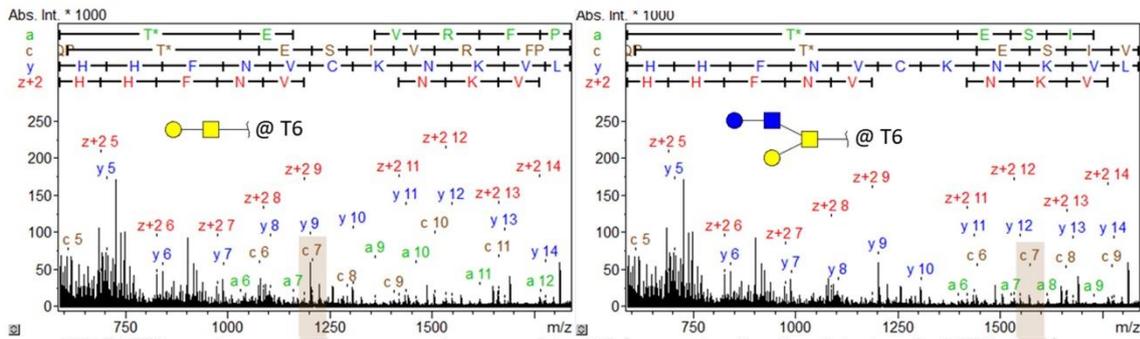
Next Generation MALDI-TDS spectrum of 29 kDa bovine CA II

POSTER SESSION B

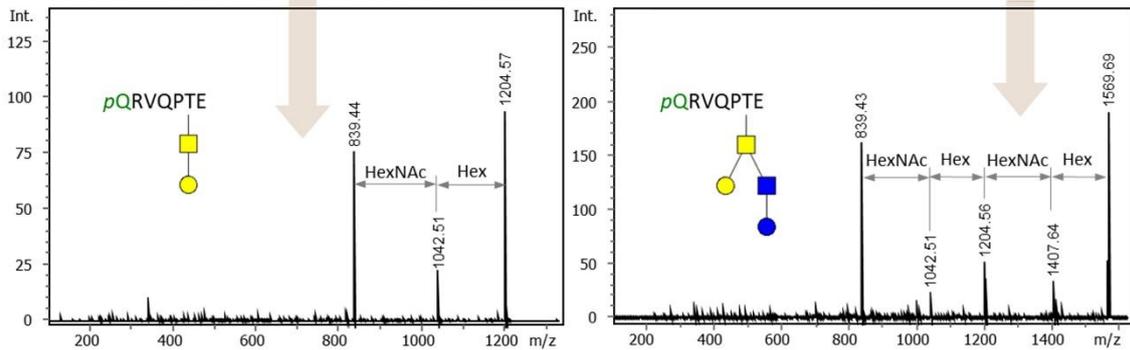
Wednesday 31 August 2022 from 14:00 to 15:30 hours  
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Intact mass region (2+) representing core 1 and core 2 O-glycosylated RBD glycoforms



MALDI-TDS spectra zoomed on mass region comprising ISD fragments c6 – c8 matched against RBD glycoforms carrying either core 1 (center, left) or core 2 O-glycosylation, with Thr-6 as proposed O-glycosylation site



TIMS-T<sup>3</sup>-Sequencing spectra acquired from N-terminal ISD fragments c7 providing further evidence for the presence of core 1 and core 2 O-glycosylation at Thr-6

MALDI-TDS spectra: recombinant SARS-CoV-2 S-glycoprotein RBD HEK293 expression product

Poster number: FP-PB-003

## AUTOMATED HIGH RESOLUTION MRNA DIGEST MAPPING OF LC-MS AND LC-IM-MS DATA

Abstract ID: 175

Presenting author: Johannes Vissers, Waters Corporation

### Introduction

mRNA has evolved into being an important modality with potential, as shown with the start of in-human clinical trials for a cancer treatment and the authorization of mRNA vaccines for COVID-19. The rapid development of mRNA based vaccines and therapeutics is supported by advances in analytical methodologies. An important aspect is conformation of identity, purity and modification of a therapeutic mRNA through sequence mapping. LC-MS has the advantage of direct molecular detection of mRNA fragments over conventional techniques, including the detection and localization of impurities and important attributes, like end-capped residues and polyA tail modifications. A comprehensive workflow for mRNA bottom-up characterization using a single platform is presented, comprising LC, UV, MS and IMS measurements, followed by digest component processing using dedicated informatics tools.

### Methods

Two synthetic firefly luciferase mRNA sequences were denatured and digested with RNase T1. The resulting digests were subjected to ion-pairing RP chromatography without further cleaning prior to LC-MS analysis in negative ionization mode using various IMS-enabled oa-ToF geometries. The synthetic mRNA sequences were *in-silico* digested using development software with a given enzyme, modifications, and number of missed cleavages. As a result, a list of digestion components, within a specified  $m/z$  range, was generated and imported as a target component library in dedicated analysis software. Components resulting from the digestion of the luciferase mRNAs sequences were automatically assigned and mapped.

### Preliminary data (results)

Ion-pairing RP was applied for the separation and annotation of firefly luciferase mRNA RNase T1 digest fragments using conventional LC-oa-ToF-MS instrumentation at 10,000 FWHM mass resolution. A typical separation result in the form of a TIC, run in triplicate, is shown in Figure 1, illustrating elution of mRNA digest products between 0 and 25 min, the polyA mRNA tail and intact mRNA, when present and detailed schematically in Figure 2, around 35 - 40 min, and intact RNase T1 at 50 min and beyond. Hence, within a single LC-MS run, characterization of both the constituting and resulting components of an mRNA-digest mapping experiment are feasible.

Automated annotation of the digest fragment was achieved through the use of custom, development software, since unlike bottom-up proteomics workflows, where a plethora of informatic solutions for data processing solutions exist, options for mRNA mapping are limited. The user interface of the custom tool is shown in Figure 2, which allows for the *in-silico* digestion of mRNA sequences using a customizable set of parameters. The generated output can be used to create compound identification target list for LC-MS peak annotation. The latter is demonstrated in Figure 2 as well, where the TIC peaks are annotated with mRNA-digest fragments. In this particular instance, the data were collected using IMS assisted DIA with the oa-ToF analyzer operating at 30,000 FWHM. Digestion efficiency, as will be demonstrated, can be readily obtained by evaluation of coverage and UV signal of digest components vs. remaining, intact mRNA.

### Please explain why your abstract is innovative for mass spectrometry?

Development of dedicated chromatographic methods and informatics tools for the enhanced analysis of enzymatically digested mRNA by means of LC-MS and LC-IM-MS mapping.

### Co-authors:

Maissa Gaye, Waters Corporation  
Jonathan Fox, Waters Corporation  
Ana-Maria Rotaru, Waters Corporation  
Emanuela Petreanu, Waters Corporation



Poster number: **FP-PB-004**

## **FAST AND ROBUST QUANTITATION OF HOST CELL PROTEINS WITH SUB PPM SENSITIVITY USING DIAPASEF AND VIP-HESI**

Abstract ID: **218****Presenting author: Stuart Pengelley, Bruker Daltonics GmbH & Co. KG**

### **Introduction**

Residual Host Cell Proteins (HCPs) present in biopharmaceutical drug preparations can be detrimental to the drug quality or efficacy, and it is therefore important to remove as many HCPs as possible in the final product. This is usually done using a combination of purification steps, and HCP clearance is monitored throughout the pipeline. Mass spectrometry has emerged as an alternative to the more established ELISA method, and has several benefits such as sample flexibility and the ability to identify individual HCPs. Here we apply parallel accumulation – serial fragmentation combined with data-independent acquisition (diaPASEF(1)) in a robust analytical-flow configuration. Sensitivity is enhanced using the Vacuum Insulated Probe Heated-ESI (VIP-HESI) ion source, allowing HCP detection and quantitation at the sub ppm level.

### **Methods**

Dynamic Universal Proteomics Standard 2 (UPS2, Sigma) was spiked at 1 in 100 into NISTmAb (Merck) and digested under native conditions as previously reported (ref). Peptides were separated using a 15 min gradient (200  $\mu$ L/min) on a CSH C18 1.7  $\mu$ m 2.1 x 100 mm column (Waters) with the Elute UHPLC interfaced with the timsTOF Pro 2 via the VIP-HESI ion source (all Bruker). Data were acquired using data independent acquisition (dia-PASEF) and spectra were searched against a mouse and UPS PASEF spectral library in Spectronaut and quantified using the Top3 method.

### **Preliminary data (results)**

Data-independent mass spectrometry methods allow complex samples to be screened using short gradients by matching spectra against a library of spectra acquired using data-dependent acquisition. This method is very attractive for the biopharmaceutical industry because libraries can be established for the host cells, then applied for rapid, high throughput HCP screening. In this work, native digests of NISTmAb spiked with equimolar UPS1 (Sigma) were analysed with a 150 min gradient and PASEF data were processed in Spectronaut to generate a spectral library composing of 1297 peptides. diaPASEF data was acquired in triplicate using a 15 minute gradient. The resulting spectra were matched against the spectral library via ion mobility profiles and relative retention time, resulting in the identification of UPS2 proteins across >5 orders of magnitude. UPS2 proteins were detected between approximately 3000 and 0.01 ppm relative to the NISTmAb. Quantitation was performed on the MS2 fragments. A regression line was calculated from this data and used for calculating the amounts of NISTmAb HCPs present in the sample. 140 NISTmAb HCPs were identified and quantified in all replicates between 0.03 ppm and 158 ppm (ng/mg), with ion mobility recorded as an additional identification metric. This demonstrates that the combination of diaPASEF, analytical flow rates and the optimal heated ionization conditions provided by VIP-HESI permits sensitive, sub-ppm HCP screening with short run times, and is ideally suited to downstream biopharma QC applications.

### **Please explain why your abstract is innovative for mass spectrometry?**

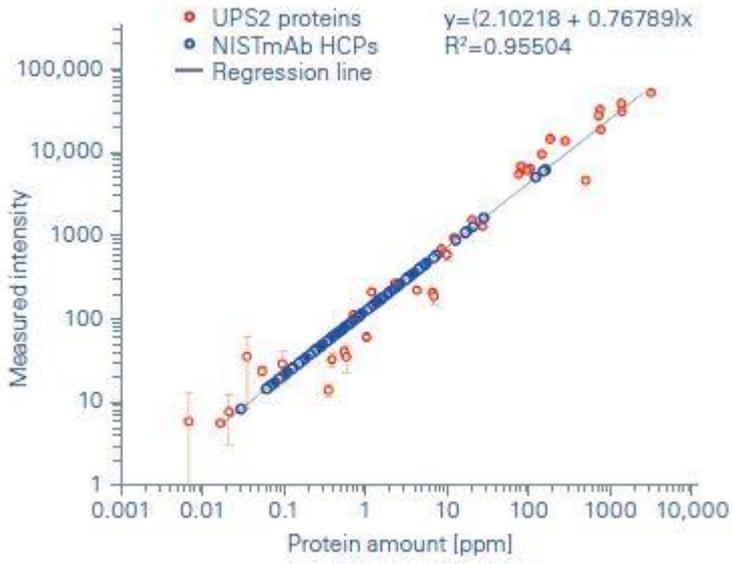
Sub-ppm quantitation of HCPs using a robust hardware configuration which includes VIP-HESI ion source. diaPASEF also provides reliable mobilograms even at 0.1 ppm for peptide validation with CCS values

### **Co-authors:**

*Eckhard Belau, Bruker Daltonics GmbH & Co. KG*  
*Waltraud Evers, Bruker Daltonics GmbH & Co. KG*  
*Christian Albers, Bruker Daltonics GmbH & Co. KG*  
*Detlev Suckau, Bruker Daltonics GmbH & Co. KG*  
*Eric van Beelen, Bruker Daltonics GmbH & Co. KG*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Quantitation of NISTmAb HCPs based on UPS2 regression line

Poster number: **FP-PB-005**

## **DIFFERENTIATION OF ISOBARIC 3'-/5'- OLIGONUCLEOTIDE CLIPPING PRODUCTS BY TRAPPED ION MOBILITY SPECTROMETRY**

Abstract ID: **418**

**Presenting author: Christian Albers, Bruker Daltonics GmbH & Co KG**

### **Introduction**

Oligonucleotide characterization by mass spectrometry has gained significant interest recently with the increased use of DNA and RNA as research reagents as well as pharmaceutical drug molecules. Characterization of production related impurities or degradation products is of key importance during the approval process of such biopharmaceutical products. Well established tools for this characterization process are LC-MS based methods.

In our work, we use an additional technique, trapped ion mobility spectrometry (TIMS), to characterize oligonucleotide degradation products. By measuring the collisional cross sections (CCS) of the two isobaric oligonucleotide clipping products with an LC-tims-MS approach, we differentiate the molecules using inherent molecular properties.

### **Methods**

Two isobaric 9mer Oligonucleotides were analyzed. These forms are clipping products originating from a 10mer molecule with guanine as base at both termini. The 9mer oligonucleotides are obtained by loss of a guanosine nucleotide either at the 5'- or 3'-end, providing two isobaric molecules. The samples were measured with UPLC, utilizing a short gradient, connected to a MS instrument with TIMS functionality (timsTOF Pro2, Bruker) for differentiation based on the characteristics of the molecules' gas-phase behavior.

Extracted ion mobilogram (EIM) traces were created and CCS values were calculated to validate if a differentiation of these isobaric molecules is possible.

### **Preliminary data (results)**

The EIM traces for the different measured charges states were used to compare the behavior of the two isobaric molecules in the gas phase. A differentiation in the gas-phase structure was identified by measuring the ion mobility of the higher charge (-6 to -4) states of the two molecules whereas the lower charge states (-3 to -2) did not show a significant difference in ion mobility. Using triplicate injections of each sample, we demonstrated the robustness and reproducibility of the TIMS technology for CCS value calculations and molecular differentiation.

### **Please explain why your abstract is innovative for mass spectrometry?**

Utilization of oligonucleotide gas-phase structure to distinguish isobaric process impurities

### **Co-authors:**

*Stephan Seiffert, Axolabs GmbH*  
*Julia Schneider, Axolabs GmbH*  
*Ingo Röhl, Axolabs GmbH*

Poster number: FP-PB-006

## DEVELOPMENT OF A GLYCOFORM-SPECIFIC PHARMACOKINETIC NANO-LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY METHOD FOR A MONOCLONAL ANTIBODY FROM PLASMA OF RHEUMATOID ARTHRITIS PATIENTS

Abstract ID: 463

Presenting author: Annika van der Zon, University of Amsterdam, Leiden University Medical Center

### Introduction

One percent of the world population is diagnosed with rheumatoid arthritis (RA). To reduce the symptoms of this auto-immune disease, such as inflammation, pain, or stiffness, therapeutic recombinant monoclonal antibodies (mAb) can be applied in the treatment. However, the antibodies feature many different types of post-translational modifications (PTMs). The pharmacokinetic (PK) behaviour of mAbs with different PTMs, especially N-glycosylation, can significantly impact the interaction with various immune receptors in the human body and the half-life of the mAb. Therefore, it is crucial to study the PK behaviour of individual glycoforms. The aim of our project was to capture the recombinant mAb from plasma samples of patients receiving treatment and establish a middle-up method for its characterization with nano-liquid chromatography-mass spectrometry (nano-LC-MS).

### Methods

The antibody was captured from human plasma by interacting with a capturing antibody linked to streptavidin-coated magnetic beads to analyze the N-glycans. After capturing, the antibody was denatured and reduced. The free antibody light and heavy chains were separated using a C4 column. A high-resolution MS was applied to distinguish between the mAb of interest and other antibodies that could be co-captured, dopant enriched gas was used to reduce TFA adducts and ion suppression. Several parameters of the nano-LC-MS and sample preparation method were optimized, e.g., gradient program, injection volume, dopant gases, sample volume, denaturation buffer (pH), and reducing agent.

### Preliminary data (results)

The optimized method made it possible to separate the reduced antibody light and heavy chains in 25 minutes run with good chromatographic performance. 100 % acetonitrile was used as a dopant gas to increase the sensitivity. The total required sample volume was 250  $\mu$ L plasma. 100 mM Tris and 8 M guanidine-hydrochloric acid at pH 7 were required for the denaturation of the protein. The reduction was completed with 20 mM Tris(2-carboxyethyl)phosphine hydrochloric. Finally, the method was validated on recovery, matrix effect, detection limit, intra-day repeatability, and inter-day repeatability. This application met all the validation criteria. The dynamic range of the method was 2.5  $\mu$ g/mL – 10  $\mu$ g/mL. Higher concentrations should be diluted to avoid overloading of the LC columns. During the lifetime of the protein, other modifications can be occurred, such as oxidation. To determine the effect of oxidation on the analysis, oxidized samples were measured. The N-glycans of interest could still be identified and quantified. Concluded, with the optimized method, it is achievable to capture the pharmaceutical mAb from patient plasma and analyze the N-glycans on the heavy chain in a dynamic range of 2.5  $\mu$ g/mL – 10  $\mu$ g/mL by using nano-LC-MS.

### Please explain why your abstract is innovative for mass spectrometry?

With mass spectrometry, the PK behaviour of specific N-glycans can be observed from human patient samples. This may help pharmaceutical industries to produce a glycoengineered mAb with a higher half-life.

### Co-authors:

*Christoph Gstöttner, Leiden University Medical Center*  
*Manfred Wuhrer, Leiden University Medical Center*  
*Elena Domínguez Vega, Leiden University Medical Center*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

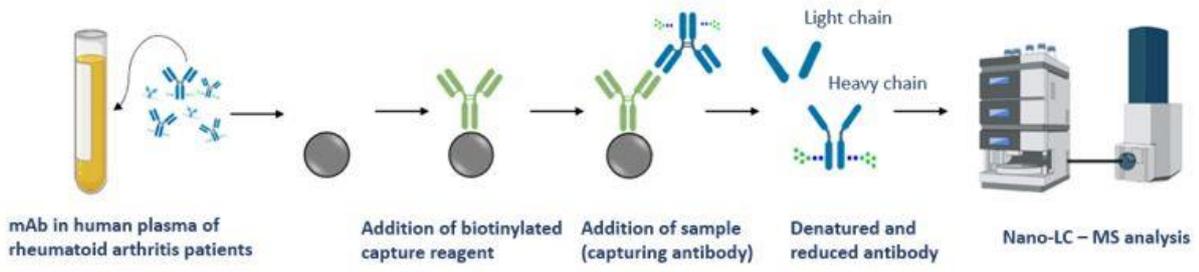


Illustration of extraction mAb from plasma and measured with nano-LC-MS

Poster number: FP-PB-007

## GLYCAN AND PROTEIN ANALYSIS OF GLYCOENGINEERED BACTERIAL E. COLI VACCINES BY MALDI-IN-SOURCE DECAY FT-ICR MASS SPECTROMETRY

Abstract ID: 512

Presenting author: Manfred Wuhrer, Leiden University Medical Center

### Introduction

Bacterial glycoconjugate vaccines have a major role in preventing microbial infections. Immunogenic bacterial glycans, such as O-antigen polysaccharides, can be recombinantly expressed and combined with specific carrier proteins to produce effective vaccines. O-antigen polysaccharides are typically polydisperse, and carrier proteins can have multiple glycosylation sites. Consequently, recombinant glycoconjugate vaccines have a high structural heterogeneity, making their characterization challenging. Since development and quality control processes rely on such characterization, novel strategies are needed for faster and informative analysis. Here, we present a novel approach employing minimal sample preparation and ultrahigh-resolution mass spectrometry analysis for protein terminal sequencing and characterization of the oligosaccharide repeat units of bacterial glycoconjugate vaccines.

### Methods

Three glycoconjugate vaccine candidates, obtained from the bioconjugation of the O-antigen polysaccharides from *E. coli* serotypes O2, O6A and O25B with the genetically detoxified exotoxin A from *Pseudomonas aeruginosa*, were analyzed by 15 T MALDI-in-source decay (ISD) FT-ICR MS. Protein and glycan ISD fragment ions were selectively detected using 1,5-diaminonaphthalene and a 2,5-dihydroxybenzoic acid / 2-hydroxy-5-methoxybenzoic acid mixture (super-DHB) as a MALDI matrix, respectively. MS/MS analysis of O-antigen ISD fragments allowed for the detection of specific repeat unit signatures.

### Preliminary data (results)

In this study, we explored MALDI-ISD FT-ICR MS for the characterization of the glycoconjugates EcoO2, EcoO6A and EcoO25B to confirm the N- and C-terminal portions of the EPA protein that was recombinantly expressed. Desalting of the samples was key for the sensitive detection of protonated or deprotonated protein fragment ions. Typically, long series of c<sup>-</sup>, y- and z<sup>-</sup>-type fragment ions were detected up to *m/z* 7000 and used for the determination of N- and C-terminal sequences with a sequence coverage of 12%, 8% and 14% for EcoO2, EcoO6A and EcoO25B, respectively. Ultrahigh-resolution measurements allowed for a reliable identification of ISD fragment ions even in *m/z*-region below *m/z* 1000 which is typically dominated by MALDI matrix cluster ions.

The bacterial vaccines were also analyzed by MALDI-ISD FT-ICR MS with super-DHB and without desalting of the samples. This allowed for the suppression of the ISD fragment ions generated from the protein moiety enhancing instead the signal of sodiated polysaccharide fragment ions. B ions corresponding to up to 11 repeat units (RU) could be detected using different acquisition methods and depending on the analyzed glycoconjugate. B ions corresponding to 1RU and 2RU were further characterized by CID MS/MS for corroboration of the repeat unit composition.

The developed strategy requires minute sample amounts, avoids the use of chemical derivatizations, and comes with minimal hands-on time allowing for fast corroboration of key structural features of bacterial glycoconjugate vaccines during early and late-stage development.

### Please explain why your abstract is innovative for mass spectrometry?

A new, direct and fast strategy for the characterization of complex glycoconjugate bacterial vaccines carrying O-antigen polysaccharides by ultrahigh-resolution MALDI FT-ICR MS was developed.

### Co-authors:

Chakkumkal Anish, Bacterial Vaccine Discovery & Early Development, Janssen Vaccines and Prevention B.V.  
Renzo Danuser, Janssen Vaccines AG

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



*Viktoria Dotz, Bacterial Vaccine Discovery & Early Development, Janssen Vaccines and Prevention B.V.*

*Elena Domínguez-Vega, Leiden University Medical Center*

*Ali Al Kaabi, Janssen Vaccines AG*

*Michel Beurret, Bacterial Vaccine Discovery & Early Development, Janssen Vaccines and Prevention B.V.*

*Simone Nicolardi, Leiden University Medical Center*

Poster number: **FP-PB-008**

## CHARACTERIZATION OF MRNA VACCINE 5' AND 3' END PRODUCTS USING BIOPHARMAFINDER 5.0

Abstract ID: **545****Presenting author: Robert L Ross, Thermo Fisher Scientific**

### Introduction

Messenger RNA (mRNA) transcripts represent a new class of therapeutics. By co-opting the cells bio-machinery, mRNA treatments can produce the enzymes and proteins necessary to alleviate suffering. Production of these treatments necessitates strict quality control at different steps of production. 5' capping and 3' poly adenylation are two critical parameters in mRNA production whether in vitro or in vivo. Enzymatic digestion followed by LC-MS/MS is the gold standard for analytical RNA measurements, what has lagged is the necessary software for processing data. Here we reported an IPRP-LC-HRAM MS/MS workflow with a dedicated software solution for automated data processing and annotation of 5' and 3' end products from mRNA.

### Methods

Synthetic mRNA was digested with RNase H (NEB) using a complementary biotinylated probe (25-mer) containing two chimeric DNA bases (IDT-DNA) or RNase T1. Poly A samples were purified post digestion with oligo d(T) magnetic beads. Samples were separated on a Thermo Scientific™ Vanquish™ Horizon™ UHPLC then analyzed on a Orbitrap™ Fusion™ Lumos™ or Exploris™ 240 mass spectrometer

Thermo Scientific™ Biopharma Finder™ 5.0 software was used for identification and characterization of digestion products via a dedicated oligonucleotide analysis workflow.

### Preliminary data (results)

RNase H digestion: Full MS spectra were acquired at resolution setting of 120,000 at 200 m/z and data dependent MS/MS fragmentation acquired at a resolution setting of 30,000 at 200 m/z providing isotopic resolution for all identified components. Collision energy and dynamic exclusion parameters were investigated to determine suitable values to provide optimal fragmentation spectra across a wide range of sequence lengths and charge states. For the poly(A) tail, data was acquired in MS only.

Data analysis was performed to identify and map digestion fragments to the mRNA sequence. Digestion fragment identification was confirmed utilizing MS/MS confirmation with spectrum annotation performed automatically within the processing software. Initial results demonstrate the high confidence identification of digestion fragments generated by the mRNA digest.

Confidence scores for digestion fragment identification are provided by the data processing software based on identified the fragment m/z values as well as a comparison of the MS/MS fragmentation spectra to a predicted MS/MS fragmentation model. In addition to the stringent acceptance criteria for confidence scores, additional acceptance criteria were applied such as a minimum intensity threshold to further increase identification confidence. Using this approach, sequence mapping coverage greater than 90% was obtained for each of the mRNA samples analyzed.

### Please explain why your abstract is innovative for mass spectrometry?

Automated characterization of mRNA capping and poly(A) tail.

### Co-authors:

*Qian Qi, Thermo Fisher Scientific*  
*Martin Zeller, Thermo Fisher Scientific*  
*Keeley Murphy, Thermo Fisher Scientific*  
*Min Du, Thermo Fisher Scientific*

Poster number: FP-PB-009

## THE POTENTIAL OF INTACT CELL MASS SPECTROMETRY AS A MONITORING TOOL FOR FERMENTATION PROCESSES

Abstract ID: 624

**Presenting author: Cristian Zanetti, TU Wien, Institute of Chemical Technologies and Analytics, Getreidemarkt 9/164, 1060 Vienna, Austria**

### Introduction

MALDI-Intact Cell Mass Spectrometry (MALDI-ICMS) is a helpful technique for discriminating/identifying microorganisms (MALDI Biotyping); it is used in clinics for rapid pathogen identification in case of hospital-acquired infections (e.g., *Staphylococcus aureus*), enabling quick response and intervention. Industrial fermentations exploit microorganisms for synthesizing products of interest; while the failure of such fermentations is cost-intensive, process characterization can help decision-making for process optimization. We present a MALDI-ICMS method applied to an *E. coli* fermentation process; by using multivariate data analysis, we exploited time-dependent spectral changes to build a model capable of monitoring the fermentation progress. This approach will allow better early-stage process characterization and prediction of process outcome.

### Methods

*E. coli* fermentation samples were washed by pelletation and resuspension in an MS-compatible buffer ( $\text{NH}_4\text{HCO}_3$ , pH 7), equalized to the same  $\text{OD}_{550}$  and mixed with MALDI matrix at a ratio of 1:2 (sinapinic acid/ferulic acid, 0.5 % trifluoroacetic acid, 70 % acetonitrile). The mixture was spotted on a MALDI target, once dried it was inserted in a MALDI-TOF instrument (UltrafleXtreme, Bruker), spectra were acquired in the linear positive ion mode, in the mass range of 2-20 kDa. Data was preprocessed using R packages, peak intensities were saved in a matrix and further used for statistical evaluations applying different approaches.

### Preliminary data (results)

*E. coli* samples from fed-batch fermentors of different sizes (mL and L volumes) were analyzed by MALDI ICMS. Samples were collected from parallel fermentation processes at different timepoints, prepared and spotted in replicates on a MALDI target. An automated acquisition was executed in the positive linear mode to accumulate 1000 laser shots for each spot. ICMS data were preprocessed using R to obtain an intensity/data-matrix usable for subsequent statistical analyses. Principal Component Analysis (PCA) of large volume data showed low biological variability and shows the potential of PCA to separate fermentation phases (batch, feed, induction). We applied Partial Least Square Regression (PLSR), which was trained by labeling the data with respective fermentation times. Model cross-validation revealed a very low prediction error of less than 5% compared to total fermentation time. To ensure that an appropriate number of MALDI spots were used, outcomes were compared for models trained with data from three and eight replicates. Depending on process demands, three replicates can be good enough. The PLSR model's robustness for different fermentation volumes was evaluated. ICMS data from all fermentations, small and large volumes, were combined; this led to a PLSR prediction error below 5% compared to the total fermentation time. Yet, small-size mutlifermmentor samples are usually used for screening in industrial processes. Using the small volume dataset to predict the large volume process led to an increase of the prediction error, but was shown to be a valid method to foresee the outcome of an industrial scale fermentation processes.

### Please explain why your abstract is innovative for mass spectrometry?

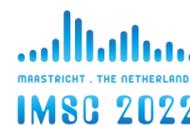
MALDI-ICMS is a powerful tool to build prediction models from small-scale fermentations to estimate outcomes for large scale production.

### Co-authors:

Christopher Stephan, Boehringer Ingelheim RCV GmbH & Co KG, Dr. Boehringer-Gasse 5-11, 1121 Vienna, Austria  
Alexandra Foettinger-Vacha, Boehringer Ingelheim RCV GmbH & Co KG, Dr. Boehringer-Gasse 5-11, 1121 Vienna, Austria

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Martina Marchetti-Deschmann, TU Wien, Institute of Chemical Technologies and Analytics, Getreidemarkt 9/164, 1060 Vienna, Austria*

Poster number: **FP-PB-010**

## **CHARACTERIZATION OF ANTIBODY-DRUG CONJUGATES (ADC) USING 2-DIMENSION LIQUID CHROMATOGRAPHY (2D-LC) AND NATIVE MASS SPECTROMETRIC TECHNOLOGIES**

Abstract ID: **724****Presenting author: Rebecca Konietzny, Agilent Technologies**

### **Introduction**

Antibody drug conjugates (ADCs), a fast-growing class of biomolecule, comprise a monoclonal antibody (mAb) conjugated to a small molecule drug through synthetic linkers. The ratio of the conjugated drug to mAb (drug-to-antibody ratio or DAR) is one of the critical quality attributes for ADC development because it can affect efficacy and safety. To characterize the ADC molecules, a typical 2D-LC/MS approach whereas Hydrophobic Interaction Chromatography (HIC) and Reversed-phase column were used. However, many degraded ADC products were detected under the organic and acid solvent condition.

In this presentation, we present a novel 2D-LC approach to overcome this obstacle, which utilizes HIC, Multiple Heart-cutting (MHC) and subsequent desalting/separation using Size Exclusion Chromatography (SEC) on-line with native MS analysis.

### **Methods**

For the research material tested in this study, a cysteine-conjugated ADC sample (Brentuximab vedotin) was used for method optimization and characterization. LC/MS analyses were conducted on a 2D-LC system coupled with a LC/Q-TOF system equipped with Jet Stream source. Various columns (HIC, PLRP-S and SEC) were used. Samples in denaturing, reduced and native conditions were analyzed using various MS data acquisition modes. All MS data of the ADC and its impurities were processed using MassHunter Acquisition, Qualitative Analysis, and BioConfirm software.

### **Preliminary data (results)**

Our analytical results indicated that the different ADC molecules were separated successfully in the first dimension (HIC) chromatography and the DAR values of each of the peaks were determined (up to 8). The LC/MS results demonstrated: 1) Under the denaturing condition (PLRP-S column used in the 2<sup>nd</sup> dimension), the majority of the intact ADCs were dissociated into various components including light chain mAb, heavy chain mAb and with various drug conjugations. 2) Under the native LC/MS analytical condition (SEC column in the 2<sup>nd</sup> dimension), the intact ADC molecules were preserved. This technique confirmed the ADCs with different drug-to-antibody ratio (DAR) supported by the accurate mass determination of the ADC conjugate. Additionally, different HIC solvent conditions were evaluated to study both separation of the ADC peaks as well as the ability to preserve the native structure for MS analysis.

### **Please explain why your abstract is innovative for mass spectrometry?**

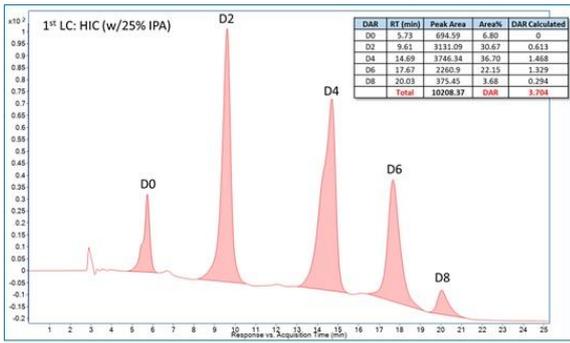
Characterization of antibody drug conjugates (ADC) by 2D-LC/Native MS analysis.

### **Co-authors:**

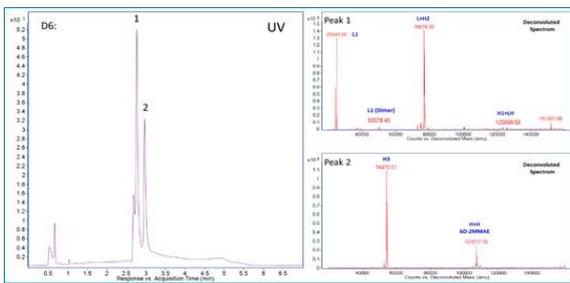
*David Wong, Agilent Technologies*  
*Sarah Stow, Agilent Technologies*  
*Christian Klein, Agilent Technologies*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



HIC separation and DAR determination of brentuximab vedotin.



2nd dimension liquid chromatogram and MS deconvoluted spectrum DAR6.

Poster number: FP-PB-011

## GREAT PROMISE OF NATIVE SCX-MS FOR MAB AND ADC HETEROGENEITY CHARACTERIZATION

Abstract ID: 726

**Presenting author: Séverine Clavier, Sanofi R&D, BioAnalytics department, Global CMC development**

### Introduction

Monoclonal antibodies (mAbs) and Antibody-drug-conjugates (ADCs) today represent one of the largest classes of newly approved drugs in the treatment of cancer and auto-immune diseases. These biotherapeutics produced in cells are heterogenous molecules. For naked mAb source of heterogeneity are post-translational modifications (PTMs) such as oxidation, deamidation, isomerization, glycation and N-glycosylation of the constant region (Fc domain). Conjugation of small molecules, either cytotoxic compounds or receptor ligands further increase the complexity of the biotherapeutics then named ADCs. Elucidation of charge variants by mass spectrometry (MS) has historically been a particularly challenging task. Indeed, due to the nonvolatility and high ionic strength of conventional buffer used for their separation, identification by MS had to be performed after labor-intensive offline fractionation.

### Methods

In addition to the conventional Reverse Phase (RP) and denaturing Size Exclusion Chromatography (SEC) classically used for the intact mass measurement of biotherapeutics, Strong Cation eXchange (SCX) with a BioResolve SCX column was directly coupled to an high resolution mass spectrometer. Ionic strength and pH conditions were screened on different biomolecules to maximize charge variants separation for mAb according to their isoelectric points and of ADC according to their drug loads. Electrospray ionization source conditions were also carefully tuned for each molecule.

### Preliminary data (results)

Application of this native SCX-MS to all biotherapeutics currently in development in Vitry-sur-Seine Sanofi R&D site required significant method parameters adjustments depending on the molecule. This allowed us to get a good understanding of the method great potential and remaining limits. It was particularly interesting to see how this method could help detecting PTMs and mispaired species for non-conventional mAb format. We also were able to obtain a good separation of cysteine linked ADCs according to their drug load which is normally a very challenging task due to the non-covalent association of the heavy and light chains of these mAbs following drug coupling on cysteines residues.

### Please explain why your abstract is innovative for mass spectrometry?

This novel hyphenation method allowing native MS analysis of proteins has the potential to greatly ease and improve the characterization work of biotherapeutics molecules which have always more complex scaffolds.

### Co-authors:

*Nelly Lechat, Sanofi R&D, BioAnalytics department, Global CMC development*  
*Florence Cordier, Sanofi R&D, BioAnalytics department, Global CMC development*  
*Hélène Le Borgne, Sanofi R&D, BioAnalytics department, Global CMC development*  
*Séverine Beauvisage, Sanofi R&D, BioAnalytics department, Global CMC development*

Poster number: FP-PB-012

## A HIGH-EFFICIENCY WORKFLOW FOR THE MONITORING OF ANTIOXIDANT ADDITIVES IN SINGLE-USE SYSTEMS

Abstract ID: 739

Presenting author: Siegrun Mohring, Thermo Fisher Scientific

### Introduction

Single-Use Systems (SUS) are increasingly used in the biopharmaceutical industry for production of biologicals. Despite their advantages, these polymeric assemblies receive attention from end-users as well as regulatory agencies because they are a potential source of contamination due to extractable and leachable (E&L) compounds. One primary source of E&L compounds encountered in SUS usage are antioxidants and their derivatives, which can migrate into the processing liquid. These substances could potentially jeopardize bioprocessing performance, cause cell growth inhibition, loss of expensive cell lines and reduce yields. For this reason, their monitoring and characterization is essential. Here we describe a complete workflow from sample preparation to data interpretation for the monitoring of such compounds based on LC-MS detection.

### Methods

This work represents our efforts in addressing the challenge of detection and monitoring of extractable anti-oxidants (and their derivatives) *via* accelerated solvent extraction (ASE) technique for extraction from SUS components, coupled with ultra-high performance liquid chromatography (UHPLC) separation and detection by high-resolution accurate mass (HRAM) mass spectrometry using an Orbitrap-based MS. Data were processed in Compound Discoverer for unbiased peak detection and compound annotation.

### Preliminary data (results)

Using common SU component materials *e.g.*, polycarbonate (PC), polyvinyl chloride (PVC), polypropylene (PP), polyetherimide (PEI), *etc.* as testing samples, common antioxidants and their derivatives were extracted by ASE. The extraction efficiency of ASE is calculated to be 26 times higher than Soxhlet extraction. Following ASE, UHPLC-HRMS was utilized to characterize the extraction samples. Orbitrap-based HRAM analysis is found to be an efficient and powerful tool for extractables structural elucidation and identification. Under the optimized chromatographic separation conditions, more than 100 additives and degradation products could be confidently identified by HRMS with Compound Discoverer against multiple data sources, such as spectral libraries and relevant compound databases.

### Please explain why your abstract is innovative for mass spectrometry?

This UHPLC-HRAM-MS setup and associated data processing paves the way for the streamlining of risk assessment of antioxidant extractables, and facilitates the design of next generation materials and process innovations.

### Co-authors:

Weifeng Lin, Thermo Fisher Scientific  
Edward J Jex, Thermo Fisher Scientific  
Kate J Comstock, Thermo Fisher Scientific  
Beibei Huang, Thermo Fisher Scientific  
Sven Hackbusch, Thermo Fisher Scientific

Poster number: **FP-PB-013**

## **GLYCOSYLATION PROFILING OF CETUXIMAB SUBUNITS WITH CZE-MS**

Abstract ID: **740**

**Presenting author: Yue Ju, Bruker Daltonics**

### **Introduction**

Monoclonal antibody cetuximab has four N-glycans with two on each heavy chain located in the Fc and CH1 domain. Previous studies have shown that the Fab glycans are complex and multiply sialylated. This complex glycosylation profile makes the molecule challenging to characterize in detail by traditional reverse phase analysis due to related glycoforms coelution. ZipChip is a microfluidic device integrating capillary zone electrophoresis (CZE) with electrospray ionization. ZipChip coupled with high resolution mass spectrometry provides charge-based selectivity which can assist with glycoforms separation. This can be used to improve domain specific characterization of proteins with complex glycosylation such as N-glycans.

### **Methods**

Reconstitute the IdeS protease in 50 mM ammonium bicarbonate and mix with 2 mg/mL cetuximab for digestion. Incubate the mixture at 37° C for 30 minutes. To the digested sample add DTT to target 10mM addition and incubate at 37° C for another 30 minutes. Cetuximab subunit sample was then buffer exchanged into BGE. 500 V/cm field strength was applied over the chip and 1 nL sample was injected for each analysis. Bruker timsTOF Pro 2 mass spectrometer was employed for the MS detection with settings tuned for intact and subunit mAb analysis.

### **Preliminary data (results)**

With the metabolite background electrolyte and HS chip, Cetuximab subunit light chain, Fc and Fd species were well separated. Five major peaks were observed within a ~2 min separation window corresponding to the 3 domains of interest and related heterogeneities. In this CZE-MS experiment, basic species migrate before the acidic species. A positive 128 Da mass shift was observed from the first two migrated electropherogram peaks with highly conserved glycosylation pattern. Incomplete C-terminal lysine processing was suggested to be the cause for the Fc basic variant. Furthermore, these respective Fc related peaks were split in 2 additional variants. This was explained by glycan heterogeneity revealed by high resolution MS data. The light chain migrated secondly and Fd species elute last. The Fd subunit glycoforms were also separated in 2 individual peaks on the total ion chromatogram. A positive mass shifts of 145 Da was observed for the second peak. This 145 Da mass delta can be attributed to sialylation and corresponds to the mass difference between a galactose and a N-glycolyl neuraminic acid. A peak corresponding to a doubly sialylated Fd was not detected, although its existence was suggested by published intact mass analysis data.

The sample will be further evaluated with a more resolving higher pH BGE to investigate the presence of glycan with multiple sialylation on the Fd subunit and compare the measured glycan distribution with published intact mass measurements of cetuximab by CE-MS and LC-MS.

### **Please explain why your abstract is innovative for mass spectrometry?**

CZE zipchip source in combination with high resolution QTOF mass spectrometer to analyze subunit glycosylation profile

### **Co-authors:**

*Aditya Kulkarni, 908 Devices*  
*Guillaume Tremintin, Bruker Daltonics*  
*Eric van Beelen, Bruker Daltonics*

Poster number: FP-PB-014

## GC-MS AS AN EFFECTIVE TECHNIQUE FOR NITROSAMINE ANALYSIS IN RANITIDINE MEDICATIONS THROUGH EMPLOYING SPME-HS STRATEGY

Abstract ID: 758

Presenting author: Fahad S. Aldawsari, SFDA

### Introduction

Nitrosamine impurities have unexpectedly emerged in pharmaceuticals since 2018. These impurities were reported in drugs valsartan, ranitidine, metformin and rifampin. In each drug, nitrosamine analysis demonstrated a challenging issue particularly during sample preparation and instrument selection. For example, Gas Chromatography Mass Spectrometry (GC-MS) could be used for nitrosamine analysis in valsartan, while it was inadvisable in the case of ranitidine. The method of choice for nitrosamine analysis in ranitidine was Liquid chromatography Mass Spectrometry (LC-MS). In this study, we demonstrate the benefit of using Solid Phase Micro-Extraction (SPME) for the analysis of nitrosamine (NDMA) in ranitidine products. SPME technique bypassed ranitidine involvement in the overestimation of NDMA levels in drugs, as heat in the GC-MS could lead to NDMA generation from ranitidine as a chemical.

### Methods

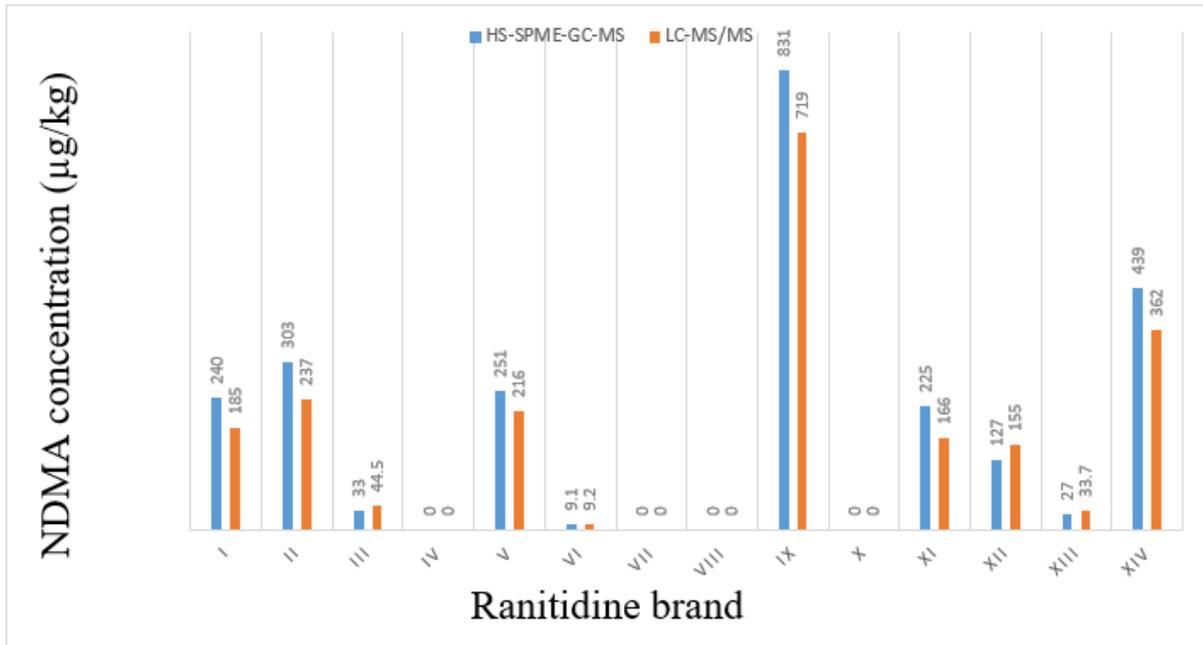
Ranitidine samples were analyzed using both LC-MS and SPME-HS-GC-MS. LC-MS method was implemented according to published protocol. We used Agilent LC coupled with AB Sciex (Q-trap 6500). Mobile phase was water and methanol in gradient elution and calibration curve utilized NDMA levels of 1-100 µg/L. For SPME-HS-GC-MS, Shimadzu GC-MS/MS with was used. Column was DB WAX 0.5 µm, 30 m and 0.25mm. The fiber used was PolyAcrylate (PA) 85 µm from PAL Systems. Ion source temperature was 230 °C and interface temperature was 220 °C. Ions were 74.0 *m/z* (quantifier) and 42.0 *m/z* (qualifier).

### Preliminary data (results)

Samples were analyzed using two different instruments. The recommended method (LC-MS) exhibited LOD and LOQ of 0.3 µg/L and 1.0 µg/L, respectively. Wide range of NDMA levels were observed in the tested samples with four samples showing levels below detection limits. Importantly, many laboratories adopted LC-MS as a method of choice while GC-MS was discouraged. We tried GC-MS using ranitidine as a standard and we confirmed its ability to generate NDMA as a consequence of high temperature. The advantage of SPME-HS is to remove ranitidine during sample processing and hence avoiding its involvement into the GC-MS analysis. In SPME-HS-GC-MS method, we obtained LOD and LOQ of 1 µg/L and 5 µg/L, respectively. We repeated the analysis of the same commercial ranitidine samples using the implemented SPME-HS-GC-MS and results demonstrated high correlation regards the detected NDMA levels between the two techniques. Importantly, negative samples showed agreements between the two methods illustrating the ability of SPME-HS-GC to avoid providing false positive results compared to the conventional GC-MS. We were the first to report the applicability of SPME-HS as an effective tool for the analysis of nitrosamines in medicines.

### Please explain why your abstract is innovative for mass spectrometry?

LC-MS was the sole technique for nitrosamine analysis in ranitidine. Bypassing high temperature, SPME-HS with GC-MS proved to be an alternative technique for the analysis of nitrosamine in ranitidine medication.



NDMA results comparison using LC-MS and SPME-HS-GC-MS instruments.

	QC1 (5 µg/L)	QC2 (15 µg/L)	Standard Check (20µg/L)
1	3.46	14.04	21.6
2	4.72	13.7	20.29
3	5.6	14.38	19.11
4	4.58	14.79	20.99
Mean	4.59	14.23	20.5
SD	0.88	0.47	1.07
RSD	19.13	3.28	5.21

GC-MS System check for quality control (QC) and instrument stability

Poster number: FP-PB-015

## UTILIZATION OF SPME FOR DETECTION OF PCA IN PARACETAMOL PRODUCTS

Abstract ID: 760

Presenting author: Yahya M. Alshehri, SFDA

### Introduction

*p*-chloroanilines (PCA) are impurities encountered in Active Pharmaceutical Ingredient (API) manufacturing and can be carried over in the final pharmaceutical product. Pharmacopeias set maximum limits for PCA in API. Recently, some reports claimed PCA presence in paracetamol medication. Potential contaminated batches of paracetamol were received for analytical investigation. Samples comprised of 41 batches representing 8 finished products, from 5 different manufacturers. Developing a fast and universal screening method was challenging due to variety of matrices of drug products. Several testing approaches (HPLC, GC-MS and LC-MS) have been attempted to evaluate samples of complex matrices including multiple components products and several dosage forms. Within the explored instruments, GC-MS equipped with on-line Solid Phase Micro-Extraction (SPME) offered better advantages for screening procedure.

### Methods

GC-MS/MS from Shimadzu, equipped with AOC Autosampler headspace SPME. Column was DB wax 30 m and oven set from 60 to 260 °C over 24 minutes. Injector and ion source were 250 and 200 C°. Ions were 127>>65 as Target, and 127>>92 as Reference Ions. Samples placed into 20-mL headspace vials with 5-mL of NaCl solution. Carbon WR SPME fiber was used. Samples were placed into oven set at 80 C° and with continuous shaking for 35 minutes. Later, the SPME fiber was placed into the injection port 260 C° for 30 minutes.

### Preliminary data (results)

Lack of selectivity was the major limitation by using HPLC for the detection procedure without prior extraction procedure. Further, HPLC uses multiple organic solvents and it's slightly a time-consuming procedure. In contrary, LC-MS offered better selectivity and specificity but still needs some additional purification particularly for multicomponent effervescent products which contained colorants and flavoring ingredients.

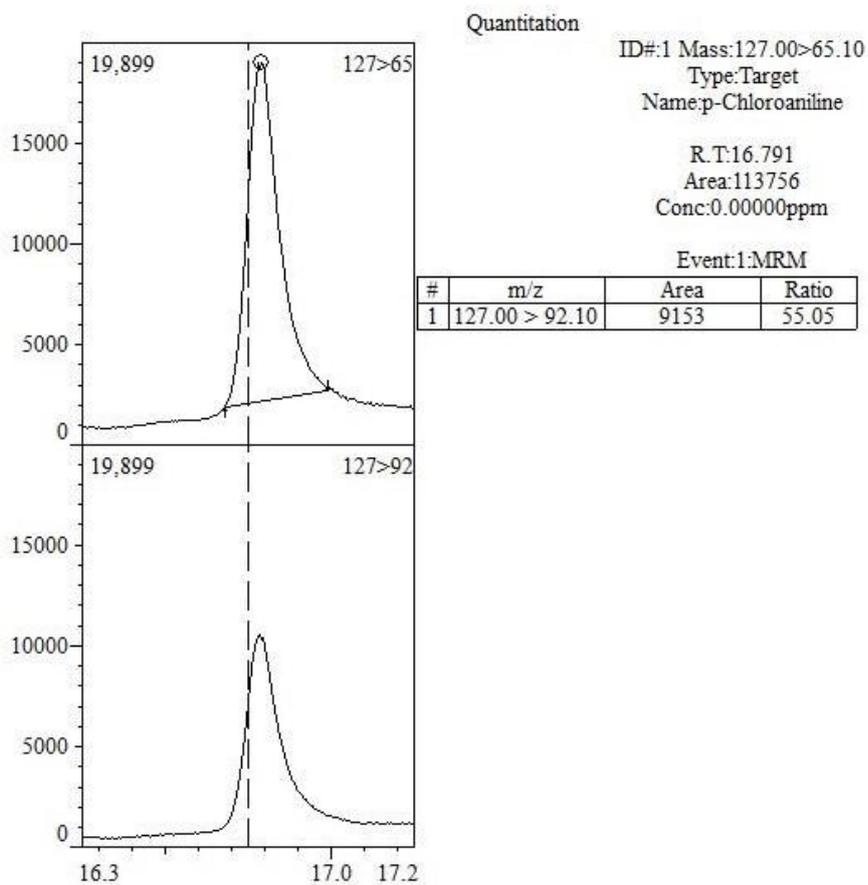
Utilizations of SPME with headspace offered advantages over conventional extraction procedures, thanks to the Contact-less extraction procedure from the headspace of the vial. It was found that PCA can be easily partitioned in the gas phase from the liquid phase under temperature and continuous shaking conditions. Moreover, PCA was strongly absorbed in SPME fiber and required higher temperature to be desorbed. The current condition is going to be optimized and validated to include shorter analysis time as the current protocol is considered long compared to other extraction procedure. Aside from that, the challenge of carry-over of PCA required longer post-run cleaning at higher temperature up to 280 C°. Nevertheless, the current work offers several advantages. Firstly, it is fully automated extraction procedure including on-line injection with minimum human contribution. Secondly, it may overcome the challenging samples and complex matrices owing to the contact-less extraction from the headspace. Lastly, this approach is considered eco-friendly since no organic solvents have been used and the SPME fiber is regenerated and reused for different samples.

### Please explain why your abstract is innovative for mass spectrometry?

HS-SPME-GC-MS/MS offered advantageous detection for PCA in paracetamol products compared to both HPLC and LC-MS.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Represented chromatogram for PCA using HS-SPME-GS-MS/MS

Poster number: FP-PB-016

## LISDM: ENZYME-FREE PROTEIN CLEAVAGE FOR BOTTOM-UP ANALYSIS ON THE NEW BEATBOX PLATFORM

Abstract ID: 874

Presenting author: Leander Runtsch, PreOmics GmbH

### Introduction

In the widely used *bottom-up* mass spectrometric analysis, proteins are cleaved into peptides prior to analysis. For this, different enzymes and chemicals can be employed. Predominantly, the serine protease trypsin is used, which cuts C-terminal to arginine and lysine. While this specificity is suitable for many applications, it has its limits when a high sequence coverage or analysis of post-translational modifications is needed. Especially proteins with too few or too many cleavage sites are then challenging, since only peptides within a certain range of length can be analyzed reliably. Here we present LiSDM (Liquid Sample Dissociation by Magnetomovement), a novel method of protein hydrolysis that does not rely on an enzyme or chemical, making use of our recently introduced tissue homogenization platform, the *BeatBox*.

### Methods

Samples containing NIST monoclonal antibody (mAb) reference material 8671 were digested with trypsin or incubated in the *BeatBox*, PreOmics' new tissue lysis instrument, applying experimental, high-energy settings within the MSPU (Magnetic Sample Processing Unit) with novel and optimized consumables. The resulting peptides were then purified using our iST technology and analyzed on a *TimsTOF pro* mass spectrometer by *Bruker* in DDA-PASEF mode. On-line separation of peptides prior to MS analysis was performed on an *EASY-nLC 1200 HPLC* by *Thermo Fisher Scientific* equipped with a  $C_{18}$  column by employing a 40 min acetonitrile gradient. MaxQuant 2.0.1.0 was used for data analysis.

### Preliminary data (results)

We discovered that incubation of protein samples on the *BeatBox* using experimental, high-energy MSPU settings leads to fragmentation of the peptide bonds. As an example, Fig. 1 shows the number of identified unique peptides of an antibody reference that was treated this way compared to a tryptic digestion. As can be seen, incubation in the MSPU generates a much higher number of unique peptide species. Indeed, our experiments with this new method of protein hydrolysis suggest a cleavage mechanism that is mostly unspecific of amino acid composition and might be affected more by other parameters such as local structural rigidity. A lot of the peptides generated belong to clusters sharing the same N-terminal amino acid and featuring peptides of incrementally increasing length. While we are showcasing a reference antibody here, we observed the same effect for a variety of different proteins.

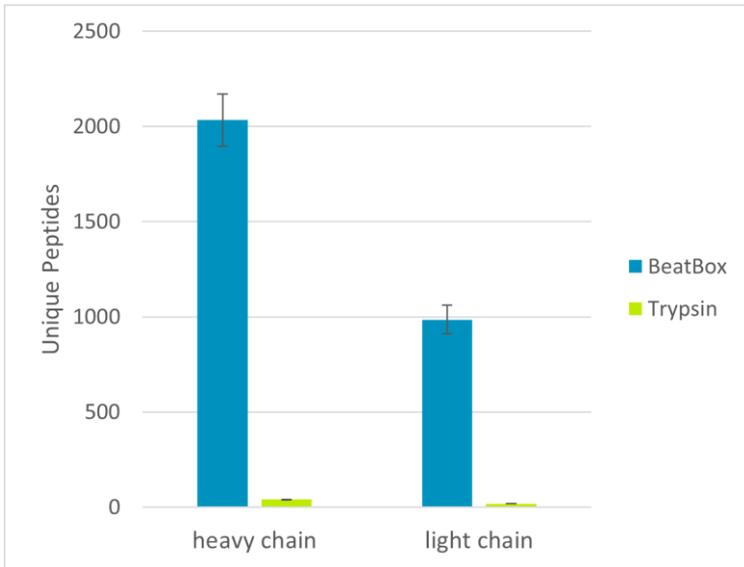
As a high number of unique peptides is not advantageous in itself, Fig. 2 additionally shows a comparison of the sequence coverage of the antibody digested with trypsin or hydrolyzed in the MSPU of a *BeatBox*. This parameter is much more important in many analyses. Here, the "mechanical" digestion in the *BeatBox* platform yields a slightly (light chain) or even considerably higher (heavy chain) coverage than a tryptic digest. For both chains, identification of almost the complete protein sequence was accomplished. In addition to eliminating the need for a protease, this signifies the advantage of our newly developed method over conventional approaches.

### Please explain why your abstract is innovative for mass spectrometry?

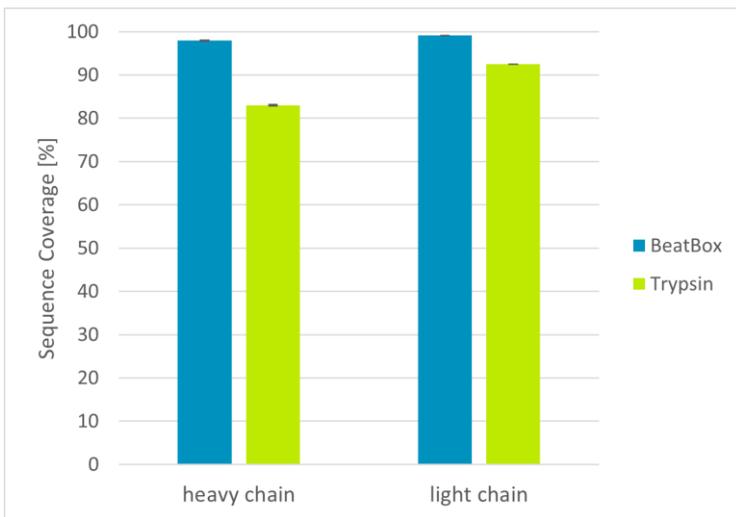
The protein cleavage method shown here, termed LiSDM, eliminates the need for and has considerable advantages over proteases in *bottom-up* analyses and is thus highly important to a broad audience.

### Co-authors:

Ankit Sinha, Max Planck Institute of Biochemistry  
Katrin Hartinger, PreOmics GmbH  
Jasmin Johansson, PreOmics GmbH  
Sebastian Johansson, PreOmics GmbH  
Matthias Mann, Max Planck Institute of Biochemistry  
Nils Kulak, PreOmics GmbH



Identified mAb unique peptides for BeatBox hydrolysis and tryptic digest.



mAb sequence coverage for BeatBox hydrolysis and tryptic digest.

Poster number: **FP-PB-017**

## **OPEN ACCESS LC-MS ANALYSIS OF INTACT RECOMBINANT PROTEINS AND ANTIBODIES**

Abstract ID: **935**

**Presenting author: Adam Hold, UCB Biopharma UK**

### **Introduction**

The availability of liquid chromatography-mass spectrometry (LC-MS) data for intact large molecule therapeutics is an invaluable tool for the accelerated development of innovative biopharmaceuticals. A robust Open Access LC-MS system generates rapid, critical data on the purity and identity of a molecule whilst liberating the analyst's time. We present our latest iteration of this technique combining minimal sample preparation, an easy-to-use software interface and a robust chromatography system which generates a single page report for users with no LC-MS experience.

### **Methods**

Use of Waters® OpenLynx™ open access application manager with a Waters® Acquity Xevo® G2 QToF LC-MS system in combination with a TFA modified solvent system and a Waters® BioResolve™ Reversed Phase (RP) Monoclonal Antibody (mAb) Polyphenyl Column.

### **Preliminary data (results)**

More than 3000 intact protein and antibody samples are analysed per year through the Open Access LC-MS system by multiple users.

### **Please explain why your abstract is innovative for mass spectrometry?**

A user-friendly workflow applicable for the rapid reporting of LC-MS data on intact proteins and antibodies.

Poster number: FP-PB-018

## INSTANT REDUCTION OF ALL INTER- AND INTRACHAIN S-S BONDS IN MABS BY ELECTROCHEMISTRY-MS

Abstract ID: 959

Presenting author: Jean-Pierre Chervet, Antec Scientific

### Introduction

An improved electrochemical method is presented that achieves full reduction of both inter- and intrachain disulfide bonds in a set of monoclonal antibodies based on their intact mass and on MS/MS analysis. The system uses an electrochemical flow cell positioned online between a chromatography system and a mass spectrometer to give direct information on pairs of heavy and light chains in an antibody. The complete reduction of the intrachain disulfide bridges is important as the redox state affects the intact mass of the antibody chain. Disulfide bonds also hamper MS/MS fragmentation of protein chains and thus limit the confirmation of the amino acid sequence of the protein of interest if not fully reduced.

### Methods

An electrochemical flow cell ( $\mu$ PrepCell-SS) controlled by a ROXY Exceed (Antec Scientific) was used for reduction. Separations were performed on a 100 mm x 150  $\mu$ m, C4, 300 Å column using a Ultimate 3000 Nano LC system. The sample was trapped and desalted (Pepmap 0.3mm x 5mm, C4, 300 Å). Gradient was 0.1% FA (A) and 0.08% FA, 80% acetonitrile in water (B), 4% to 90% (B) in 25min at 1  $\mu$ L/min. Post-column, 19  $\mu$ L/min 1% FA, 50% acetonitrile was added as makeup flow, to total 20  $\mu$ L/min. Orbitrap Fusion Lumos was used as MS (Thermo Scientific).

### Preliminary data (results)

In this poster, we demonstrate that a selection of monoclonal antibodies (mAbs) such as Bevacizumab (Roche), Panitumumab (Amgen), Pembrolizumab (Merck), Cetuximab (Eli Lilly and Co), Adalimumab (Abbott) and Alemtuzumab (Genzyme) could be fully reduced by electrochemical reduction after chromatography of the intact molecule. Both heavy and light chains were released until no signal could be observed from the intact mAb. The intact masses observed in deconvoluted MS spectra were consistent with the reduction of both inter- and intrachain disulfide bridges. Furthermore, the analysis of MS/MS spectra of the light chains confirmed the reduction of the disulfide bonds in the electrochemical cell, and for heavy chains such MS/MS data were obtained from a Fab fragment. The only aspect that was varied was the applied voltage in the electrochemical cell. In general, potentials around 1000 mV were optimal for complete reduction. The post-column reduction setup with makeup flow allows for conventional conditions during separation but full control of the reduction environment. Furthermore, this post-column configuration result in co-elution of reduced chains that were still connected by a disulfide bridge during chromatography. While this is not beneficial when performing an analysis on a single purified mAb, the design allows for the separation of more complex samples prior to reduction. In this way, the pairing between protein chains in the mixture could be revealed. The current work demonstrates that it is feasible to reduce electrochemically all inter- and intrachain disulfide bridges in various mAbs instantaneously.

### Please explain why your abstract is innovative for mass spectrometry?

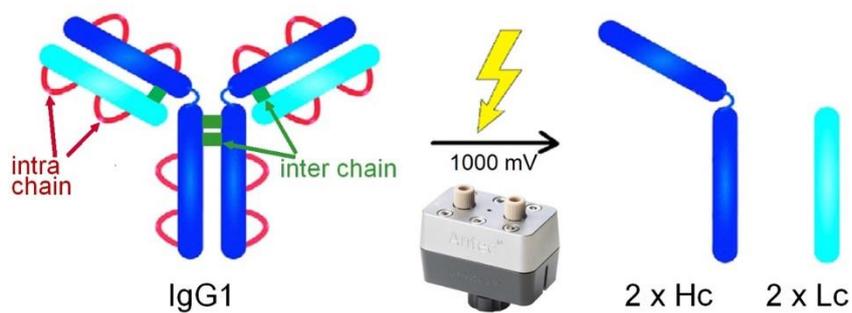
Instant and complete reduction of mAbs without denaturing or reducing agents using Electrochemistry-MS

### Co-authors:

Martijn M. Vanduijn, Erasmus Medical Center  
Theo M. Luider, Erasmus Medical Center  
Hendrik-Jan Brouwer, Antec Scientific

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Schematics electrochemical reduction of a mAb (IgG1)

Poster number: FP-PB-019

## ONLINE ELECTROCHEMICAL REDUCTION OF MABS FOR RAPID LC-MS ANALYSIS

Abstract ID: 1005

Presenting author: Hendrik-Jan Brouwer, Antec Scientific

### Introduction

In this poster, we will demonstrate the successful reduction of inter- and intrachain disulfide bonds of various monoclonal antibodies (mAbs) with an inline electrochemical flow cell coupled to a liquid chromatography-mass spectrometry (LC-MS) system. The addition of a trap/release column in the chromatographic set-up allowed the analytical separation and mass spectrometry analysis to be unmodified with run times of only 23 min. The study demonstrates the complete reduction of intact mAbs to the corresponding light and heavy chain (Lc, and Hc) subunits. Middle-up subunit analysis by electrochemical reduction coupled to HRAM LC-MS can be carried out from intact antibodies without the need for enzymatic digestion, specific reducing agents, or specific denaturing agents.

### Methods

A  $\mu$ -PrepCell SS cell with a ROXY Exceed Potentiostat (Antec Scientific), was used for the reduction, controlled by Chromeleon software (Thermo Scientific). The temperature was 20 °C for partially and 60 °C for the fully reduced experiments. 1  $\mu$ L of sample was injected onto the  $\mu$ -PrepCell with products trapped on a MAbPac column (50 mm length), after column switching samples were washed onto a MAbPac column (100 mm length). Separation occurred with a linear gradient. LC-MS analysis was acquired on a Vanquish Flex Duo UHPLC system coupled to a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific).

### Preliminary data (results)

Electrochemical reduction of both the intra- and interchain disulfide bonds in different mAbs such as Rituximab, Nivolumab, Denosumab, and Cetuximab, could be carried out using electrochemical reduction inline with an LC-MS system. Increasing the electrochemical potential of the electrochemical cell resulted in more complete disulfide bond reduction. Tertiary structure of mAbs was shown to reduce electrochemical efficiency but denaturing the antibodies by thermostating the electrochemical flow cell at 60 °C, using the oven of the ROXY Potentiostat, increased the reduction efficiency. The LC-MS system required no modification to the separation and mass spectrometry methods other than the introduction of the electrochemical cell. The reduction of antibodies can be carried out inline with the addition of an electrochemical cell into the chromatographic flow path reducing the intact mAbs. The developed workflow reduces an antibody down to light chain and heavy chain subunits without the need for addition of enzymes or specific denaturing agents. The electrochemical reduction workflow can be used for the analysis of previously digested fabricator samples as well as intact antibody species. Glycoforms of each antibody were shown to be unaffected, even at the maximum level of reduction. Overall, the data showed completely reduced light and heavy chain formation for the online analysis of intact mAbs, and completely reduced light chain, Fd, and Fc/2 subunits when coupled with offline IdeS digestion. Selectivity of the disulfide bond electrochemical reduction by tandem MS could yield useful information about the formation of non-uniform disulfide bonding structures within antibodies.

### Please explain why your abstract is innovative for mass spectrometry?

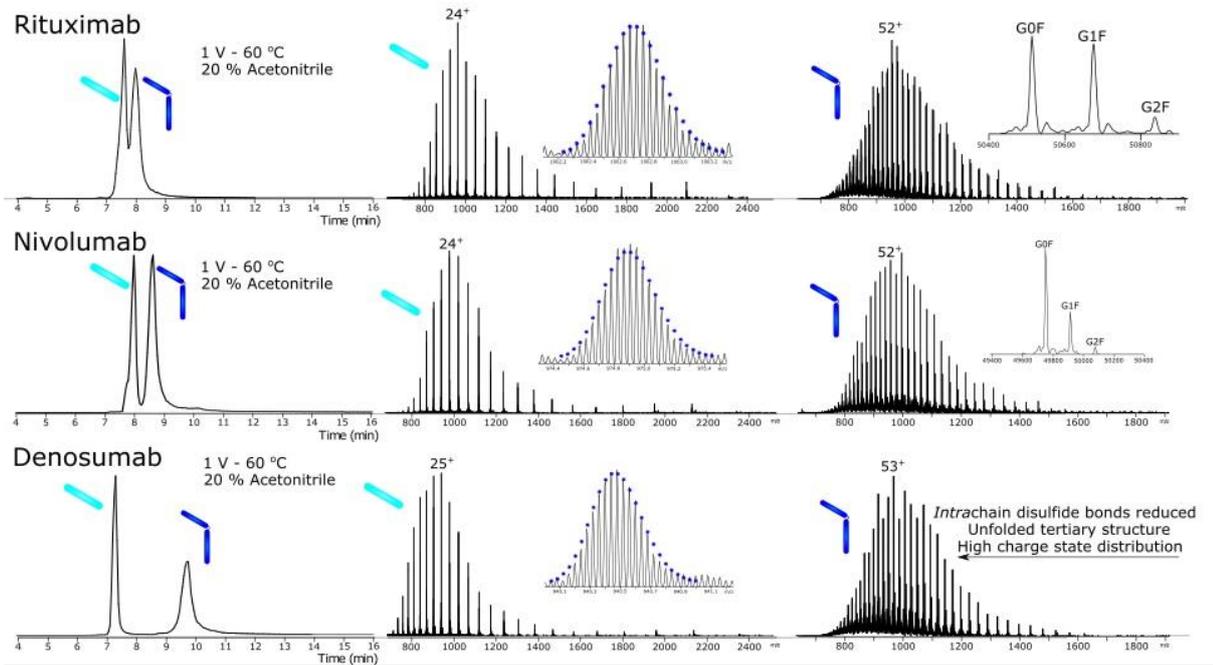
Instant reduction of S-S bonds in mAbs by online coupling of Electrochemistry with LCMS, without denaturing or reducing agents

### Co-authors:

Tomos Morgan, National Institute of Bioprocessing Research and Training (NIBRT)  
Jonathan Bones, National Institute of Bioprocessing Research and Training (NIBRT)  
Craig Jakes, National Institute of Bioprocessing Research and Training (NIBRT)  
Ken Cook, Thermo Fisher Scientific  
Jean-Pierre Chervet, Antec Scientific

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Rapid electrochemical reduction of all inter-and intrachain disulfide bonds

Poster number: FP-PB-020

## LOW-PG/ML QUANTIFICATION OF COMPLEX DISULFIDE-RICH PEPTIDES IN RAT PLASMA USING MICROFLOW LC-MS/MS

Abstract ID: 1023

Presenting author: Ferran Sánchez, SCIEX

### Introduction

Cyclic peptides exhibit enhanced biological activity compared to traditional peptides, given their disulfide-rich composition, which confers structural stability and conformational rigidity. As a result, have become crucial therapeutic candidates and successful therapeutic agents in cardiovascular diseases. With the current advancement of cyclic peptide therapeutics, there is an equivalent drive toward the development of highly robust and sensitive quantitative methods. Existing LC-MS bioanalytical methods lack the sensitivity necessary primarily due to their complex tertiary structure and high baseline interference with the application of single MS mode. In this study, low-pg/mL quantification was achieved at an LLOQ of 0.01 ng/mL.

### Methods

Rat plasma was protein precipitated and the supernatant was diluted 1:1 (v/v) with water. Human atrial natriuretic peptide (human ANP) and a labeled cyclic peptide were then spiked into the biological matrix. To generate the calibration curves, serial dilution with processed plasma was performed. A microflow LC system operated in trap-and-elute mode and 20  $\mu$ L of sample was loaded for analysis. Flow for analyte trapping: 50  $\mu$ L/min, whereas for analyte separation: 5  $\mu$ L/min. Sample analysis was performed in positive MRM mode. Collision energy, source and MS parameters were optimized to achieve sensitive MS/MS quantification.

### Preliminary data (results)

In this workflow, a sensitive LC-MRM method was developed for the quantification of disulfide-rich peptides in rat plasma. Human ANP is composed of 28 amino acids and 1 disulfide bridge between cysteine residues. Human ANP was spiked into processed rat plasma to generate a calibration curve for concentrations ranging from 0.01 ng/mL to 200 ng/mL. Measurements at each concentration were performed in triplicate.

To determine the lower limit of quantification (LLOQ), the %CV needed to be below 20% with an accuracy between 80% and 120% of the nominal concentration. For the concentrations above the LLOQ, the %CV needed to be below 15% with an accuracy between 85% and 115% of the nominal concentration.

For human ANP, an LLOQ of 0.01 ng/mL was achieved. The matrix blank showed no indications of matrix interferences at the retention time of the analyte. The linear range evaluated was between 0.01 ng/mL to 200 ng/mL. The linear dynamic range (LDR) of the human ANP calibration curve spanned 4.3 orders of magnitude. All calculated concentrations were between 85% and 115% of the nominal value, including at the LLOQ. The %CV was less than 12.5%, demonstrating high reproducibility of the developed assay.

Overall, a highly sensitive method for the quantification of disulfide-rich peptides was developed with excellent accuracy and precision. For human ANP, quantification at low-pg/mL levels was successfully achieved.

### Please explain why your abstract is innovative for mass spectrometry?

Development of a highly sensitive method for quantification of complex disulfide-rich cyclic peptides using microflow LC-MS/MS.

Poster number: FP-PB-021

## A NON-TARGETED 4D-METABOLOMICS WORKFLOW FOR QUALITY CONTROL OF CHIRAL HEPARIN DISACCHARIDE BY LC-TIMSTOF AND METABOSCAPE®

Abstract ID: 1028

Presenting author: Peter Abrahamsson, Bruker

### Introduction

Heparin is a highly sulfonated polysaccharide applied in clinic for treatment of several diseases. Heparin disaccharide I-S tetrasodium salt purchased from various vendors have shown differences in target-binding activity as large as 10-fold, although 100% HPLC purity is provided by vendor's certificate of analysis (COA). The biological activity is closely related to its molecular structure, e.g., chirality. Trapped ion mobility spectrometry (TIMS) has the ion mobility separation power to differentiate enantiomers of heparin disaccharide. MetaboScape® is an integrated software supporting non-targeted screening, compound identification, and statistics. In this study, a 4D-Metabolomics data acquisition and analysis workflow is used to determine the differences among heparin disaccharide samples with ranging target-binding activities, which can be utilized for quality control of this material.

### Methods

Heparin disaccharide I-S tetrasodium salt standards were purchased from five different vendors. 1.0 mg/mL stock solution of each sample was prepared in water/acetonitrile. Sample solutions were used for both direct infusion and reverse phase liquid chromatography. LC-MS acquisitions were performed by Elute UHPLC wC18 column and timsTOF Pro 2 with TIMS enabled. Data analysis was conducted in DataAnalysis 5.3. Nontargeted feature detection was achieved by MetaboScape® 2022. Principal component analysis and partial least squares regression were conducted to identify possible contaminant compounds which are trending with reduced biological activity. Compounds were annotated with spectral libraries and SmartFormula.

### Preliminary data (results)

By direct infusion, two major peaks were observed from the extracted ion mobilogram (EIM) of  $m/z = 665.9064 \pm 0.002$ , the protonated ion of heparin disaccharide I-S tetrasodium salt, indicating more than one enantiomer present in the samples. A previously established 4D-Metabolomics method with  $m/z$  20-1300 and mobility range of  $1/K_0$  0.45-1.45  $V \cdot s/cm^2$  was used. Further optimization on accumulation time, duty cycle, ramp time and ion mobility range can improve ion mobility separation resolution.

LC-MS acquisitions were performed by injecting each sample in triplicate for non-targeted and statistical analysis. LC-MS base peak chromatogram (BPC) overlays showed differences between samples at several retention time regions. To further determine the compounds responsible for difference in biological activity, untargeted analysis was performed using MetaboScape® to extract and annotate features, followed by statistical analysis.

Principal component analysis was carried out using MetaboScape®, more than 50% variance is covered by PC1 and PC2, indicating difference compositions in samples obtained from different vendors despite similar HPLC purity results from vendor COAs. Partial least squares regression was carried out to find the correlation between detected feature intensities and the relative target-binding activities. Compounds of particular interest have intensities trending or reverse-trending with biological activities. To identify unknown components, features are annotated by the SmartFormula module and MetaboBASE 3.0 Spectral Library.

Correlating the target-binding activity with 4D profiles of heparin disaccharide from different vendors highlights the application of timsTOF and MetaboScape® to provide guidance in quality control of heparin disaccharide materials.

### Please explain why your abstract is innovative for mass spectrometry?

Demonstrated a 4D-Metabolomics workflow for fast chiral separation and impurity identification for heparin disaccharide by timsTOF and MetaboScape®

## **Session: Food, Nutrition & Agriculture**

Poster number: **FP-PB-022**

### **METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF ANTIBIOTICS AND VETERINARY DRUGS IN MILK, ANIMAL FEEDS AND ANIMAL TISSUES BY QSIGHT LC-MS/MS**

Abstract ID: 11

**Presenting author: Jingcun Wu, PerkinElmer Health Sciences Canada Inc.**

#### **Introduction**

Antibiotics and veterinary drugs are used in animal production to improve animal health. However, improper use of drugs can lead to residue violations in products and cause health risks. Regulatory agencies around the world have established maximum residue levels (MRLs) for these drugs in foods and animal feeds. To ensure food safety and enforce regulations, reliable analytical methods are needed for monitoring drugs in different sample matrices. In this study, two methods were developed for the analysis of 48 antibiotics and veterinary drugs in milk, animal feed and animal tissue samples. The methods were validated by spiking different concentrations of analytes in sample matrices. All the analytes could be determined with LOQ below the MRLs by QSight LC-MS/MS.

#### **Methods**

Samples were ground and homogenized before use. Due to different properties of analytes, two methods were developed and validated: For polar analyte analysis, such as aminoglycoside antibiotics in milk, a mixed mode LC method was used to improve analyte retention and a trichloroacetic acid - acetonitrile extraction method was used to enhance analyte extraction efficiency. For less polar analytes, a reversed phase UHPLC method was used for analyte separation and an acidified acetonitrile extraction was applied for sample preparation. All analytes were measured by QSight LC-MS/MS.

#### **Preliminary data (results)**

To evaluate method extraction efficiency (analyte recovery) and sample matrix effects, three sets of calibration curves were built for all analytes: reagent only (RO), matrix-matched (MM, by post spike), and matrix-based (MB, by prior spike). Matrix effects were evaluated by comparing the slopes of calibration curves obtained by RO with slopes obtained by MM. Significant matrix effects (up to 90%) were observed for all samples due to less sample clean up. Analyte recovery or method extraction efficiency was studied by comparing the slopes (or responses) obtained by MB calibration with MM calibration, good enough recoveries (60 to 90%) were obtained for most analytes in all sample matrices. Method accuracy was evaluated based on the relative recovery of analytes using the MB calibration method or using the MM calibration with correction factors for extraction efficiency. Good method accuracy (75 to 120%) was obtained for all analytes in all sample matrices. The methods used simple sample preparation procedures and demonstrated high sensitivity and selectivity with good precision (RSD% < 20%) for all analytes in all samples studied.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Stay-clean source for complex matrices with less maintenance; automated polarity switch provides high sensitive detection for multiple analytes in both positive and negative modes; dual ionization source (ESI and APCI).

#### **Co-authors:**

*Feng Qin, PerkinElmer Health Sciences Canada Inc.*

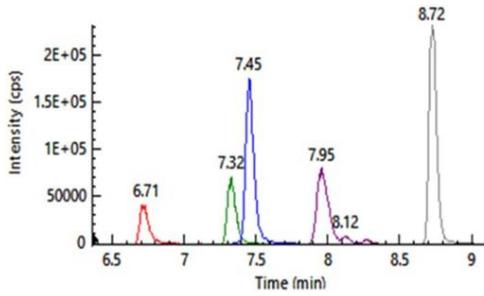
*Derek Mattern, PerkinElmer Inc.*

*Philippe Boniteau, PerkinElmer Inc.*

*Francisco Ferron, PerkinElmer Inc.*

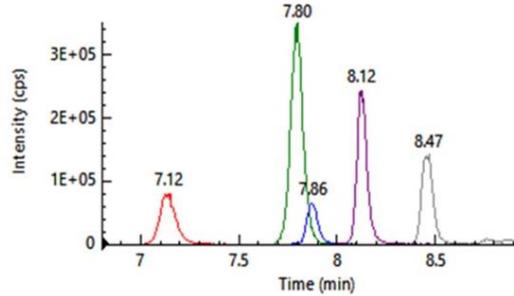
Ignazio Garaguso, PerkinElmer Inc.  
 Roberto Bozic, PerkinElmer Inc.

(A) C18 Column



Analyte	RT (min)
Malachite Green	6.71
Crystal Violet	7.32
Brilliant Green	7.45
Leuco Crystal Violet	7.95
Leuco Malachite Green	8.72

(B) Epic Polar



Analyte	RT (min)
Leuco Crystal Violet	7.12
Leuco Malachite Green	7.80
Malachite Green	7.86
Brilliant Green	8.12
Crystal Violet	8.47

Figure 1. Analysis of Drugs Using Columns with Different Selectivity

Repeatability of the Method (181 Injections of a Spiked Chicken By-Product)

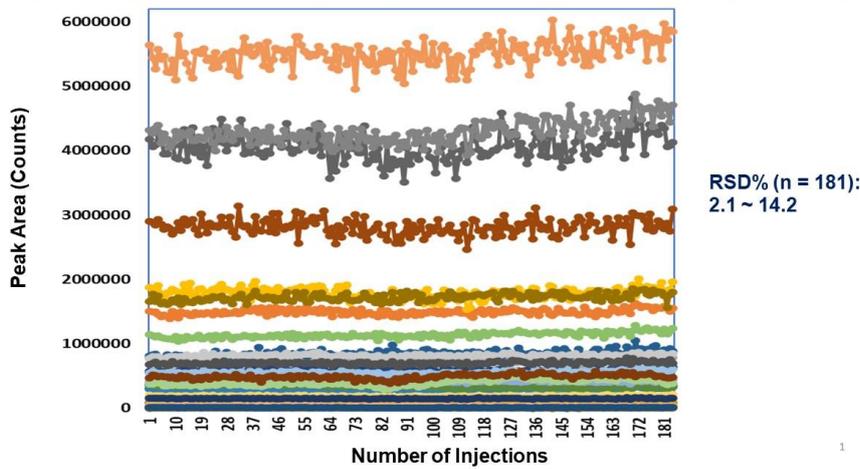


Figure 2 Repeatability of the Method (181 Injections of Samples)

Poster number: **FP-PB-023**

## **ANALYSIS OF 400 PESTICIDES IN TEA VIA LC/MS/MS: SIMPLE SAMPLE PREPARATION AND APCI TO IMPROVE ANALYTE COVERAGE**

Abstract ID: **31****Presenting author: Alexander Kasperkiewicz, PerkinElmer Health Sciences Canada, Inc.**

### **Introduction**

As labs implement consolidated methods for multiple matrix and analyte classes, compound lists increase to hundreds or thousands of targets. In this work, a simple extraction approach was coupled with ESI and APCI modes on an LC/MS/MS instrument for the analysis of over 400 analytes extracted from black tea (347 compounds via ESI and 51 compounds via APCI along with internal standards). Excellent method performance (defined as accuracy of 70 – 120 % and precision of RSD < 20 %) was achieved for over 93 % of analytes, including compounds normally analyzed by GC/MS such as trifluralin, chlormephos, chlormephos, chlorobenzilate and others. This approach can allow the consolidation of a pesticide method to a single instrument or reduce the workload of a complementary GC/MS method.

### **Methods**

A simple solid-liquid extraction sample preparation procedure with stable isotope dilution was employed: 1 g of the ground and sieved tea sample was weighed into a 15 mL centrifuge tube before extraction with 5 mL of acetonitrile and 0.1 % formic acid. Samples were centrifuged and filtered prior to analysis via APCI and ESI methods on one instrument. All experiments were carried out using a QSight LX50 UHPLC coupled to a QSight 420 triple quadrupole mass spectrometer via a divert valve. All instrument control, method development, and data processing were completed using the Simplicity 3Q software suite.

### **Preliminary data (results)**

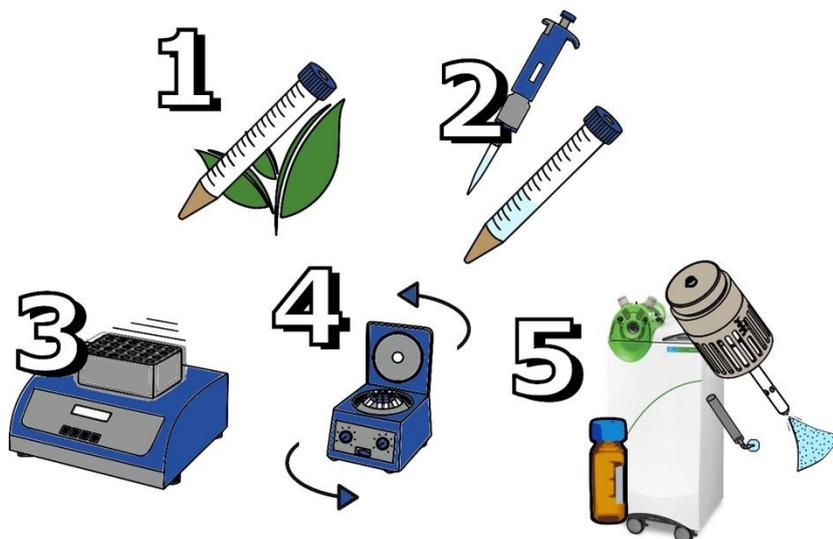
Method validation produced encouraging results: Of the 401 compounds validated, 93 % (371 compounds) displayed accuracy values of 70 to 120 % along with sub-20 % RSD values at the 100 ng/g validation level with 3 % (15 compounds) within 60 to 140 %. Of the 11 compounds which resulted in recoveries of below 60 %, 8 compounds (dodine, formetanate, propamocarb, ethirimol, imazalil, dodemorph, pymetrozine, cyromazine) were found to have constant recoveries at 5 concentration levels spanning 5 to 500 ng/g (cyromazine at 100 – 500 ng/g), with reproducibility across 2 experiments. Of the 51 compounds analyzed by APCI, 33 analytes displayed limits of quantitation of 10 ng/g or less, including thiometon, bromophos, trifluralin, pentachloronitrobenzene, and chlormephos. An investigation into absolute matrix effects revealed that 97 % of compounds displayed signal suppression/enhancement values of between 70 - 120 %.

### **Please explain why your abstract is innovative for mass spectrometry?**

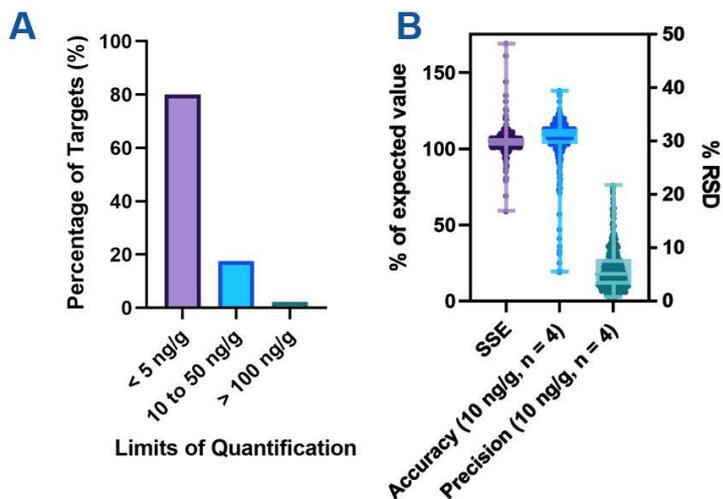
Several novel APCI transitions are utilized to analyze pesticides via LCMSMS which would otherwise require GCMS for analysis, supporting the use of one LCMSMS instrument for multiresidue pesticide analysis.

### **Co-authors:**

*Avinash Dalmia, PerkinElmer, Inc.*  
*Feng Qin, PerkinElmer Health Sciences Canada, Inc.*  
*Derek Mattern, PerkinElmer, Inc.*  
*Francisco Ferron, PerkinElmer, Inc.*  
*Philippe Boniteau, PerkinElmer, Inc.*  
*Ignazio Garaguso, PerkinElmer, Inc.*  
*Roberto Bozic, PerkinElmer, Inc.*



Analytical procedure employed for the analysis of pesticides from black tea: 1) 1 g of tea sample measured into a 15 mL centrifuge tube along with internal standards, 2) 5 mL of acetonitrile with 0.1 % formic acid is added, 3) sample is agitated for 30 minutes during extraction, 4) sample is centrifuged before 5) filtration and analysis via APCI and ESI LC/MS/MS methods.



Summary of select method validation data, with limits of quantification (LOQs) under 5 ng/g for approximately 80 % of compounds under study (A) along with individual compound data points and corresponding box and whisker plots for matrix effects (% SSE), accuracy, and precision at the 10 ng/g validation point (for the compounds of which LOQ < 10 ng/g) (B).

Poster number: **FP-PB-024**

## **HIGH-THROUGHPUT ANALYSIS OF FRESHNESS MARKERS IN VARIOUS FOOD SAMPLES BY SIFT-MS**

Abstract ID: **48**

**Presenting author: Arnd Ingendoh, Syft Technologies GmbH**

### **Introduction**

Consumer acceptance and food safety are key for wholesalers and retailers of fresh fruit, vegetable, fish or meat products. Although consumers provide the ultimate feedback on quality, suitable instrument-based methods can provide rapid analysis, objectivity and low costs per sample, which are not always possible using human subjects.

SIFT-MS (Selected Ion Flow Tube Mass Spectrometry) is a very rapid, direct, and sensitive technique with detection limits matching those of human olfactory system and minimal samples preparation. Therefore, it is ideal for detecting food spoilage at an early stage and for wide-scale freshness screening. SIFT-MS utilizes precisely controlled chemical ionization reactions to detect and quantify trace amounts of volatile organic compounds (VOCs). Analysis occurs in real-time and with typical LODs in the sub-ppbv range.

### **Methods**

Selection of the individual reagent ion is performed with an upstream quadrupole, following controlled ion-molecule reactions with the sample gas in the flow tube chamber. The mass analysis of the product and remaining reagent ions is done in a downstream quadrupole. A library-based software processes the ion counts together with instrumental parameters to calculate absolute concentrations of the target compounds. SIFT-MS was coupled to a headspace autosampler (Gerstel). Since no front-end separation is performed, but direct analysis of all samples, the setup provides a robust, easy-to-operate solution for sensitive, quantitative screening of a large number of samples per day.

### **Preliminary data (results)**

During ripening, fruits emit a diverse range of low molecular weight compounds arising from various hormonal and metabolic processes. The relative abundance of these volatiles changes over time. Ethylene is usually of particular interest because it promotes ripening. SIFT-MS was applied for the rapid and sensitive analysis of VOC from fruits, demonstrating significantly higher throughput in an automated separation-free workflow: while GC/MS could analyze 21 samples within 24h, SIFT-MS did 128 samples, i.e. a 6x higher throughput. In fish, seafood and meat, besides trimethylamine, which is a compound with characteristically "fishy" aroma of spoilage, various other compounds like ammonia, various sulfur compounds and oxygenates have been investigated as significant markers for sample age. It was obvious that inorganic preservatives have been added to the minced beef to prolong its shelf life: this keeps the dimethyl sulfide concentration constant and moderates the production of ethyl acetate.

### **Please explain why your abstract is innovative for mass spectrometry?**

Direct and quantitative analysis of freshness markers in various food samples with a 6x higher sample throughput than conventional GC/MS.

### **Co-authors:**

*Christopher Pfaff, Syft Technologies GmbH*  
*Vaughan Langford, Syft Technologies Ltd.*  
*Mark Perkins, Anatune Ltd.*

Poster number: FP-PB-025

## LC-MS-BASED DETECTION OF SILKWORM PUPAE IN FEED WITH AND WITHOUT PRIOR IMMUNOAFFINITY ENRICHMENT

Abstract ID: 71

**Presenting author: Tobias Stobernack, German Federal Institute for Risk Assessment, Department Food Safety, National Reference Laboratory for Animal Protein in Feed, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany**

### Introduction

In order to meet the demand of an ever-growing meat consumption and to reduce deforestation, existing resources must be used efficiently and sustainable alternatives to conventional feed sources have to be considered. Due to their high nutrient content and feed conversion efficiency, insects are such an alternative. Recently, the silkworm (*Bombyx mori*) was authorized as feed for aquaculture, poultry, and non-ruminant mammals (Regulation (EU) 2021/1925).

### Methods

As its pupae are rich in protein and fat and available in large scale as sericulture by-product, it serves as an attractive substitution for higher priced fishmeal [1]. Official control to (i) prove absence in non-authorized feeds and (ii) correct labeling in authorized feeds relies on the availability of new analytical methods for the detection of *B. mori*. In addition to a DNA-based method developed previously in-house [2], we developed and validated a targeted proteomic nanoLC-hrMS method using a quadrupole orbitrap hybrid mass spectrometer (Q Exactive™ Plus, Thermo) and including an optional, magnetic bead-based immunoaffinity enrichment step.

### Preliminary data (results)

Though enrichment provided cleaner backgrounds especially in low-concentrated samples, the developed method is able to detect down to 0.05 % (w/w) silkworm pupae in feed, whether the enrichment step was included or not and independently from the experimenter. Validation further revealed no significant interference with over 30 commercially available feed and a good intra- and interday reproducibility. From a quantitative point of view the use of an internal standard is recommended. To test the method's robustness, different drying techniques, storage stability of protein extracts and peptides as well as two different trypsines were evaluated. In order to broaden its application range and to allow for easier transfer to the equipment predominant in the control laboratories, the method will be transferred to a UHPLC-coupled triple quadrupole (QqQ) instrument in the future.

[1] Huis, A., et al. (2013). *Edible insects: future prospects for food and feed security* (Vol. 171): Food & Agriculture Organization of the United Nations (FAO).

[2] Zarske, M., et al. (2021). Detection of silkworm (*Bombyx mori*) and Lepidoptera DNA in feeding stuff by real-time PCR. *Food Control*, 126.

### Please explain why your abstract is innovative for mass spectrometry?

Development of a PRM based Method for the detection of silkworm in feed

### Co-authors:

Michael Zarske, German Federal Institute for Risk Assessment, Department Food Safety, National Reference Laboratory for Animal Protein in Feed, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

A. Niedzwiecka, German Federal Institute for Risk Assessment, Department Food Safety, National Reference Laboratory for Animal Protein in Feed, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

J. Zagon, German Federal Institute for Risk Assessment, Department Food Safety, National Reference Laboratory for Animal Protein in Feed, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

A. Steinhilber, NMI Natural and Medical Sciences Institute at the University of Tuebingen, Markwiesenstr. 55, Reutlingen, Germany

O. Poetz, NMI Natural and Medical Sciences Institute at the University of Tuebingen, Markwiesenstr. 55, Reutlingen, Germany, SIGNATOPE GmbH, Markwiesenstr. 55, Reutlingen, Germany

*U. M. Herfurth, German Federal Institute for Risk Assessment, Department Food Safety, NRL for Animal Protein in Feed, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany*

Poster number: **FP-PB-026**

## **ABSOLUTE AND RELATIVE QUANTITATION OF AMYLASE/TRYPsin-INHIBITORS FROM CULTIVATED WHEAT SPECIES MODIFIED BY CRISPR-CAS9 AND RNAI**

Abstract ID: **90**

**Presenting author: Sabrina Geisslitz, Department of Bioactive and Functional Food Chemistry, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany, Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising, Germany**

### **Introduction**

Amylase/trypsin-inhibitors (ATIs) are wheat grain proteins triggering health-related disorders such as Bakers' asthma and non-celiac wheat sensitivity (NCWS) in up to 6% of the population. Gene-editing tools aim to silence the genes responsible for expression of ATIs to reduce the immunogenic potential of wheat used for bread and pasta (common and durum wheat). These efforts are facing challenges due to the high complexity of wheat's genetic background, as common wheat is hexaploid and durum wheat is tetraploid. Thus, sensitive, accurate and precise methods are required to confirm the success of silencing of ATIs in wheat and to monitor the effects on other wheat proteins. The aim of the study was to compare different methods for the absolute and relative quantitation of ATIs.

### **Methods**

Three ATIs of Bobwhite (common wheat) and two ATIs of Svevo (durum wheat) were silenced by RNAi and CRISPR-Cas9, respectively. Twelve different ATIs were quantitated using heavy isotopic labeled peptides and a LC-MS/MS-SRM method on a triple quadrupole system. For relative quantitation, data-dependent acquisition (DDA) was performed on a Q-Exactive+ and on a Bruker Amazon Speed IonTrap without labeling, and iTRAQ experiments on a Q-Exactive-HF. DDA data analyzed at the ion trap were evaluated using MASCOT-MS/MS-Ions search, DDA data from the Q-Exactive+ using MaxQuant, intensity based absolute quantitation (iBAQ) and Skyline, and iTRAQ data using ProteinPilot.

### **Preliminary data (results)**

Absolute quantitation verified silencing of the three ATIs in the mutants of Bobwhite and that of the two ATIs in the mutants of Svevo. In Bobwhite, silencing of target genes led to silencing of three other non-target ATIs in the mutants. In contrast, this was not observed in the tetraploid mutants, in which all ATIs were expressed in lower levels compared to Svevo. The relative quantitation methods demonstrated the same results for Svevo and the tetraploid mutants. In contrast, these methods detected peptides of the silenced ATIs in the hexaploid mutants at a maximum level of 5% compared to Bobwhite. Manual curation of the identified peptides using Skyline confirmed their presence in the hexaploid mutants. The largest discrepancy between absolute and relative quantitation was observed for the most abundant ATI called '0.19', which is only expressed in hexaploids, but not in tetraploid wheat. The relative quantitation of 0.19 using MaxQuant and iBAQ showed that 0.19 was not present in Bobwhite and the hexaploid mutants. Further, ProteinPilot did not use the peptides of 0.19 for relative quantitation. This uncovered a major problem of wheat proteomics. The number of ATI peptides with the attribute 'unique' is very limited, because there are several entries for all ATIs in the UniProtKB database, e.g., seven entries for 0.19 (P01085) with 100% identity. In summary, this shows the high necessity either for selective absolute methods or for the manual curation of software-based protein identification.

### **Please explain why your abstract is innovative for mass spectrometry?**

Wheat proteomics is facing challenges due to the high genetic complexity, the close relationship to other cereals and the incomplete, redundant protein database requiring sensitive, precise, accurate LC-MS/MS methods.

### **Co-authors:**

*Shahidul Islam, State Agricultural Biotechnology Centre, College of Science, Health, Engineering & Education, Murdoch University, Western Australia*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Lukas Buck, Department of Bioactive and Functional Food Chemistry, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany*

*Francesco Camerlengo, Department of Agricultural and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy*

*Clemens Grunwald-Gruber, BOKU Core Facility Mass Spectrometry, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria*

*Francesco Sestili, Department of Agricultural and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy*

*Stefania Masci, Department of Agricultural and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy*

*Stefano D'Amico, Institute for Animal Nutrition and Feed, AGES - Austrian Agency for Health and Food Safety, Vienna, Austria*

Poster number: FP-PB-027

## GC-MS BASED METABOLITE PROFILING OF RED SEaweEDS REVEALS THE SIMILAR BIOCHEMICAL FEATURES IN THE SPECIES BELONGING TO DIFFERENT TAXONS

Abstract ID: 125

Presenting author: Claudia Birkemeyer, University of Leipzig, Institute of Analytical Chemistry

### Introduction

Metabolomics of marine seaweeds is still not a well-studied field of plant biochemistry. Despite the growing interest to different algal products, broadly used in food industry, medicine etc., there are only few red algal species which biochemical composition is thoroughly investigated. Meanwhile, it is known that red seaweeds generally have more complex metabolite profiles compared to those of the other marine macrophytes, and even the individual red algal species possess a number of specific metabolites. Here we used a GC-MS based metabolomics approach to study and compare the patterns of low-molecular-weight metabolites of the representatives of the different taxonomic groups of red algae.

### Methods

Fifteen red algae species representing the orders Ahnfeltiales, Corallinales, Palmariales, Gigartinales, and Ceramiales were collected near the western coast of the White Sea. Algal thalli were extracted with 100% methanol, dried and derivatized prior GC-MS analysis on a 6890/5973N GC-MS (Agilent Technologies, Böblingen, Germany) with 1 mL/min He flow, splitless injection at 250°C and standard electron impact ionization. AMDIS 2.65 was used for peak deconvolution. Sixty authentic standards, GMD and NIST14 mass spectral libraries were used for peak identification. Peak integration of extracted ion chromatograms at specific retention times was accomplished using Xcalibur 3.0 software.

### Preliminary data (results)

After analysis of the whole dataset of 15 species, 546 compounds were detected in the red algal extracts, from which about a third was identified. All samples contained relatively high amounts of soluble carbohydrates and free amino acids (including nine non-proteinogenic ones: pipercolic acid,  $\beta$ -alanine, ornithine etc.). The dominating metabolites were specific red algal storage compounds and osmolytes floridoside ( $\alpha$ -D-galactopyranosyl-(1-2)-glycerol) and/or digeneaside ( $\alpha$ -D-mannopyranosyl-(1-2)-D-glycerate), hexitols and cholesterol. Several species or groups of species accumulated particular metabolites such as trehalose, sucrose, aconitic acid, glyceric acid etc. Interestingly, several specific organic acids (e.g., aminomalonic, citramalic, and 2-methylcitric acids) were also found in the extracts.

Discriminant (sPLS-DA) analysis of the dataset showed that all studied species could be grouped into three big clusters according to their metabolite profiles. Notably, several representatives of different orders and even subclasses demonstrated similar biochemical composition and were assigned to the same cluster. Thus, the first cluster combined the gigartinalean and corallinalean algae as accumulating the highest amounts of floridoside, sugars, polyols, and lipid metabolites. The algae of the second cluster (Ceramiales and Palmariales) contained lesser diversity and amounts of sugars, but accumulated free amino acids, organic acids, and phenolic compounds. The third cluster included only *Ahnfeltia plicata* (Ahnfeltiales) generally exhibiting a metabolite profile close to that of gigartinalean algae, which tended to specifically accumulate trehalose,  $\alpha$ -tocopherol, cholesterol, and alanine.

Research was supported by the Russian Foundation for Basic Research (project 20-04-00944) and the MS-UL core facility of the University of Leipzig.

### Please explain why your abstract is innovative for mass spectrometry?

Metabolic taxonomy of the hitherto little investigated red seaweeds by GCMS metabolite profiling proved a valuable tool for ongoing taxonomic revisions for species of this phylum.

### Co-authors:

Nikolay Yanshin, St. Petersburg State University, Faculty of Biology  
Elena Tarakhovskaya, St. Petersburg State University, Faculty of Biology

Poster number: FP-PB-028

## UNTARGETED AND TARGETED PROTEOMIC ANALYSIS OF WHEAT RELATED TO BAKING QUALITY

Abstract ID: 190

Presenting author: Christine Kaemper, Karlsruhe Institute of Technology

### Introduction

Wheat is one of the most important cereals in the human diet. It stands out for its special baking properties, which enable it to be processed into bread, cake and other baked goods. The parameter 'baking quality' is influenced by various factors including the quality and quantity of proteins in the grains, known as gluten. Gluten can be divided into gliadins and glutenins whereby the amount and the ratio of the various gliadin and glutenin protein types is crucial for its quality. Therefore, it is essential to gain knowledge about the exact gluten composition. The aim of this work is to set up high-throughput bottom-up proteomics methods (untargeted and targeted) to comparatively analyse a large number of wheat flour samples and extend their phenotypic description.

### Methods

The proteins were extracted from the wheat flours in a three-stage extraction process (Figure 1). Extract 1 contains mainly enzymes, puroindolines and other salt-soluble proteins, while extract 2 is enriched in gliadins and extract 3 in glutenins. The extracts were further processed separately. After reduction and alkylation, the samples were digested. Four different digestive enzymes were tested: trypsin, chymotrypsin, pepsin and thermolysin, as well as the combinations trypsin + chymotrypsin and trypsin + thermolysin. The work-up was finished by purification by solid phase extraction. The peptides were investigated with untargeted LC-MS/MS to maximize numbers of identified peptides.

### Preliminary data (results)

The sample set is the Bavarian MAGIC (multiple advanced generation intercross) wheat population (BMWpop). BMWpop consists of 394 inbred lines that capture 72 % of the allelic diversity of the German wheat breeding gene pool. With its high variability in terms of baking quality, BMWpop is well suited to identify proteins that are associated with, e.g., high loaf volume.

The extraction step was performed with three different extraction solutions to ensure a comprehensive protein extraction. Trypsin was found to be most suitable for the digestion of extract 1 and 3. In general, extract 2 was digested more poorly, because gliadins have many repetitive amino acid sequences with a high proportion of glutamine and proline. For extract 2, all enzymes led to similar results. However, the omega-gliadins could not be identified with trypsin. Apparently, the other enzymes or their combinations were more suitable to represent the entire gliadin spectrum, as all gliadin types were identified.

The purification step was carried out in 96-well plates. This way, three extracts of 10 samples in triplicate can be processed in parallel, reducing the work-up time from 16 h to 8 h. The untargeted LC-MS/MS-method was optimised to a run time of 80 minutes. In the samples examined so far, 3,500 - 7,000 peptides were identified, corresponding to up to 3,800 proteins from 1,600 different protein groups. Based on these results, a targeted method will be set up to quantitate proteins related to baking quality.

### Please explain why your abstract is innovative for mass spectrometry?

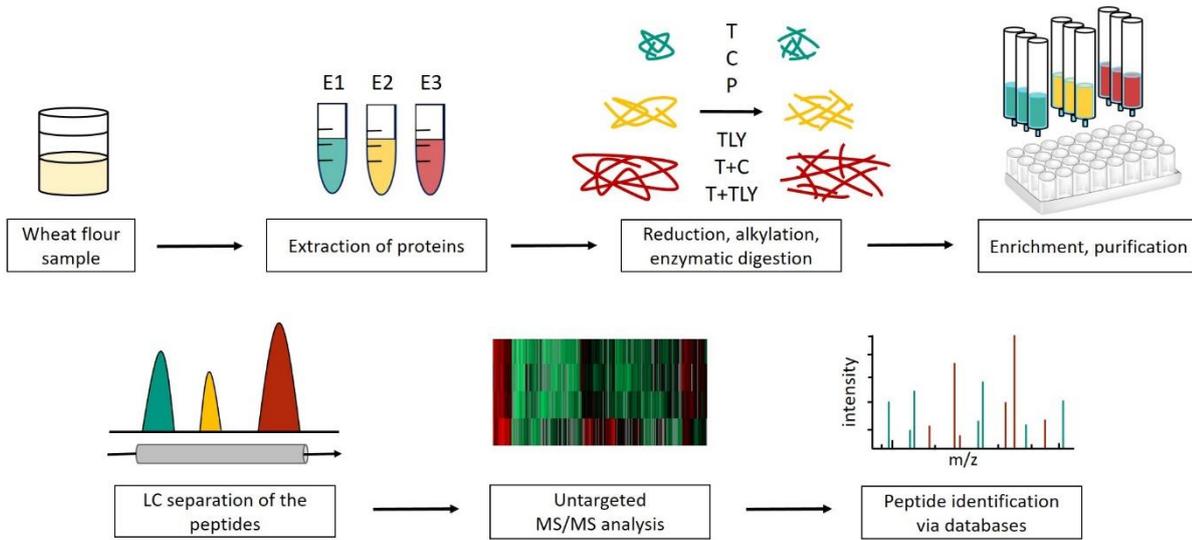
The implementation of an untargeted high-throughput proteomics method for wheat flour samples was successful and can be used for the investigation of large sample sets such as the BMWpop.

### Co-authors:

Sabrina Geisslitz, Karlsruhe Institute of Technology  
Katharina Scherf, Karlsruhe Institute of Technology

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



E1: Extract 1 containing enzymes, puroindolines & other salt-soluble proteins; E2: Extract 2 containing gliadins; E3: Extract 3 containing glutenins;  
T: Trypsin; C: Chymotrypsin; P: Pepsin; TLY: Thermolysin.

Work-up and subsequent untargeted LC-MS/MS measurement of wheat samples.

Poster number: FP-PB-029

## TARGETED LC-MS/MS METHOD FOR THE QUANTIFICATION OF BARLEY-SPECIFIC AMYLASE/TRYPsin-INHIBITORS IN DIFFERENT BEERS AND STAGES OF THE BREWING PROCESS

Abstract ID: 284

**Presenting author: Sarah Joestl, Karlsruhe Institute of Technology, Leibniz Institute for Food Systems Biology at the Technical University of Munich**

### Introduction

Apart from gluten proteins, amylase/trypsin-inhibitors (ATI) are postulated as putative triggers for different wheat related disorders, like baker's asthma or non-coeliac wheat sensitivity (NCWS). NCWS is an inflammatory disease with numerous gastrointestinal and extraintestinal symptoms, like bloating or headache. Its pathogenesis still remains insufficiently characterized and no specific biomarkers for diagnosis have been identified. So far, ATI were mainly quantitated in wheat flours. It is known that ATI also pass into grain-based foods during processing. The aim of the current study was the development of a method for the absolute quantification of barley-specific ATI in different beer types, e.g., light or gluten-free beers and during the beer brewing process.

### Methods

Beer proteins were reduced, alkylated and digested with trypsin. A solid-phase extraction with C-18 cartridge material was used for purification. Peptides were analyzed with a targeted LC-MS/MS approach (Figure 1). Ten different barley ATI-types were quantitated by means of stable isotope dilution analysis (SIDA). Precision, limit of detection and recovery were determined for method validation. The method was used on a sample set comprising different beer types, e.g. lager beer, white beer, gluten-free beers, stout and beers made of alternative malts like millet or rice. Additionally, samples from different steps of the brewing process were analyzed.

### Preliminary data (results)

With the newly developed method it is possible to quantitate ten barley-specific ATI in beer. Precision, recovery and limits of detection underlined the good performance. The different beer types in the sample set also showed differences in their ATI compositions. Lager beers had a range of 0.03 µg/mL – 40.26 µg/mL. All gluten-free beers contained less ATI. Especially in gluten-free beers made of alternative malts, no ATI were detectable. Among ATI-types, the BDAI, which is also known for its beer foam stabilizing properties, had the highest contents. The BDAI-type content was over ten times higher than each of the other ATI-types. The contents of all ATI-types first increased during the beer brewing process until the fermentation step. Then, they decreased, so that the ATI-content was lower in the final beer than at the beginning of the brewing process. This trend could be observed for the different ATI-types as well as for the total ATI-content. The results are an important step towards ATI quantification in processed foods, such as beer, and the production of ATI-reduced alternatives suitable for NCWS-patients.

### Please explain why your abstract is innovative for mass spectrometry?

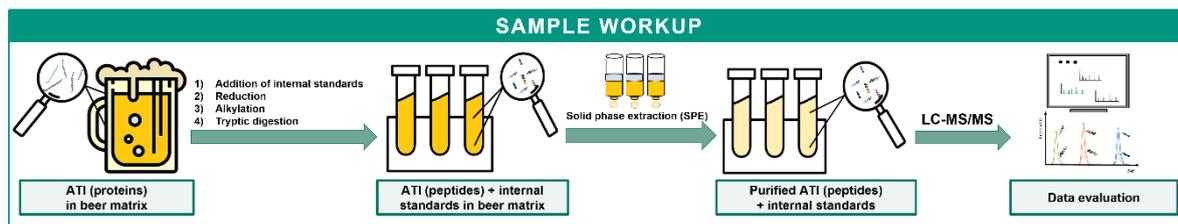
A targeted LC-MS/MS approach was successfully implemented for the quantification of barley-specific ATI-types by means of SIDA in samples of different beers and stages of the brewing process.

### Co-authors:

*Sabrina Geisslitz, Karlsruhe Institute of Technology*  
*Katharina Anne Scherf, Karlsruhe Institute of Technology*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Workup flow for the quantification of ATI in beer samples.

Poster number: **FP-PB-030**

## **BENCHMARK OF A NEW AUTOMATED SAMPLE PREPARATION PLATFORM FOR PROTEOMICS APPLIED TO BEEROMICS SAMPLES**

Abstract ID: **322****Presenting author: Christopher Bolcato, Thermofisher Scientific**

### **Introduction**

Sample preparation plays a major role in the quality of results for any proteomics analysis when studying the human biological samples, animal models, tissue, or any other sample type. Utilizing an automated sample preparation platform significantly reduces the possibility of introducing variabilities into the results. In this study, a new automated sample preparation platform for proteomics samples was tested for the characterization of yeast proteome in the context of understanding the effects of a commercial yeast strain in combination with timed dry hopping during the production of hazy beer. The automated sample preparation platform enables standardized, hands-off, reproducible, and high throughput preparation of the samples

### **Methods**

A label-free quantitative method using the Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer was utilized in this study. Proteins from hazy beer samples were acetone precipitated for cleanup. Proteins were then reduced, alkylated, and digested to peptides using an automated sample preparation platform. Peptides were then cleaned up and 2ug of each digest was loaded to facilitate equivalent amounts per analysis. Data were processed using Thermo Scientific™ Proteome Discoverer™ software. In addition, software from Protein Metrics was utilized orthogonally for interpretation of beer proteome digest complexity as well as searching for novel post-translational modifications

### **Preliminary data (results)**

A global proteomics approach allowed for the determination of proteins across different beer samples made from a single yeast strain (OYL-011, Omega Yeast), wheat, barley, and varying dry hopping addition times. In addition, the presence of hop-derived post-translational modifications targeted as a novel aspect of this study. The new automated sample preparation platform has significantly increased the experiment throughput with high reproducibility leading to accurate LFQ results. Digestion efficiency was >90%, reduction/alkylation efficiency was >90%, and nonspecific modification <1%. The manual time the user had to spend was <20min. More importantly, the CV between the samples was below 10%. The proteomics of hazy beer based on varying hop addition times during fermentation are shown to generate unique protein expression patterns through label-free protein quantification. Data analysis confirmed the identification of 1430 proteins with high confidence. Data comparing proteins present in samples dry hopped at 168hr vs 24hr show 51 proteins with a negative fold change and 88 proteins with a positive fold change demonstrating that there is proteomics effect on the turbidity and flavors of Hazy beers as a function of the length of the dry hopping process. This study adds an understanding of how recipes for producing beers with different organoleptic properties can be tunable. In addition, this platform offers an exciting opportunity to increase access to proteomics technologies to newcomers to the field due to its ease of use and additionally increases the throughput and reproducibility in proteomics sample preparation.

### **Please explain why your abstract is innovative for mass spectrometry?**

Deep proteome characterization using an automated sample preparation platform

### **Co-authors:**

*Amirmasoor Hakimi, Thermofisher Scientific*  
*Eric Tague, Thermofisher Scientific*  
*David Horn, Thermofisher Scientific*  
*Laura Burns, Omega Yeast*  
*Lance Shaner, Omega Yeast*  
*Daniel Lopez Ferrer, Thermofisher Scientific*

Poster number: **FP-PB-031**

## **SEARCHING FOR BLOWFISH POISON (TETRODOTOXIN) IN SWEDISH SHELLFISH WITH ULTRA-PERFORMANCE HYDROPHILIC INTERACTION LC-MS/MS**

Abstract ID: **387**

**Presenting author: Aida Zuberovic Muratovic, Uppsala University / Swedish Food Agency**

### **Introduction**

Tetrodotoxin (TTX) is one of the most potent natural toxins known, with lethal doses in human that range from 1.5–2.0 mg. TTX acts as a blocker of the voltage-gated sodium channels in nerve cell membranes thereby preventing muscles from contracting as a result of absent nervous stimulation (paralysis). TTX has previously been most common in tropical waters where it is best known from the pufferfish (Tetraodontidae family) from which it was firstly discovered and named. During the last decade, several studies have shown the geographical spread of TTX and its presence in other marine species including European bivalves from southern as well as mid-European waters (The Netherlands and UK).

### **Methods**

The water-soluble and heat-stable TTX is accumulated through filter-feeding which can lead to human poisoning upon consumption of contaminated shellfish since the toxin remains active even after heating. This rises a food safety concern and the question of TTX presence in Swedish shellfish gains an increasing importance. In this study, the occurrence of TTX is investigated in 376 bivalve samples (mussels and oysters) collected along the Swedish west coast in summer 2019 and 2021. Analyses are carried out with an in-house validated method using ultra-performance hydrophilic interaction liquid chromatography with tandem mass spectrometry (UP-HILIC-MS/MS) detection.

### **Preliminary data (results)**

With the UP-HILIC-MS/MS, as the state-of-the-art approach in (at the moment non-EU regulated) monitoring of one of the most potent marine biotoxins, this is the first study to report whether the TTX is present in Swedish bivalves or not.

### **Please explain why your abstract is innovative for mass spectrometry?**

UP-HILIC-MS/MS analysis in this study provides new information on the occurrence of TTX and analogs in Swedish bivalves. The data collected will contribute to a more reliable risk assessment.

Poster number: FP-PB-032

## A COMPREHENSIVE METHOD TO ELUCIDATE PYOVERDINES PRODUCED BY FLUORESCENT PSEUDOMONAS SSP. BY UHPLC-HR-MS/MS

Abstract ID: 420

**Presenting author:** Karoline Rehm, University of Zurich, Department of Chemistry

### Introduction

Microbial secondary metabolites represent a rich source for drug discovery, plant protective agents and biotechnologically relevant compounds. Among them are iron-chelating molecules called siderophores that play key roles in bacterial community assembly. Some siderophores such as certain pyoverdines can act as plant protective agents due to their pathogen control properties. These biologically active pyoverdines are commonly not identified due to their complex chemical structure and the lack of simple and rapid analytical procedures despite great interest. They are solely produced by fluorescent *Pseudomonas* members and consist of different peptide chains specific to each bacterial species often incorporating unusual amino acids (see Figure).

### Methods

A high-throughput UHPLC-MS/MS pipeline was developed for the structural elucidation of pyoverdines using a Q Exactive MS. Liquid bacterial cultures were purified by a small-scale solid phase extraction (SPE) and directly submitted to liquid chromatography. All ion fragmentation (AIF) generated mass spectra containing characteristic fragments of the biological precursor of pyoverdine, ferribactin, leading to the revelation of the mass of secreted pyoverdines. Targeted MS/MS experiments at multiple collision energies accomplished the full structure elucidation of the pyoverdine peptide chains. The interpretation of MS/MS spectra was simplified using a mass calculator and a fragmentation predictor programmed in Excel.

### Preliminary data (results)

Our method was optimized and successfully validated using four well-known pyoverdines with various peptide chains. The extraction procedure as well as the instrument settings were shown to be suitable for a general analysis of pyoverdines and its derivatives. The method robustness and applicability were demonstrated by the analysis of 13 unknown pyoverdines secreted by sampled bacterial cultures. Among these, 4 novel pyoverdine peptide chains were discovered that were not previously reported in literature.

### Please explain why your abstract is innovative for mass spectrometry?

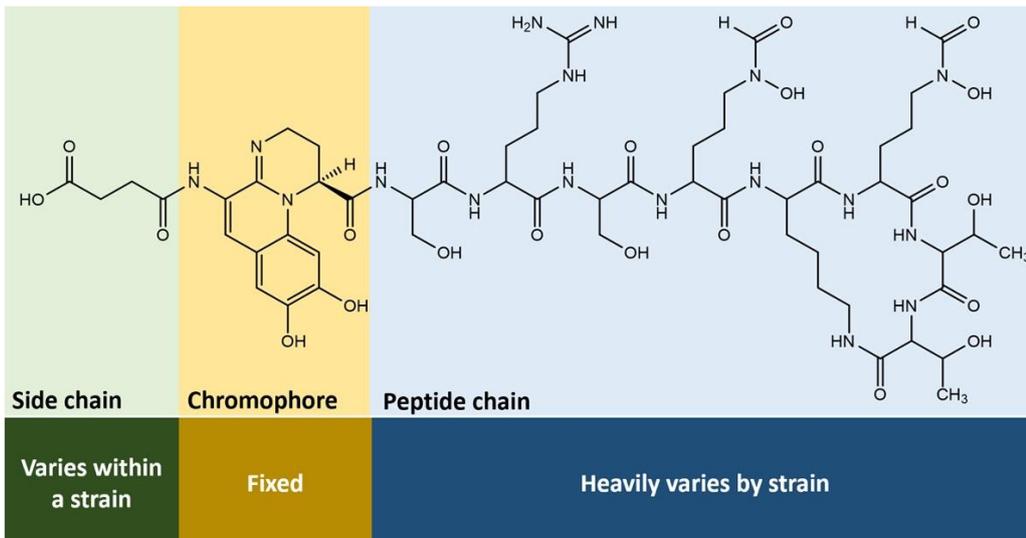
A simple analytical workflow, from sample preparation to MS/MS interpretation, was developed and validated on a Q Exactive MS for the elucidation for pyoverdines facilitating siderophore research.

### Co-authors:

*Vera Vollenweider, University of Zurich, Department of Quantitative Biomedicine*  
*Rolf Kümmerli, University of Zurich, Department of Quantitative Biomedicine*  
*Laurent Bigler, University of Zurich, Department of Chemistry*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Structural elements of pyoverdines secreted by fluorescent *Pseudomonas* spp.

Poster number: FP-PB-033

## FIA-HRMS FINGERPRINTING SUBJECTED TO CHEMOMETRICS TO ADDRESS FOOD CLASSIFICATION AND AUTHENTICATION ISSUES

Abstract ID: 429

**Presenting author:** Oscar Núñez, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain., Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921 Santa Coloma de Gramanet, Spain.

### Introduction

The rise of food fraud practices, affecting a wide variety of goods and their specific characteristics (e.g., quality or geographical origin), demands analytical methodologies to ensure consumers protection. Chromatographic and related techniques—such as capillary electrophoresis (CE), gas chromatography (GC), and liquid chromatography (LC)—with spectroscopic detection or coupled to mass spectrometry (MS), and combined with chemometrics, have proven excellent capacity to address these issues through fingerprinting strategies. However, the need for more rapid high-throughput analytical approaches has focused the attention on direct MS techniques. FIA–HRMS fingerprinting was proposed to address specific food authentication issues through chemometrics by studying the geographical origin of Spanish red wines and paprika, the distinction of olive oil from other vegetable oils, and the assessment of oil quality.

### Methods

Red wine samples were directly analyzed, but paprika and vegetable oil samples were previously processed by solid-liquid extraction (SLE) and liquid-liquid extraction with low-temperature partition (LLE-LTP), respectively. Moreover, a 50:50 (v/v) mix composed of water acidified with 0.1% formic acid (v/v) and acetonitrile were used as the carrier at 150  $\mu\text{L}\cdot\text{min}^{-1}$  for 1.5 min. Regarding HRMS detection, each sample was acquired in positive and negative electrospray ionization (ESI) modes, using a Q-Orbitrap mass analyzer, working in full-scan MS mode ( $m/z$  range from 100 to 1500).

### Preliminary data (results)

Raw data was treated using the mzMine 2.53 software and the subsequent FIA–HRMS fingerprints (samples  $\times$  ion signal intensity values as a function of  $m/z$ ) were subjected to principal component analysis (PCA), partial least squares regression-discriminant analysis (PLS-DA), and soft independent modeling of class analogy (SIMCA) using Solo 8.6 chemometrics software from Eigenvector Research (Manson, WA, USA). A total of four data matrices consisting of negative ionization, positive ionization, and low-level (LLDF) and mid-level data fusion (MLDF) FIA–HRMS fingerprints were evaluated.

HRMS allowed sample characterization by the putative identification of some of the most common ions. Moreover, it conferred great selectivity and molecular coverage, leading to rich fingerprints, resulting in satisfactory results, especially when using either negative ionization, positive ionization, or LLDF data. In this context, excellent classification accuracy values were reached by the built PLS-DA models after external validation (above 78.9%). Similarly, good assignment results were also obtained when validating the corresponding SIMCA models (above 75.4%). Furthermore, the successful application of the MLDF strategy to some of the studied food authentication cases also suggested the eligibility of targeted profiling approaches, focusing on specific compounds, to assess them.

### Please explain why your abstract is innovative for mass spectrometry?

A rapid and high-throughput screening method based on FIA-HRMS was successfully developed and assessed to solve food classification and authentication issues with an instrumental analysis time below 1.5 min/sample.

### Co-authors:

*Guillem Campmajó, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain., Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921 Santa Coloma de Gramanet, Spain.*

*Javier Saurina, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain., Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921 Santa Coloma de Gramanet, Spain.*

Poster number: FP-PB-034

## HIGH-THROUGHPUT FIA-MS FINGERPRINTING METHODS IN THE CLASSIFICATION AND AUTHENTICATION OF COFFEE AND TEA BEVERAGES.

Abstract ID: 434

**Presenting author:** Oscar Núñez, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain., Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921 Santa Coloma de Gramanet, Spain.

### Introduction

Food products are very complex matrices, which makes the quality of these products an issue of great interest in our society. Considering the complexity of the food chain, the adulteration of food and beverages is increasing, causing food fraud cases. In this field, drinks are food products that can be very easily adulterated. The present work will focus on the authentication and fraud detection of two of the most popular and worldwide consumed beverages, coffee and tea, that are also among the most adulterated food products. Both coffee and tea contain a high number of bioactive substances, contributing to their antioxidant activities, that can also be employed as markers to solve authentication issues.

### Methods

54 coffee samples of different origin and varieties (together with 69 possible coffee adulterants including chicory, barley, and wheat, rice, cornmeal, rye, and oatmeal flours), and 87 tea samples of different varieties (together with 21 chicory samples) were directly analyzed with the proposed FIA-MS method after coffee/tea brewing and filtration. 10  $\mu$ L of sample were injected using 0.1% formic acid aqueous solution:methanol 1:1 (v/v) as carrier at 150  $\mu$ L/min. Full-scan acquisition ( $m/z$  100-550) in a quadrupole-linear ion trap MS instrument and ESI in both positive and negative ionization modes were employed to obtain FIA-MS fingerprints.

### Preliminary data (results)

High-throughput FIA-ESI-MS fingerprints in both positive and negative ESI mode were obtained in less than 1.5 min/sample and resulted in excellent sample chemical descriptors to address the characterization and classification of coffee and tea beverages by partial least squares regression-discriminant analysis (PLS-DA) according to their production region and variety. Besides, perfect discrimination (100% classification rates) between coffee or tea beverages versus their potential adulterants (chicory, flours, etc.) was also accomplished.

In addition, several fraudulent adulteration cases such as arabica coffees adulterated with chicory, flours, or barley, and tea beverages of different varieties (red, white, oolong, black and green) adulterated with chicory, were evaluated by creating blended mixtures with adulterant levels from 2.5 to 90%. The obtained FIA-MS fingerprints resulted to be excellent chemical descriptors to detect frauds and quantify the adulterant level by partial least squares (PLS) regression. PLS calibration, cross-validation, and prediction errors in the range of 2.5 to 15% were obtained, showing the good performance of the proposed strategy to address and solve coffee and tea authentication issues, by means of a fast and high-throughput methodology.

### Please explain why your abstract is innovative for mass spectrometry?

A fast and high-throughput screening FIA-ESI-MS fingerprinting method (1.5 min/sample, direct beverage analysis) was proposed to authenticate coffee and tea beverages and to detect and quantify adulterations.

### Co-authors:

*Josep Pons, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain.*

*Àlex Bedmar, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain.*

*Nerea Núñez, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain.*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



*Javier Saurina, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain., Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921 Santa Coloma de Gramanet, Spain.*

*Sònia Sentellas, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, E-08028, Barcelona, Spain., Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921 Santa Coloma de Gramanet, Spain., Serra Hùnter Lecturer, Generalitat de Catalunya, 08007 Barcelona, Spain.*

Poster number: FP-PB-035

## COFFEE AUTHENTICATION BY HS-SPME-GC-MS AND CHEMOMETRICS. APPLICATION TO THE DETECTION AND QUANTIFICATION OF ADULTERATION IN COFFEE SAMPLES

Abstract ID: 439

**Presenting author:** Nerea Núñez, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. E-08028, Barcelona, Spain.

### Introduction

Recently, given the complexity of the food chain, food adulteration is increasing causing food fraud cases. In this field, beverages are very easily adulterated through practices such as flavor supplementation or the addition of additives to increase their volume. This work is based on the detection of fraud in one of the most popular beverages in the world: Coffee.

Actually, it is known that Coffee contains an elevated number of bioactive substances that contribute to an important antioxidant activity, known for its beneficial health effects. The content of that substances can vary depending on the origin region, weather, or the roasting degree, among other factors. As a consequence, bioactive compound fingerprinting can be used as a source of analytical data to address coffee authentication.

### Methods

HS-SPME-GC-MS using two capillary columns (a polar WAX and a non-polar DB5) was proposed to achieve metabolomic information of the analyzed coffee samples. 185 coffee samples of different geographical origins and varieties were directly analyzed without any sample treatment. In addition, Robusta and Arabica coffees were adulterated with Chicory and Robusta coffee, respectively, in percentages of adulteration between 15% and 85%, to test the feasibility of the proposed method to detect and quantify adulteration levels.

HS-SPME-GC-MS metabolomic fingerprints were subjected to chemometrics to characterize and classify the different coffee samples and to quantify the adulterant percentages in coffee.

### Preliminary data (results)

The obtained HS-SPME-GC-MS metabolomic fingerprints with both capillary columns showed to be good coffee chemical descriptors to be exploited for the characterization, classification authentication of coffee beverages by chemometrics. Very good discrimination between the different coffee samples and chicory (a frequently used coffee adulterant), soluble coffee and ground coffee beans, coffees from different geographical origin (Vietnam, Cambodia, and Costa Rica), and different coffee varieties (Arabica, Robusta and mixtures of Arabica and Robusta) was accomplished by partial least square regression-discriminant analysis (PLS-DA), with classification ratios between 90-100%.

In addition, the coffee adulteration studies carried out by partial least squares (PLS) regression demonstrated the good capability of the proposed methodology for the detection and quantitation of the adulterant levels in coffee beverages down to 15% adulteration. Calibration and prediction errors below 8% were obtained in all the adulteration cases evaluated.

### Please explain why your abstract is innovative for mass spectrometry?

A feasible HS-SPME-GC-MS metabolomic fingerprinting method able to authenticate coffees, and to detect and quantify adulterations in coffee beverages was developed, without requiring any sample manipulation prior to analysis.

### Co-authors:

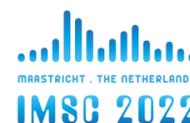
*Erica Moret, Department of Agro-Food, Environmental and Animal Sciences, University of Udine. Via Sondrio 2/a, Udine, Italy.*

*Paolo Lucci, Department of Agro-Food, Environmental and Animal Sciences, University of Udine. Via Sondrio 2/a, Udine, Italy.*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



*Javier Saurina, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. E-08028, Barcelona, Spain. , Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921, Santa Coloma de Gramanet, Spain*

*Oscar Núñez, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. E-08028, Barcelona, Spain. , Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921, Santa Coloma de Gramanet, Spain*

Poster number: FP-PB-036

## MASS SPECTROMETRIC CHARACTERIZATION OF BIOACTIVE COMPOUNDS RECOVERED FROM AGRI-FOOD WASTES

Abstract ID: 448

**Presenting author:** Aina Mir Cerdà, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain, , Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain

### Introduction

The food industry generates a considerable amount of waste, both solid and liquid, during food production processes. In the specific case of juice production from fruit and vegetables, the resulting wastes are mainly composed of skins, seeds, and stems where most of the bioactive compounds, such as polyphenols, are concentrated. For this reason, within the framework of the circular economy, a special interest in reusing these wastes arises.

Polyphenols are plant metabolites with more than one phenol group in their structure. They are beneficial for human health due to their high antioxidant character, neutralizing the formation of free radicals involved in oxidation processes. Several studies have correlated the intake of these compounds to positive effects in diseases such as cancer, diabetes, hypertension, among others.

### Methods

A preliminary study has been performed to evaluate the antioxidant capacity of 27 different matrices obtained from solid wastes of the agri-food industry. Polyphenols have been extracted from these samples by solid-liquid extraction using green extraction solvents (ethanol/water mixture). The resulting extracts have been analyzed by liquid chromatography coupled to mass spectrometry (LC-MS) to carry out the identification of the main polyphenols of the studied matrices. Compounds have been separated by reversed-phase mode using a C18 column, under an elution gradient program generated from 0.1% (v:v) formic acid aqueous solution and acetonitrile as the components of the mobile phase.

### Preliminary data (results)

Results of the estimation of the antioxidant capacity by the FRAP and Folin-Ciocalteu assays have shown that the matrices with the highest antioxidant capacity are mandarin tree leaves, nut and almond hulls, pomegranate, blueberries, kale, coffee and orujo residue, and olive tree leaves. As a result, such matrices have been selected as potential sources of bioactive polyphenolic compounds.

Identification and quantification of the principal polyphenols present in the matrices have been achieved by LC-MS using both low- and high-resolution mass spectrometric techniques. The most representative polyphenols are hesperidin (maximum of 718.0 mg·kg<sup>-1</sup> found in oranges) and quercetin (maximum of 786.0 mg·kg<sup>-1</sup> found in oranges) in fruits, acid ferulic (maximum of 18.2 mg·kg<sup>-1</sup> found in kale) in vegetables and rutin (maximum of 328.0 mg·kg<sup>-1</sup> found in mandarin tree leaves), chlorogenic acid (maximum of 276.0 mg·kg<sup>-1</sup> found in coffee residue), p-coumaric acid (maximum of 67.2 mg·kg<sup>-1</sup> found in almond hulls) and vanillin acid (maximum of 33.4 mg·kg<sup>-1</sup> found in almond hulls) in other agricultural wastes.

### Please explain why your abstract is innovative for mass spectrometry?

Polyphenol identification in agri-food wastes is especially difficult due to its complexity and diversity. In this work, different MS modes were combined to identify and quantify up 38 compounds.

### Co-authors:

*Mercè Granados, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain;*

*Javier Saurina, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain,; Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain*

*Sònia Sentellas, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain,; Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain, Serra Hünter Lecturer, Generalitat de Catalunya, 08007 Barcelona, Spain*

Poster number: FP-PB-037

## IDENTIFICATION AND QUANTIFICATION OF POLYPHENOLS IN WINE LEES EXTRACTS BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

Abstract ID: 453

**Presenting author:** Aina Mir Cerdà, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain, Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain

### Introduction

Residues resulting from winemaking processes, such as lees wastes, are rich sources of polyphenols displaying a high antioxidant activity. Polyphenols are molecules with a great added value in industries such as cosmetics, pharmaceuticals and agri-food so that the revalorization of these agri-food industry by-products offers great opportunities in the framework of a circular economy.

This work aims at characterizing the polyphenolic content of lees extracts obtained from solid-liquid extraction processes using green extraction solvents (e.g. water and ethanol/water mixtures) compatible with food fortification and pharmaceutical applications. Sample extracts have been analyzed by liquid chromatography with UV detection (LC-UV), and coupled to mass spectrometry (LC-MS) using both low- and high-resolution mass spectrometric techniques (LRMS and HRMS, respectively).

### Methods

The chromatographic separation relies on reversed-phase mode using a core-shell C18 column and 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as the components of the mobile phase. The elution gradient was optimized to achieve a good resolution for the main peaks of the chromatogram. Polyphenols were detected by UV at 280, 330, and 370 nm, and by MS in negative mode using electrospray as the ionization source. Depending on the MS instrument, different acquisition modes were used, including Full Scan, Multiple Reaction Monitoring (MRM) and Data-Dependent Acquisition (DDA).

### Preliminary data (results)

The most remarkable peaks in the UV chromatograms, attributed to hydroxycinnamic acids, were tentatively identified based on MS and MS/MS characterizations, and further confirmed using the corresponding standards as the reference. It was found that the most abundant polyphenols in the extract were caffeic acid –with concentrations ca. 20 mg L<sup>-1</sup>–, trans-coumaric acid, cis-coumaric acid, caffeic acid, and *p*-coumaric acid –with concentrations of 5 mg L<sup>-1</sup> or less–. These species were especially relevant from the point of view of revalorization issues because of their great concentrations as they could be recovered and purified. Other compounds occurring at lower concentrations were also identified, including gallic acid, astilbin, rutin, and syringic acid, among others.

### Please explain why your abstract is innovative for mass spectrometry?

Combining different MS modes, up to 24 compounds were successfully confirmed and quantified in lees extracts, a waste matrix scarcely studied and that exhibited high complexity.

### Co-authors:

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Poster number: **FP-PB-038**

## **CHARACTERIZATION OF DARK CHOCOLATES BASED ON POLYPHENOLIC PROFILING OBTAINED BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY**

Abstract ID: **456**

**Presenting author: Sonia Sentellas, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain, Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain, Serra Hünter Lecturer, Generalitat de Catalunya, 08007 Barcelona, Spain**

### **Introduction**

The popularity of dark chocolate has increased greatly in recent years not only because it is considered a delicatessen, but also due to some interesting healthy properties. Some of the bioactivities attributed to dark chocolate –such as antioxidant, anti-inflammatory, and anti-carcinogenic properties– are mainly related to its high polyphenolic content. Hence, the development of reliable methods for chocolate characterization is required to guaranty the nutritional and functional features through the profiling of bioactive compounds as well as to ensure the authenticity of these products. Cocoa is the main ingredient of chocolate and its derivate products.

This work aims at characterizing dark chocolates based on their geographical origin, cocoa variety and cocoa content using alkaloid (caffeine and theobromine) and polyphenolic composition as the data.

### **Methods**

A set of 42 chocolates from 4 different bean varieties (Criollo, Forastero, Nacional and Trinitario) and 3 different geographical origins (Africa, America and Asia) were analyzed. Samples were first defatted with chloroform and the resulting residues were further extracted with a methanol/water 60:40 (v:v) solution. Extracts were analyzed by reversed-phase high-performance liquid chromatography with UV and mass spectrometry detection (HPLC-UV-MS). Data consisting of the compositional profiles of alkaloids and polyphenols were analyzed by principal component analysis (PCA) and partial least squares – discriminant analysis (PLS-DA) as exploratory and classification methods, respectively.

### **Preliminary data (results)**

Representative chocolate samples and standard mixtures were analyzed in both negative (for polyphenols) and positive ionization modes (suitable for alkaloid/xanthine derivatives) in order to confirm the identification of the main peaks. More than twenty species were found among which theobromine, caffeine, catechin, epicatechin, gallic acid, caffeic acid and procyanidins B2 and C1 were the most abundant bioactive phytochemicals.

Compositional profiles were used as the data to carry out further sample characterization studies based on chemometric methods. Results indicated that African samples display high levels of flavanols while American samples are richer in alkaloids. Regarding cocoa varieties, epicatechin, catechin and procyanidins B2 and C1 are more abundant in Forastero and Nacional derived chocolates, caffeine and theobromine in Criollo, and Trinitario is characterized by the high levels of flavonols and poor catechin content.

### **Please explain why your abstract is innovative for mass spectrometry?**

The HPLC-UV-MS method developed was used for the identification and quantification of the most relevant phytochemicals. 21 compounds were determined and used as the data in characterization and authentication studies.

### **Co-authors:**

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Poster number: FP-PB-039

## DETERMINATION OF QUATERNARY AMINE POLAR PESTICIDES USING IMPROVED CATION EXCHANGE SEPARATION TECHNOLOGY COMBINED WITH SUPPRESSED CONDUCTIVITY AND TANDEM MASS SPECTROMETRY DETECTION

Abstract ID: 461

Presenting author: Wai-Chi Man, ThermoFisher Scientifics

### Introduction

Pesticide contamination in food, including ionic polar pesticides, is a potential health risk and a growing public concern. Quaternary amines, such as paraquat and diquat, are applied as desiccants in grain fields for faster harvest and to prevent fungal contamination, thereby are a higher contamination risk to the food chain. Quaternary amine determinations are challenging due to similar chemical structures and strong affinity to cation-exchange chromatography. In addition, oat cereals are challenging samples with poor recoveries of added standards. Here we demonstrate direct determinations of four quaternary amine polar pesticides in oat cereals using a high-performance cation-exchange column designed to resolve quaternary amine pesticides and inorganic cations. The polar pesticides are serially detected by suppressed conductivity and tandem mass spectrometry.

### Methods

The IC-MS/MS method applied to diluted supernatant, centrifuged, acid-extractions of oat cereals (acid-modified version 12 of the EUVL-FV QuPPE method). Chlormequat, mepiquat, paraquat, and diquat quaternary amines were separated using electrolytically generated methanesulfonic acid gradient at 0.3 mL/min at 40 °C. The column effluent carrying the separated pesticides, passes through electrolytic suppressor, neutralizing the eluent, and were detected by suppressed conductivity and tandem MS using SRM. Chlormequat and mepiquat were detected and quantified as singly charged precursor and product cations. Doubly charged paraquat and diquat cations were used for quantification with the singly charged cations for confirmation.

### Preliminary data (results)

The determinations of quaternary amine pesticides in oat cereals were successfully determined using IC-MS/MS with modified acid-methanol extractions. The four quaternary amine polar pesticides were well resolved from each other, other cations, and the sample matrices using the new cation exchange column and detected by MS/MS. In suppressed conductivity detection, the sample matrices dominated the chromatograms. Trace amounts, 0.2-1.7 µg/Kg, of the four quaternary amine pesticides were measured in oat cereals, well below the EU limits of 0.02 – 15 mg/kg. Recoveries of spiked in reagents were 85-118%. Sensitivities, as judged by the limits of detection, were less than single digit µg/L.

### Please explain why your abstract is innovative for mass spectrometry?

The quaternary amine pesticides especially paraquat and diquat were successfully resolved from oat cereals extracts, using IC-MS/MS detected down to µg/Kg concentrations.

### Co-authors:

Terri Christison, ThermoFisher Scientifics  
John Madden, ThermoFisher Scientifics  
Jeff Rohrer, ThermoFisher Scientifics

Poster number: **FP-PB-040**

## **ULTRA-HIGH SENSITIVITY QUANTIFICATION OF VETERINARY DRUG RESIDUES IN PRODUCTS OF ANIMAL ORIGIN**

Abstract ID: **502**

**Presenting author: Jack Steed, SCIEX**

### **Introduction**

The use of pharmacologically active substances in veterinary settings has been scrutinized for several years due to their sometimes inappropriate or intensive application. Therefore, these substances must be limited to mitigate negative consequences. One way to implement controls is to perform analytical testing in products of animal origin. Several compounds found in these by-products have a maximum residue limit (MRL) to minimize their use, and some compounds are prohibited due to their inherent toxicity. To limit these compounds within the food industry, it is important to achieve LOQ values that are as low as is reasonably possible.

Here, we present a method for analyzing over 180 compounds used in the veterinary industry that can achieve LOQ values as low as 0.005 ng/mL.

### **Methods**

A standard solution was provided at 1 µg/mL, a dilution series was prepared to cover 0.005–100 ng/mL.

The pork, milk or chicken samples were prepared as per specified protocol. Post-spike sample preparation was then performed.

Chromatographic separation was performed using a Phenomenex Kinetex Polar C18 (2.6 µm, 100 x 2.1 mm). A triple quadrupole MS system was operated in scheduled multiple reaction monitoring (sMRM) mode using electrospray ionization (ESI) with fast positive and negative switching.

### **Preliminary data (results)**

As regulations continue to tighten around food testing, it has become increasingly challenging to achieve the necessary levels of sensitivity during analysis while maintaining a high level of accuracy and precision. Within this method, LOQ values down to 0.005 ng/mL have been achieved. When spiked into matrices, LOQs of 0.01 µg/kg in pork and chicken and 0.005 µg/kg in milk were reached. This high level of sensitivity allows routine laboratories to further dilute their samples to minimize any matrix effects observed. The linear range of each compound analyzed has been assessed, with ranges spanning up to 4 orders of magnitude and *r* values >0.99. sMRM acquisition helps ensure that both quantifier and qualifier transitions can be measured to increase the specificity of the analysis without the need to compromise on data quality. In addition, chromatographic separation is important to minimize the number of compounds analyzed at any one time. This allows for a balance between the cycle time of the mass spectrometer and the dwell time for each analyte.

In addition to MRLs, minimum method performance requirements (MMPRs) are recommended by the EU for some prohibited compounds, which are summarized here. In these instances, the sensitivity of the analysis is paramount to ensure the MMPR is achieved or exceeded. In this method the MMPR has been met or improved upon, showing that even for the most difficult to analyze compounds, this method can achieve or improve on the recommended levels of sensitivity.

### **Please explain why your abstract is innovative for mass spectrometry?**

An ultra-sensitive and robust method enables the quantification of veterinary drugs using LC-MS/MS in routine laboratories, improving on current regulations.

### **Co-authors:**

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*Jianru Stahl-Zeng, SCIEX*

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*Santosh Kapil Kumar Gorti, SCIEX*

Poster number: FP-PB-041

## AUTOMATED IDENTIFICATION OF POTENTIAL PESTICIDES RESIDUES IN FRUIT SAMPLES USING HRMS DATA

Abstract ID: 504

**Presenting author: Ismael Zamora, Lead Molecular Design, S.L., Pompeu Fabra University**

### Introduction

In food safety and related fields, High Resolution Mass Spectrometry techniques applied for multiresidue analysis had become an alternative to the historical routine procedures involving triple quadrupole instruments. This evolution was mainly driven by the possibility to interrogate hundreds or thousands of compounds without a prior individual study of all of them. However, due to the big amount of information that can be generated during the data acquisition, the later data processing and data analysis steps can be quite time demanding. In this presentation we will show how this late step could be automatized using Chemical Monitoring workflow included in MassChemSite 3.1

### Methods

For chromatographic analysis, Thermo Fisher Scientific Vanquish Flex Quaternary LC (Thermo Scientific Transcend™, Thermo Fisher Scientific, San Jose, CA, USA) was used. The chromatographic system is coupled to a hybrid mass spectrometer Q-Exactive Orbitrap Thermo Fisher Scientific (Exactive™, Thermo Fisher Scientific, Bremen, Germany) using an electrospray interface (ESI) (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA) in positive-negative mode. ESI parameters were as follows: spray voltage, 4 kV; sheath gas (N<sub>2</sub>, 95%), 35 (adimensional); auxiliary gas (N<sub>2</sub>, 95%), 10 (adimensional); S-lens RF level, 50 (adimensional); heater temperature, 305 °C; and capillary temperature, 300 °C.

### Preliminary data (results)

Strawberry, white grape and orange samples providing from Almería (Spain) greenhouses were acquired in the University of Almería and processed using the Chemical Monitoring data workflow included in MassChemSite 3.1. Data was interrogated against an in-house pesticide database generated by literature search including up to 1500 different pesticides. From the total, up to 10 different pesticides were detected in all the samples in less than five minutes of data processing.

The identification step was performed using the MS and MSMS information: MS was used to detect the pesticide in the sample, while fragmentation information was used to finally elucidate the structure of the detected pesticide, by means of a computational fragmentation of the detected pesticide and a later assignation to the MSMS data provided by the instrument. The fitting among computed and experimental fragments is reported as "score" which can be used to discriminate among other structural isobaric compounds associated to the same chromatographic peak.

Data analysis and reporting were done in ONIRO server after an automatic uploading of the raw data. Later filtering steps were applied and tracked by the application for further inspection. Additionally, a final report was generated automatically once the experiment was reviewed. Data generated during the acquisition remained on the server for later use or further re-analysis.

### Please explain why your abstract is innovative for mass spectrometry?

MassChemSite 3.1 and ONIRO are valuable tools perform an automatic pesticide identification in food samples.

### Co-authors:

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Poster number: FP-PB-042

## ANALYSIS OF XENOBIOTICS IN PLANTS WITH MASS SPECTROMETRY IMAGING METHODS

Abstract ID: 507

Presenting author: Akhila Ajith, University of Manchester

### Introduction

With the increasing global demand for food, the need to produce newer crop-protection products is also growing for efficient and productive farming. For the commercial production of agrochemicals, it is pivotal to understand their distribution and metabolism in the plant system. Traditionally, in the industry, the surface distribution of agrochemicals has been detected with autoradiography. However, the need to synthesize radiolabeled compounds and the risks associated with handling such radiolabeled compounds are points of concern. Mass Spectrometry Imaging (MSI) represents an effective alternative to autoradiography for molecular-specific surface analysis, being an untargeted method with relatively simple sample preparation. The aim of this study is to investigate sample handling and analysis methodology to optimise MSI protocols for the analysis of crop-protection products applied to plants.

### Methods

Secondary ion mass spectrometry (SIMS) and Matrix-assisted laser desorption ionization (MALDI) were applied to study the surface chemical distribution of selected agrochemicals following their application on various plant surfaces, including grape skin, tomato and wheat leaves. Time-of-Flight (ToF) SIMS was performed with polyatomic primary ion beams including C60+ and (H2O)n+ (n>10,000) on a buncher-ToF instrument (Ionoptika Ltd. J105) equipped with cryostage. MALDI-ToF-MS was performed with a 355 nm nanosecond laser operating at 10 kHz (Bruker Rapiflex). Data were acquired in positive and negative ion mode from calibration standards and plant materials treated with crop-protection products.

### Preliminary data (results)

**Figure 1** shows an example of a ToF-SIMS calibration plot for the fungicide Azoxystrobin, spin-coated from methanol solution onto a silicon wafer. Using a 40 keV C60+ primary ion beam, the detected secondary ions include [M+H]+ at  $m/z$  404, and the diagnostic fragments at  $m/z$  372, 329 and 301. A limit of detection of ~1 nmol was estimated under these conditions. **Figure 2** shows the 3D distribution of Azoxystrobin applied to grape skin illustrating that the compound reaches a peak concentration below the surface. The inset ToF-SIMS image shows the signal of the Azoxystrobin fragment at  $m/z$  372 in red in the image with an endogenous grape skin component at  $m/z$  175 shown in blue. The signal of the Azoxystrobin fragment is concentrated within a 500 × 500 μm<sup>2</sup> etch crater corresponding to the depth-profile measurement. We will present and compare data from complementary MSI techniques including ToF-SIMS, MALDI-ToF-MS and ambient ionisation methods.

### Please explain why your abstract is innovative for mass spectrometry?

MSI of agrochemicals in plants is a relatively novel topic with great implications in the agritech industry in terms of effective sample preparation and reproducible imaging protocols.

### Co-authors:

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Nicholas Lockyer, University of Manchester

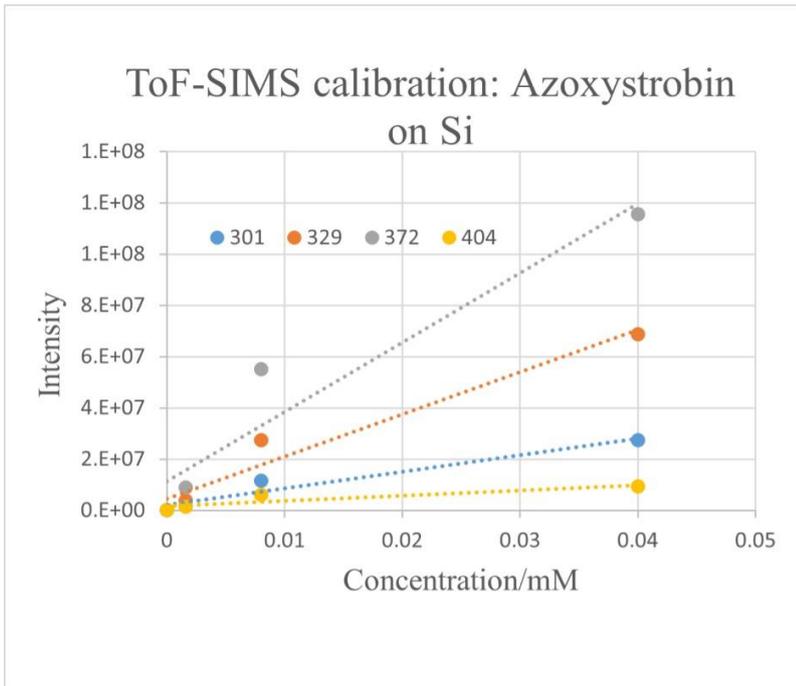


Figure 1

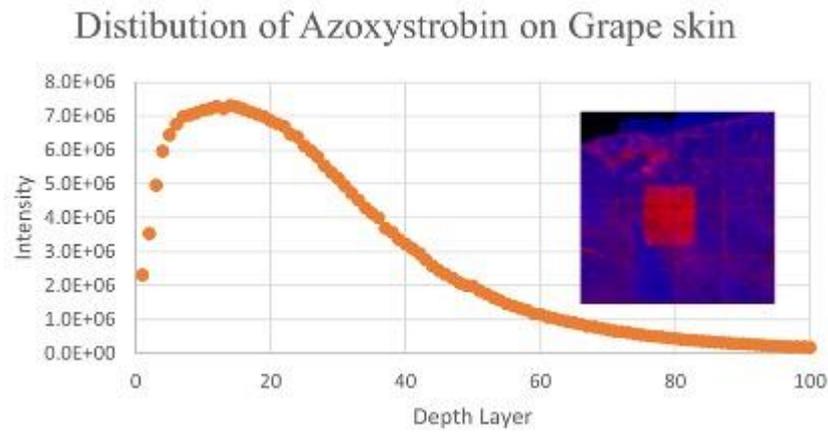


Figure 2

Poster number: **FP-PB-043**

## **DEALING WITH COMPLEXITY IN PEPTIDE ANALYSIS WITH UNTARGETED MASS SPECTROMETRY**

Abstract ID: **519**

**Presenting author: Gijs Vreeke, Laboratory of Food Chemistry, Wageningen University & Research**

### **Introduction**

Automated mass spectrometry approaches from proteomics are used to characterise peptides for food applications and in protein digests. In these approaches,  $m/z$  signals in MS spectra are converted to a list of candidate peptides, considering the mass error of the instrument. Peptide annotations and confidence in these annotations are then based on the MS/MS spectra. Low reproducibility in repeat analyses has been reported even for annotations with high confidence, independently of the mass spectrometer and processing software used. When analysing protein hydrolysates (in food) it is important to determine criteria that yield highly reproducible annotations of peptides, regardless of the various sizes and concentrations of the peptides. This study provides a structured approach to determine these data processing criteria.

### **Methods**

Tryptic hydrolysates of  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and  $\beta$ -casein were analysed in a UPLC coupled to ESI-Q-TOF with an untargeted MSe method. The MS spectra were analysed manually and automatically. Quantification of peptides was done absolutely and label-free based on UV<sub>214</sub> absorbance. Different parameters were calculated to evaluate the completeness of the analysis. The hydrolysates were mixed to evaluate the robustness in analysis of complex samples.

### **Preliminary data (results)**

A lock mass with two components was introduced resulting in an average mass error of 1 ppm. Processing filters were set to ensure reliable annotations based on MS/MS fragmentation, while maintaining maximum amount of information. Peptides in the individual hydrolysates with an MS intensity above the limit of annotation represented 99% of total MS intensity and were 100 % consistently annotated between four replicates. Amino acid and peptide sequence coverages for the individual protein hydrolysates were 99-100 % and 89-95 %, respectively. The molar concentration for peptides above the LOA from absolute quantification with UV had a relative standard deviation of 4 %. Mixing the hydrolysates resulted in a loss of 11% of the peptide annotations above the LOA and lower reproducibility (97%) for the remaining annotations, as well as more co-eluting peptides. Calculated concentrations of co-eluting peptides in mixed hydrolysates varied  $37 \pm 21$  % from the value for single hydrolysates.

### **Please explain why your abstract is innovative for mass spectrometry?**

The proposed approach aims to describe the peptides with high confidence and repeatability in an untargeted MS analysis. It allows complete description of the peptide composition even in mixed hydrolysates.

### **Co-authors:**

*Peter Wierenga, Laboratory of Food Chemistry, Wageningen University & Research*

Poster number: **FP-PB-044**

## COMPOSITIONAL ANALYSIS OF ESSENTIAL OIL AND SOLVENT EXTRACTS OF NORWAY SPRUCE SPROUTS BY ULTRAHIGH-RESOLUTION MASS SPECTROMETRY

Abstract ID: **603****Presenting author: Olufunmilayo Omolara Mofikoya, University of Eastern Finland**

### Introduction

Spruce sprouts also known as buds or shoots, are an interesting, yet limitedly available natural resource. They are highly enriched with vitamin C and other antioxidants and thus are used as a dietary supplement or to make different culinary products. The harvesting season of spruce sprouts is only a couple of weeks around mid-May to early June. To evaluate spruce sprout potential in different pharmaceutical and nutraceutical applications, a deeper understanding of their chemical composition is needed. In this study, the analysis of essential oil and different solvent extract was performed with Fourier transform ion cyclotron (FT-ICR) mass spectrometry, combined with atmospheric pressure photoionization (APPI) and electrospray ionization (ESI).

### Methods

The sprout samples were collected from the Ylä-Valtimo region in eastern Finland in May. The fresh sprouts were stored in the cold room (4 °C) to avoid the loss of volatile components. The essential oil and solvent extracts were obtained by using a simple steam distillation apparatus and a continuous Soxhlet extractor with different solvents (acetone, toluene, methanol, hexane, and dichloromethane). The obtained extracts were characterized by using a 12-T Bruker solarix XR FT-ICR mass spectrometer, equipped with ESI and APPI ion sources.

### Preliminary data (results)

ESI/APPI FT-ICR MS analysis of the essential oil and solvent extracts of spruce sprouts revealed their complex chemical nature. Up to 3200 unique molecular formulae were assigned to the peaks in a single mass analysis experiment. (Figure 1) The use of both ESI and APPI provided complementary compositional insight across all the extracts and allowed identification of more than 200 compounds. The identified metabolites belong to different chemical classes: terpenes, phenolic compounds, fatty acids, amino acids, and sterols.

Negative-ion ESI preferentially ionized polar, oxygen-containing compounds like acids, phenols, and carbohydrates. Quinic acid ( $m/z$  191.05611) was the most abundant compound found in the polar extracts (Figure 2). A few examples of other acids ionized by ESI were shikimic acid, gallic acid, salicylic acid, and cinnamic acid. Another class of compounds present were nitrogen-containing compounds such as amino acids and piperidine alkaloids. These compounds were preferentially detected with positive-ion ESI. The identified piperidine alkaloids included pinidinone, 1,6-hydropinidine, pinidinol, and dehydropinidinone. These compounds were abundant in the water extract.

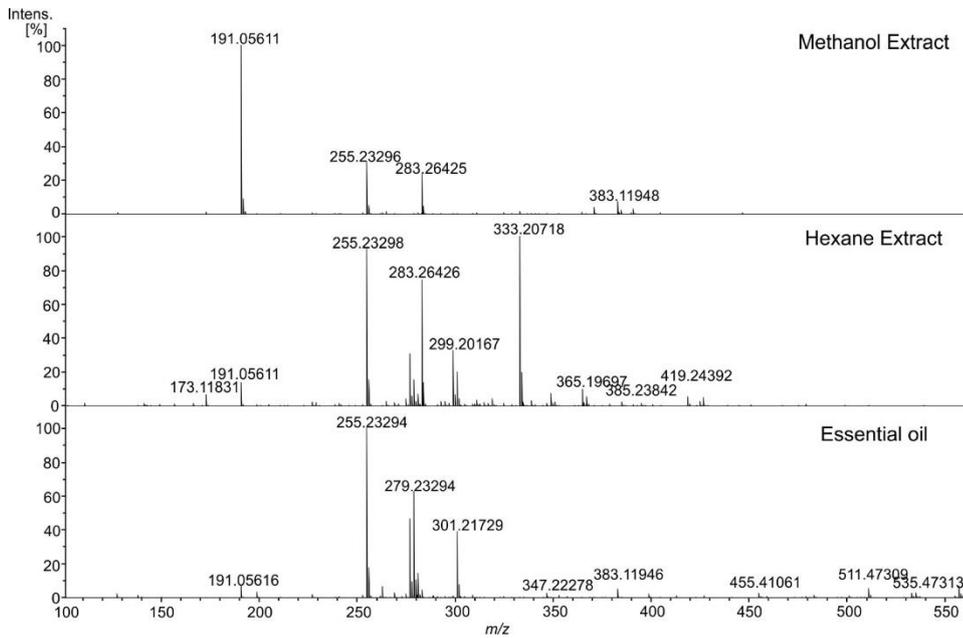
Positive-ion APPI allowed detection of less polar compounds like neutral lipids, phenolics, and unsaturated hydrocarbons. The spruce sprout essential oil was highly enriched with monoterpenes and sesquiterpenes while the lipophilic extracts were dominated by diterpenes and resin acids. Flavonoids, such as catechin and kaempferol, were quite abundant in the hydrophilic extracts. Sterols were another class of compounds efficiently ionized by APPI.

### Please explain why your abstract is innovative for mass spectrometry?

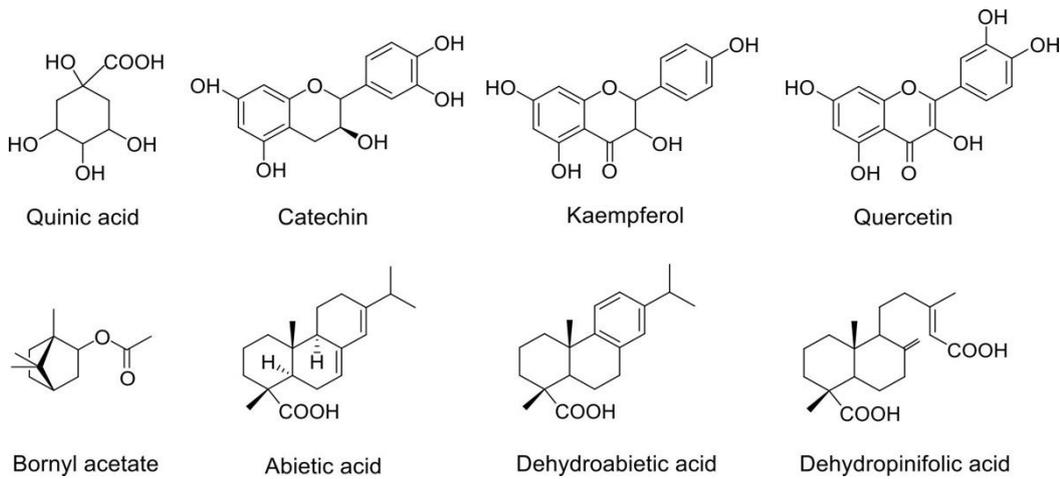
The use of direct-infusion high-resolution FT-ICR MS coupled with ESI and APPI provided complementary compositional information about the rich chemistry of spruce sprout extracts.

### Co-authors:

*Marko Mäkinen, University of Eastern Finland**Janne Jänis, University of Eastern Finland*



Negative-ion ESI mass spectra of some selected spruce sprout extracts.



Structures of some compounds found in the spruce sprout extracts.

Poster number: FP-PB-045

## LC-MS BASED ANALYSIS OF FATTY ACID AND OXYLIPIN PATTERN IN OMEGA-3-PUFA SUPPLEMENTS

Abstract ID: 605

**Presenting author: Elisabeth Koch, Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal**

### Introduction

Numerous studies show that a sufficient supply with the long-chain polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) is essential for human health. It reduces the risk of coronary heart disease, enables optimal brain and visual function, and their oxidation products are believed to have anti-inflammatory effects. Thus, the German Nutrition Society and the American Heart Association recommend a daily intake of at least 250 mg EPA and DHA by consuming a minimum of 1-2 servings of (oily) fish per week or by using omega-3-PUFA supplements. However, to date reports about the accuracy of EPA and DHA declaration and primary oxidation state of these supplements are inconsistent and new quality parameters beyond peroxide values are needed.

### Methods

We developed a combined LC-MS based method for simultaneous quantification of precursor fatty acids and their oxidation products from one sample [Ostermann/Koch, Prostag Oth Lipid M (2020) 146; Koch, Anal Bioanal Chem (2021) 413]. The sample preparation is simple and comprises alkaline saponification of n3-rich oils diluted in *iso*-propanol, analysis of fatty acids directly from the hydrolysate and subsequent extraction of oxylipins by solid phase extraction. The optimized chromatographic separation and MS detection using scheduled selected reaction monitoring (SRM) mode allows the analysis of > 200 oxylipins and 41 fatty acids.

### Preliminary data (results)

Analysis of oxylipins and their precursor fatty acids can be performed from one sample without additional sample preparation using two LC-MS based approaches. Simple cleavage of esterified fatty acids and purification by solid phase extraction for oxylipins makes both accessible to LC-MS analysis. MS detection of the low fragmenting fatty acids was a challenge that could be solved by using *pseudo*-SRM. The selective determination of free fatty acids in presence of other lipids can be achieved by LC-MS analysis. Eleven omega-3-PUFA supplements based on fish, algae and krill oil which are available over-the-counter were then examined. The determined EPA and DHA content was in line with manufacturer declaration – however, the overall fatty acid profile was diverse. It appears technically possible to achieve an oxidation rate as well as a free fatty acid content of < 0.1% in refined oils, whereas significantly higher values were present in unrefined krill oil. Interestingly, algae oil had high concentrations of the terminal hydroxylation products of EPA and DHA (20-HEPE, 22-HDHA), which were low in the other oils. Our results show that LC-MS based fatty acid and oxylipin analysis i) is relevant for assessing the quality of the supplements, ii) provides information on the production of the oils, as well as iii) allows an evaluation of authenticity.

### Please explain why your abstract is innovative for mass spectrometry?

- *Pseudo*-SRM as valuable tool for low fragmentating molecules.
- New sample preparation allowing simultaneous quantitative analysis of free and total fatty acids as well as oxylipins.

### Co-authors:

Nadja Kampschulte, Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal  
Nils Helge Schebb, Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal

Poster number: FP-PB-046

## DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF CONTAMINANTS OF EMERGING CONCERN IN TREATED WASTEWATER

Abstract ID: 610

**Presenting author: Eirini Andreaidou, Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia, Jožef Stefan International Postgraduate School, Ljubljana, Slovenia**

### Introduction

Water availability and management issues are evidenced in the modern era as they are directly related to population growth, climate change and global warming. Moreover, urgent actions are considered important to deal with the large amounts of wastewater and sludge, by-products of waste water treatment. Thus, treated wastewater (TWW) and treated sludge (TS) reuse for plant irrigation and fertilization, respectively, constitute practices towards improved resource management and circular economy. Although specific parameters are set for wastewater quality for agricultural irrigation (e.g., fecal coliforms, BOD, COD, TSS, turbidity,...) and sludge disposal to agricultural land (pH and heavy metals), regulations regarding organic contaminants of emerging concern (CECs) remain unregulated in both cases. Such compounds include pharmaceuticals, pesticides, personal care products and industrial chemicals.

### Methods

This poster describes the development and validation of an analytical method for determining 32 model CECs (pharmaceuticals, antibiotics, hormones, parabens, antimicrobials, pesticides, industrial chemicals, UV filters, musks) in treated wastewater and sludge. Sample preparation involved solid phase extraction (SPE) with Oasis Prime HLB (wastewater) and ultrasonic extraction followed by SPE (sludge) and analysed by reversed-phase liquid chromatography (LC) coupled to tandem mass spectrometry (Sciex Qtrap 4500 MS). Characterized treated wastewater and sludge will be used for irrigating tomato plants in both, pot and hydroponic experiments and experiments mimicking field conditions (e.g., in lysimeters). Innovation for mass spectrometry.

### Preliminary data (results)

The aim of this study is to understand the uptake and translocation of 32 model CECs in tomato plants (*Solanum lycopersicum* (L.)) grown and irrigated with sludge and treated wastewaters. Results presented will include a full characterization of treated wastewater and sludge.

Acknowledgements: "This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956265".

### Please explain why your abstract is innovative for mass spectrometry?

These results will serve as the basis for developing mass spectrometric methods for characterizing soil and plant tissues (fruit, stem, roots, leaves).

### Co-authors:

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Poster number: **FP-PB-047**

## **ANALYSIS OF PESTICIDE RESIDUES IN CEREALS BY A NOVEL M-SOLID PHASE EXTRACTION CARTRIDGE**

Abstract ID: **696**Presenting author: **Mario F. Mirabelli, CTC Analytics AG**

### **Introduction**

A novel micro-solid-phase extraction ( $\mu$ -SPE) extraction device was recently developed and adopted for the fully automated analysis of pesticide residues in cereals. A workflow was developed that allowed, among others, automatic preparation of calibration standards, automatic sample dilution, and automatic clean-up of sample extracts. This is expected to highly increase the analytical throughput in routine laboratories, allowing to achieve a lower cost per sample, as well as faster results and reduced analytical errors associated with human factors.

### **Methods**

The system used to evaluate the novel cartridge performance and the developed methods is gas chromatography-Orbitrap mass spectrometry (GC-Orbitrap-MS). All cereal samples were extracted with the QuEChERS method, and the resulting acetonitrile phase was used for the automated  $\mu$ -SPE clean-up. The clean-up  $\mu$ -SPE method was optimized several parameters, e.g., injection volume influence on the recovery, pH, pre- and post-elution solvent, elution speed.

### **Preliminary data (results)**

The  $\mu$ -SPE clean-up efficiency was demonstrated in the removal of matrix-interfering components and in the recovery of pesticides. The sorbents used include magnesium sulfate as a desiccant agent, primary-secondary amine, C18 and graphitized carbon black. The method removed more than 70% of matrix components for five types of cereals (barley, oat, rice, rye, and wheat). The clean-up method was validated for 170 pesticides in rye, 159 pesticides in wheat, 142 pesticides in barley, 130 pesticides in oat, and 127 pesticides in rice. For all pesticides, spike recovery values were between 70 and 120%, and the repeatability was less than 20% (relative standard deviation). The limits of quantitation achieved were  $0.005 \text{ mg kg}^{-1}$  for almost all analytes, ensuring compliance with the maximum residue limits. Additional sorbents are currently being investigated to achieve maximum retention of matrix interfering compounds, yet maximizing the recovery of the target analytes.

### **Please explain why your abstract is innovative for mass spectrometry?**

This automated method is a convenient and effective solution for the cleanup of complex sample mixtures, that could be a replacement of manual dispersive SPE approaches prior to MS analysis.

### **Co-authors:**

*Elena Hakme, Denmark Technical University, DTU-Food, Analytical Food Chemistry Group*  
*Ederina Ninga, Denmark Technical University, DTU-Food, Analytical Food Chemistry Group*  
*Thomas Preiswerk, CTC Analytics AG*  
*Mette Erecius Poulsen, Denmark Technical University, DTU-Food, Analytical Food Chemistry Group*

Poster number: FP-PB-048

## FOODTRACK – STABLE ISOTOPE TOOL FOR DETERMINATION OF AUTHENTICITY AND TRACEABILITY OF FOOD

Abstract ID: 706

Presenting author: Cathrine Terro, Jožef Stefan International Postgraduate School

### Introduction

Stable isotope-ratio signatures ( $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{34}\text{S}$ ) play an increasingly important role in food forensics in three main areas of application: (i) detection of adulteration; (ii) assignment of geographical origin; and (iii) identification of mode of production, i.e. organic vs conventional farming systems. However, a common requirement in food authenticity and traceability studies is the need for a product reference database. This presentation deals with developing the FoodTrack database, which is organized to enable further multivariate data analysis, GIS applications, and modelling.

This presentation focuses on Slovenian milk and the use of stable isotopes and elemental composition to study the effect of year, season and region of production and verify the labelling on the Slovenian market [1].

### Methods

Four different ways of evaluating the results will be presented: (i) FoodTrack data visualization in a graphical mode (maps) that enable the users to see and understand trends, outliers, and patterns in the dataset; (ii) clustering - an unsupervised data mining approach to group samples according to the year and season of production; (iii) discriminant analysis to differentiate milk according to the year, season and region of production and (iv) driven soft independent modelling of class analogy (DD-SIMCA) to verify the declaration of commercial Slovenian milk samples.

### Preliminary data (results)

It was possible to discriminate milk samples according to the year, season and production region using discriminant analysis (DA) with an overall temporal prediction variability of 84.6% and 56.4% for regional differences. Prediction ability was the highest for the Pannonian (82.1%) and lowest (26.9%) for the Alpine region. The results revealed the mislabeling of three Slovenian milk products.

This approach will serve as the basis for the further development of isotopic mapping (isoscaples), providing a cost-effective extension to the isotopic dataset approach.

### References

[1] Potočnik, D., Nečemer, M., Perišič, I., Jagodic Hudobivnik, M., Mazej, D., Camin, F., Eftimov, T., Strojnik, L., Ogrinc, N. Geographical verification of Slovenian milk using stable isotope ratio, multi-element and multivariate modelling approaches. *Food Chemistry*. 2020, 326, 126958-1-126958-11. DOI: 10.1016/j.foodchem.2020.126958

### Please explain why your abstract is innovative for mass spectrometry?

Developing the FoodTrack database, stable isotope tool for determination of authenticity and traceability of food, which is organized to enable further multivariate data analysis, GIS applications, and modelling.

### Co-authors:

Robert Modic, Jožef Stefan International Postgraduate School  
Andraž Simčič, Jožef Stefan International Postgraduate School  
Matevž Ogrinc, Jožef Stefan International Postgraduate School  
Tome Eftimov, Jožef Stefan International Postgraduate School  
Doris Potočnik, Jožef Stefan International Postgraduate School  
Barbara Koroušič Seljak, Jožef Stefan International Postgraduate School  
Nives Ogrinc, Jožef Stefan International Postgraduate School

Poster number: **FP-PB-049**

## **MEASUREMENT OF POST HARVEST QUALITY OF FRUITS USING A PORTABLE MASS SPECTROMETRY DEVICE**

Abstract ID: 711

**Presenting author: Marc Claesen, Aspect Analytics NV**

### **Introduction**

Food processing supply chains are monitored to ensure safety and quality parameters such as freshness, moisture, and contamination (pesticides, bacteria, etc). This is typically conducted via random sampling of batches and testing by independent laboratories. This process, however, requires significant time and costs and hampers traceability due to the long distance between sampling and test results. Portable mass spectrometers could provide a valuable alternative for real-time, in situ food monitoring.

We have developed a handheld mass spectrometry device with atmospheric pressure rapid sampling inlet for detection and evaluation of samples in their native form (solid, liquid, or gas). This device was used to investigate post-harvest quality of fruits and vegetables based on the presence of ethylene, alcohols and esters in the gasses emitted during storage.

### **Methods**

Our hand-held device consists of a novel sampling inlet based on arrangements of silicone membrane with the heater to enhance its pervaporation, a portable mass spectrometer enclosed in a lightweight vacuum chamber. This portable device was used to measure/analyse samples in ambient conditions from containment facilities in real-time without sample preparation. The samples are measured by directly placing the sampling inlet in the containment area. The resulting mass-spectral data are processed using custom Python-based software, where the resulting regression model reports scoring of sample ripeness directly on the device.

### **Preliminary data (results)**

Approximately 100 samples containing volatiles emitted from stored fruits were analyzed using the portable mass spectrometer. A mass spectrum was generated for each sample, with a mass range of 1-200 m/z and a 1 m/z bin size. Each sample was given a score of 0-10 on ripeness of the originating fruit, with 10 being the highest, along with a timestamp. Data was further processed using a custom pipeline developed in Python.

As an initial step, various unsupervised data analysis algorithms were used to provide an initial explorative analysis of the data. NMF and PCA were used to investigate underlying trends and identify potential outliers. K-means clustering was used to discover groupings in the provided samples. Initial analyses showed clear trends in grouping along ripeness scores.

Next, relevant peaks were extracted for ethylenes, alcohols and esters known to be indicative of fruit ripeness. For each peak, univariate correlation between time after harvest and ripeness was investigated. Furthermore, a multivariate regression model based on these selected peaks was constructed, showing good predictive power for fruit ripeness. Cross validation was used to prevent overfitting of the model. Finally, the resulting regression model score was then converted into a visual cue for easy interpretation by non-expert users (sample ripeness scores), which is displayed on the device together with concentrations of relevant compounds.

### **Please explain why your abstract is innovative for mass spectrometry?**

We demonstrate a portable and user-friendly mass spectrometer for use in detecting volatile compounds emitted from fruit. This device can be used to assess food quality in real-time.

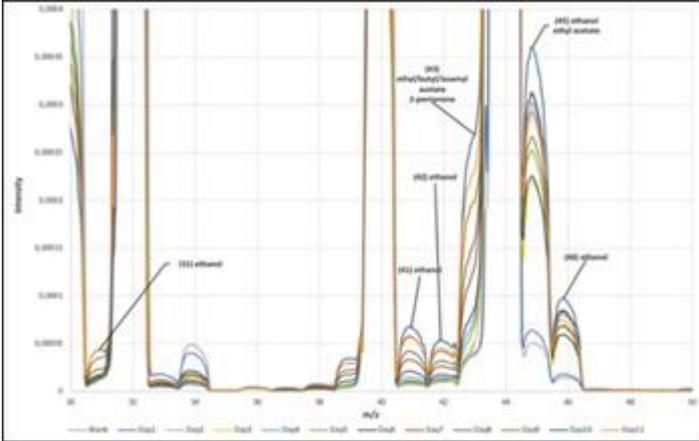
### **Co-authors:**

*Geethu Joseph, Next Generation Sensors BV*  
*Alice Ly, Aspect Analytics NV*

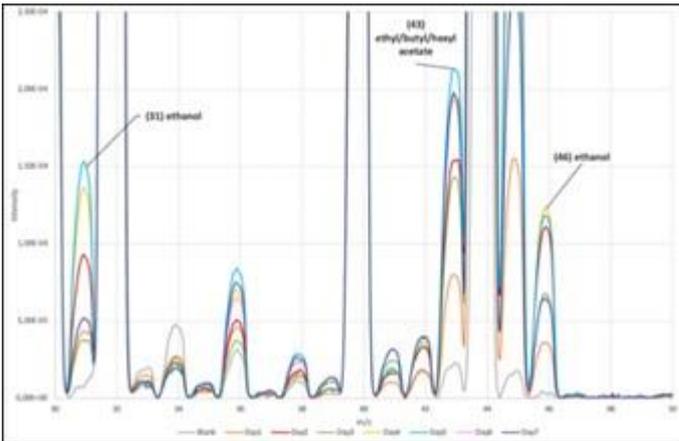
**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

Yiyuan Lin, Next Generation Sensors BV  
Sarfarazuddin AH Syed, Next Generation Sensors BV  
Nico Verbeeck, Aspect Analytics NV



Spectrum measuring volatiles in ripening bananas using portable mass spectrometer.



Mass spectrum showing various ripening compounds in pears.

Poster number: **FP-PB-050**

## **AUTHENTICATION OF VALUABLE WINE SAMPLES BY ION-SUPPRESSION PROFILING**

Abstract ID: **719****Presenting author: Christian Berchtold, FHNW**

### **Introduction**

Wine fraud is a significant problem for expensive wine traded at auctions. Up to 50% of wine traded in China (Ambler, Forbes 2017) and about 20% worldwide (Drew, The Wine Wankers, 2017) appears to be faked. There are many analytical possibilities to classify and identify wine by elemental analysis, fingerprinting of organic compounds, IR or NMR (Louis Gougeon, Food Analytical Methods 2019). However, all these techniques are laborious and need sophisticated instrumentation and personnel. As an alternative new method, the ion-suppression profiling, is introduced. This approach is based on the suppression of 29 reference markers, which are analyzed in a standard ESI-MS instrument and allow an analysis time of less than one minute per sample with very little sample preparation.

### **Methods**

30  $\mu$ L of wine was diluted in 500  $\mu$ L water-ethanol (15%) and spiked with 29 reference compounds. The compounds used as references are common compounds such as naproxen or bradykinin, which are usually not detected in wine. Flow injection was performed on an Agilent 6406 triple quadrupole as well as on several other single quadrupole instruments. The profile of peak intensities for each reference was used to build a model to distinguish the different wine samples. The models were based on LDA, KNN or neuronal networks. Several sets of wine samples were analyzed to investigate the model performance and robustness.

### **Preliminary data (results)**

In an initial experiment 31 wine bottles (28 different wine samples) were analyzed. Figure 1 shows the 2-dimensional PCA analysis of this experiment. All wine samples were already well separated, whereas the similar bottles overlapped. However, methodical drifts showed that quality controls are needed to optimize the performance and robustness of the models. Therefore, the method was further optimized and additionally tested with a set of 40 wines. The models of this experiment show a correct classification of 98.2% (LDA), 92.7 (KNN) and 99.1% (neuronal network) without mathematical correction, which is already acceptable for many cases. Injection CV appears the most critical parameter to get maximum performance of the classification independently of the model used. This value should be below 2% for the markers in average. However, a correction algorithm based on a set of 6 quality control wines, pushed the quality of identification to 100 % within this experiment, independently of the model used. Further experiments show that the concept performs well on several mass spectrometry platforms and is sufficiently robust if quality controls are used. Nevertheless, long-term stability and platform independence needs to be further investigated.

### **Please explain why your abstract is innovative for mass spectrometry?**

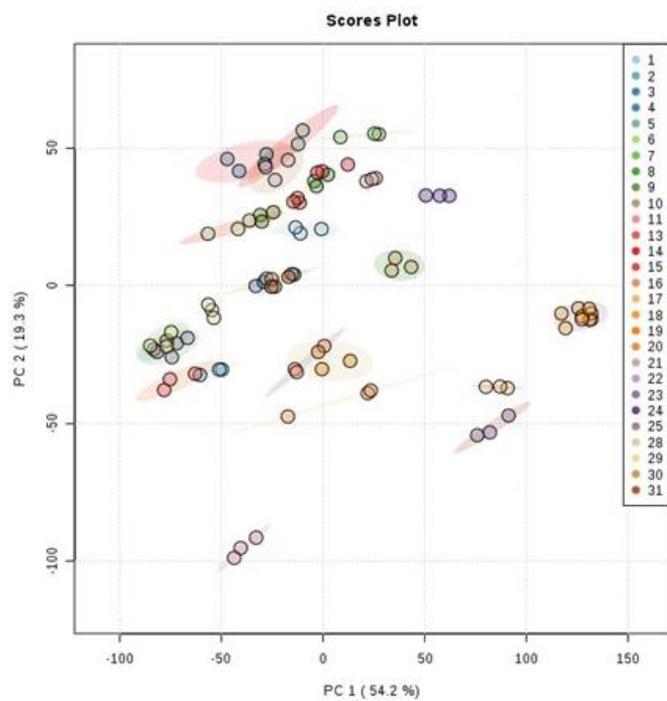
Ion-suppression profiling mass spectrometry, which is capable to identify a variety of beverages with very little effort (Patent WO 2020/229346 (A1)) is Introduced here.

### **Co-authors:**

*Frank Dieterle, FHNW*  
*Timm Hettich, FHNW*  
*Markus Ehrat, Orvinum*  
*Götz Schlotterbeck, FHNW*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



2-dimensional PCA of 31 wine bottles (28 different samples)

Poster number: FP-PB-051

## MASS SPECTROMETRY IMAGING DISCLOSED SPATIAL DISTRIBUTION OF PHYTOCHEMICALS IN RESPONSE TO MYCOTOXIN ACCUMULATION IN PIGMENTED WHEAT

Abstract ID: 730

**Presenting author:** Laura Righetti, Department of Food and Drug, University of Parma, Parma 43124, Italy

### Introduction

Pigmented wheat (*Triticum aestivum* L.) has recently gained attention for their high content in bioactive compounds, mainly phenolic acids, anthocyanins and polyphenols, that are responsible for the grain characteristic colors (blue, black, purple or red). The consumer interest in these types of colored wheat varieties is growing being considered as good sources of bioactive phytochemicals. On the other hands, in planta, the contribution of these secondary metabolites in multiple defenses roles, is mostly related to an antioxidant behavior and to the regulation of ROS-induced signaling cascades.

Anthocyanin's localization has been traditionally investigated by analyzing their content in roller-milled and pearling fractions, while in this work we aimed at optimizing their fine tissue localization using Mass Spectrometry Imaging (MSI).

### Methods

Samples from twelve varieties of pigmented bread wheat (red, black, purple and blue types), grown in Cigliano (NW Italy) were collected over two harvesting years (2019 and 2020). Three biological replicates of each sample were considered. Targeted quantification of mycotoxins and pigments was conducted following solid-liquid extraction and UHPLC-MS/MS analysis. Furthermore, transversal cross-sections obtained from the middle of the grains were analyzed using DESI XS mounted on Q-TOF mass spectrometer to visualize endogenous molecules such as anthocyanins spatial distribution.

### Preliminary data (results)

Fourteen anthocyanins, including cyanidin, pelargonidin and peonidin aglycones and glycosides, were identified and quantified. The total anthocyanins content (TAC) was found to be significantly different between the cultivars, reporting the highest TAC for black varieties (9488  $\mu\text{g}/\text{Kg}$ ) and the lowest content for red grains (209  $\mu\text{g}/\text{Kg}$ ). Our data also revealed a significant influence of the grain colour on the mycotoxin (DON, DON3Glc, AcDON, T2, HT2, ZEN, EnnB, CIT) content ( $p=0.000$ ) with the blue grain being the more contaminated (5352  $\mu\text{g}/\text{Kg}$ ) and the red grain the one accumulating less mycotoxins (715  $\mu\text{g}/\text{Kg}$ ). However, no significant correlation was found between mycotoxins accumulation and anthocyanins content. On the other hands, information on the spatial distribution of colored pigments revealed differences in the kernel outer layers localization. The accumulation of these pigments in the outer aleurone layer is considered a recently evolved trait, resulting from environmental adaptation serving as a plastic mechanism for the protection against biotic stressors, including pathogenic fungi.

The use of MSI will enable to correlate the distribution of bioactives with the accumulation of natural contaminants, such as mycotoxins, at the tissue level and get new insight into the local plant defence mechanism mediated by these phytochemicals.

### Please explain why your abstract is innovative for mass spectrometry?

Here, we reported for the first time the spatial localization of phytochemicals in colored grains using DESI MSI.

### Co-authors:

Marco Gozzi, Department of Food and Drug, University of Parma, Parma 43124, Italy

Chiara Dall'Asta, Department of Food and Drug, University of Parma, Parma 43124, Italy

Massimo Blandino, Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco 10095, Italy

Emmanuelle Claude, Waters Corporation, SK9 4AX Wilmslow, United Kingdom

Poster number: FP-PB-052

## APPLICATION OF COMPUTATIONAL TOOLS IN METABOLOMICS OF STINGLESS BEE HONEY ENDEMIC FROM SOUTH AMERICA

Abstract ID: 813

**Presenting author: Luiz Feitosa, Núcleo de Pesquisas em Produtos Naturais e Sintéticos (NPPNS), School of Pharmaceutical Sciences of Ribeirão Preto (FCFRP), University of São Paulo Av. do Café s/n (USP).**

### Introduction

Honey is a natural product from the floral nectar collected and chemically modified by bees. Stingless bees (Apidae) are found in tropical and many subtropical regions of the world. They play an ecological role as pollinators of many wild plants and are important to honey production. Brazil presents a great biodiversity of stingless bees, encompassing about 87 endemic species, which was corresponding to 20% of the estimated Neotropical species. The *Tetragonisca angustula* (popularly know as "Jataí") honey has higher biological activities, unique organoleptic characteristics and higher added value compared to traditional *A. mellifera* honey. In this way, this species can be an excellent model for pioneers metabolomics studies of Stingless bees endemic to South America.

### Methods

The methanol fractions of "Jataí" honeys were concentrated to dryness in SpeedVac, dissolved with methanol: formic acid 0.1 % and analyzed by HPLC-ESI-HRMS/MS, with a qTOF analyzer. HPLC analyses were performed in C18 column and mobile phase was water (A) and acetonitrile (B), both with formic acid 0.1% (v/v). MS/MS data was uploaded to web-based and free-access platform GNPS (Global Natural Products Social Molecular Networking) and then submitted to *in silico* tool NAP (Network Annotation Propagation). Annotations considered only molecular formulas with errors less than 10 ppm.

### Preliminary data (results)

It was verified annotations of many classes of metabolites, highlighting the occurrence of flavonoids, such as congeners of methoxylated flavones, and amine derivatives (Fig 1). Antioxidant, anti-inflammatory and antimicrobial properties were previously reported to some methoxylated flavones found in this work. These findings suggest the potential activities of stingless bee honey. In addition, the antimicrobial effects suggest that flavonoids may be important to protection of bee colonies against microbial infection. Amine derivatives, such as sphingosine and phenolamides, were previously found in samples pollen. Then, these compounds may be diffused from pollen to honey, suggesting that chemical diversity of bee products are related to botanical origin. Moreover, previous studies reported that phenolamides presented insecticide activities, suggesting the relevance of these compounds to chemical protection of plants against herbivorous insects. Therefore, this study allowed the increase of chemical diversity in stingless bee honeys, as well potential bioactive compounds. This work also opens perspectives for expanding knowledge about chemical interactions between bees, plants, and their environments.

### Please explain why your abstract is innovative for mass spectrometry?

This is the first study of honey natural products annotations from the stingless bees "Jataí" by mass spectrometry using the *in silico* tool NAP (Network Annotation Propagation).

### Co-authors:

Juliana Feres, Núcleo de Pesquisas em Produtos Naturais e Sintéticos (NPPNS), School of Pharmaceutical Sciences of Ribeirão Preto (FCFRP), University of São Paulo Av. do Café s/n (USP). , Heborá: Abelhas do Brasil, Av. Dra. Nadir Águiair . 1805, Prédio 2. Bairro Jd. Dr. Paulo Gomes Romeo Ribeirão Preto. SP . CEP 14056-680, Brazil  
Laila Salmen Espindola, Universidade de Brasília, Laboratório de Farmacognosia, Campus Universitário Darcy Ribeiro, 70910-900, Brasília, DF, Brazil.  
Thais Guaratini, Heborá: Abelhas do Brasil, Av. Dra. Nadir Águiair . 1805, Prédio 2. Bairro Jd. Dr. Paulo Gomes Romeo Ribeirão Preto. SP . CEP 14056-680, Brazil

Poster number: FP-PB-053

## EXPLOITING SELF-ASSOCIATION FOR THE EVALUATION OF ENANTIOMER COMPOSITION BY CYCLIC ION MOBILITY-MASS SPECTROMETRY

Abstract ID: 819

Presenting author: Emma Marsden-Edwards, Waters Corporation

### Introduction

The characterization of enantiomers is an important analytical challenge in the chemical and life sciences. Thorough evaluation of the purity of chiral molecules is required particularly in the pharmaceutical industry where safety concerns are paramount. Assessment of the enantiomeric composition is still challenging and time-consuming meaning that alternative approaches are required. In this study, we exploit the formation of dimers as diastereomeric pairs of enantiomers to affect separation by high resolution cyclic ion mobility-mass spectrometry. Using the example of (*R/S*)-thalidomide, we show that even though this is not an enantiomer separation, we can determine which enantiomer is in excess and obtain quantitative information on the enantiomer composition without the need for a chiral modifier.

### Methods

All experiments were performed using a cyclic ion mobility-enabled quadrupole time-of-flight mass spectrometry system. For high resolution multipass cyclic ion mobility analysis the travelling wave pulse height was set to 12 V, with a velocity of 375 m/s. The number of passes was varied by choosing appropriate 'separate' times in the instrument control software. Pre- and post-mobility voltages were minimised to preserve the dimer complexes throughout the instrument. Thalidomide solutions were introduced into the mass spectrometer by direct infusion at concentrations of 100 micromolar to promote dimer formation. NaCl was added to a final concentration of 1 millimolar.

### Preliminary data (results)

We performed multipass cyclic ion mobility experiments on quadrupole-isolated dimers of thalidomide in the form of  $[2M+H]^+$  and  $[2M+Na]^+$  from a racemic mixture. Both ions exhibited two features in the arrival time distributions (ATDs). Pure (*R*) and (*S*) thalidomide solutions exhibited ATDs with only single features which were identical and aligned with the most mobile feature from the ATD of the racemic mixture. This indicated that the racemic thalidomide dimers are in fact diastereomeric pairs of enantiomers with *R,R*, *S,S* (high mobility) and *R,S* and *S,R* (low mobility) configurations. Altering the ratio of (*R*) and (*S*) thalidomide in the mixtures lead to corresponding changes the ratio of the ATD features. This observation suggested that the mobility-separated  $[2M+H]^+$  and  $[2M+Na]^+$  dimers of thalidomide might be used to determine the enantiomer ratio (akin to enantiomer composition or enantiomeric excess) of the starting solutions. Indeed, a theoretical framework was developed to allow the direct determination of enantiomer ratio using the cyclic ion mobility data.

### Please explain why your abstract is innovative for mass spectrometry?

Determination of enantiomer ratio using ion mobility-mass spectrometry without the need for a chiral modifier.

Poster number: FP-PB-054

## METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF ANTIBIOTICS AND VETERINARY DRUGS IN MILK, ANIMAL FEEDS AND ANIMAL TISSUES BY QSIGHT LC-MS/MS

Abstract ID: 820

Presenting author: Jingcun Wu, PerkinElmer Inc.

### Introduction

Antibiotics and veterinary drugs are used in animal production to improve animal health. However, improper use of drugs can lead to residue violations in products and cause health risks. Regulatory agencies around the world have established maximum residue levels (MRLs) for these drugs in foods and animal feeds. To ensure food safety and enforce regulations, reliable analytical methods are needed for monitoring drugs in different sample matrices. In this study, two methods were developed for the analysis of 48 antibiotics and veterinary drugs in milk, animal feed, and animal tissue samples. The methods were validated by spiking different concentrations of analytes in sample matrices. All the analytes could be determined with LOQ below the MRLs by QSight LC-MS/MS.

### Methods

Samples were ground and homogenized before use. Due to different properties of analytes, two methods were developed and validated: For polar analyte analysis, such as aminoglycoside antibiotics in milk, a mixed mode LC method was used to improve analyte retention and a trichloroacetic acid - acetonitrile extraction method was used to enhance analyte extraction efficiency. For less polar analytes, a reversed phase UHPLC method was used for analyte separation and an acidified acetonitrile extraction was applied for sample preparation. All analytes were measured by QSight LC-MS/MS.

### Preliminary data (results)

To evaluate method extraction efficiency (analyte recovery) and sample matrix effects, three sets of calibration curves were built for all analytes: reagent only (RO), matrix-matched (MM, by post spike), and matrix-based (MB, by prior spike). Matrix effects were evaluated by comparing the slopes of calibration curves obtained by RO with slopes obtained by MM. Significant matrix effects (up to 90%) were observed for all samples due to less sample clean-up. Analyte recovery or method extraction efficiency was studied by comparing the slopes (or responses) obtained by MB calibration with MM calibration, good enough recoveries (60 to 90%) were obtained for most analytes in all sample matrices. Method accuracy was evaluated based on the relative recovery of analytes using the MB calibration method or using the MM calibration with correction factors for extraction efficiency. Good method accuracy (75 to 120%) was obtained for all analytes in all sample matrices. The methods used simple sample preparation procedures and demonstrated high sensitivity and selectivity with good precision (RSD% < 20%) for all analytes in all samples studied.

### Please explain why your abstract is innovative for mass spectrometry?

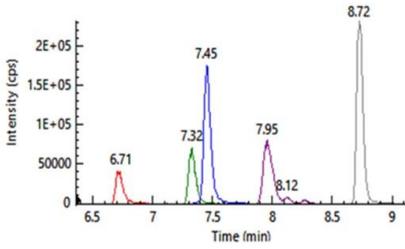
Stay-clean source for complex matrices with less maintenance; automated polarity switch provides highly sensitive detection for multiple analytes in both positive and negative modes; dual ionization source (ESI and APCI).

### Co-authors:

*Feng Qin, PerkinElmer Inc.*

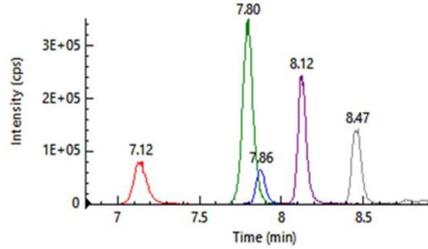
*Derek Mattern, PerkinElmer Inc.*

**(A) C18 Column**



Analyte	RT (min)
Malachite Green	6.71
Crystal Violet	7.32
Brilliant Green	7.45
Leuco Crystal Violet	7.95
Leuco Malachite Green	8.72

**(B) Epic Polar**



Analyte	RT (min)
Leuco Crystal Violet	7.12
Leuco Malachite Green	7.80
Malachite Green	7.86
Brilliant Green	8.12
Crystal Violet	8.47

Figure 1. Analysis of Drugs Using Columns with Different Selectivity

**Repeatability of the Method (181 Injections of a Spiked Chicken By-Product)**

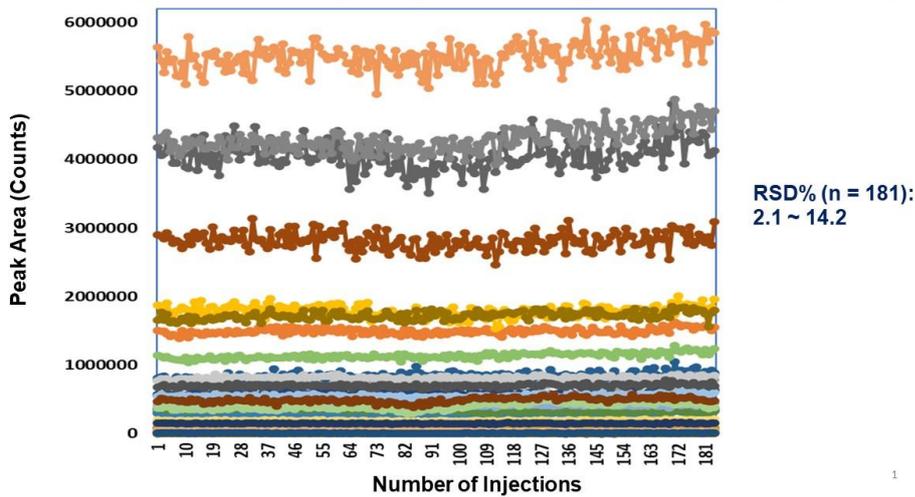


Figure 2 Repeatability of the Method (181 Injections of Samples)

Poster number: FP-PB-055

## CHARACTERIZATION OF SPARKLING WINE BASED ON POLYPHENOLIC PROFILING OBTAINED BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

Abstract ID: 829

**Presenting author: Sonia Sentellas, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain, Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain, Serra Hünter Lecturer, Generalitat de Catalunya, 08007 Barcelona, Spain**

### Introduction

Wine is a well-known and used drink obtained from the fermentation of grapes or must. The diversity and quality of the wines depend on the climate, the soil, the vineyard, its exposure to solar radiation, and the method of cultivation, which provide different organoleptic characteristics among the wine varieties. Wine is rich in polyphenols (PC) which are known mainly for their antioxidant characteristics. These secondary plant metabolites give the wine very different organoleptic characteristics such as color, taste, and smell; thus, allowing the classification of varieties based on their total content and their diversification.

### Methods

39 wine samples, belonging to different varieties and qualities, were filtered and injected into the LC-MS/MS system for polyphenol identification and quantification. A C18 column with a safety guard was used for the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis. An aqueous solution (H<sub>2</sub>O) with 0.1% formic acid (v/v) and acetonitrile were used as the mobile phase. The injection volume and flow rate were set to 5  $\mu$ L and 0.700 mL min<sup>-1</sup>, respectively. The results obtained using a targeted approach were then processed with Principal Component Analysis and Partial Least-Squares Discriminant Analysis.

### Preliminary data (results)

PCA and PLS-DA allowed the discrimination of the two wine varieties according to the type and content of PCs identified and quantified in each sample.

In fact, the content of PCs varies by sample type, while it increases or decreases linearly based on the quality. The most interesting polyphenols for the characterization and discrimination of the two types of wine are *trans*-coutaric and caftaric acids since they are present in different concentrations according to quality. In particular, the content of *trans*-coutaric acid is higher in Xarel-lo samples (about 10-13% of the total content of PCs present in the targeted list); similarly, procyanidin B<sub>1</sub> and B<sub>2</sub> are also present in a higher concentration. However, Pinot Noir is characterized by a higher content of caftaric acid (approximately 56-86% of the total content) and a higher content of acids such as *p*-coumaric, ferulic and gallic acids, and OH-tyrosol.

In conclusion, by means of LC-MS/MS analysis, it was possible to identify and quantify numerous PCs present in the samples. The subsequent processing of the data using chemometric methods made it possible to discriminate between variety and quality.

### Please explain why your abstract is innovative for mass spectrometry?

The combination of a LC-MS/MS method and different data treatment approaches allows the classification of wine based on the variety and quality.

### Co-authors:

*Eleonora Oliva, Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64100 Teramo, Italy, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028 Barcelona, Spain*  
*Aina Mir-Cerdà, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



*Barcelona, Spain, Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain*

*Javier Saurina, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, 08028*

*Barcelona, Spain, Research Institute in Food Nutrition and Food Safety (INSA), University of Barcelona, 08921 Santa Coloma de Gramenet, Spain*

Poster number: FP-PB-056

## RED COLORING CAUSED BY TWO COMMONLY USED COMPONENTS IN THE FOOD MARKET – A STRUCTURAL ELUCIDATION AND QUANTIFICATION STUDY

Abstract ID: 837

Presenting author: Waldemar Hoffmann, BASF SE

### Introduction

A growing world population and changes in dietary patterns contribute to an increased demand on nutrient-dense food. Although natural oils are widely recognized as important for balanced diet, parts of the population do not consume enough during their daily meals. The addition of oils to food or dietary supplements can close nutritional gaps. Other ingredients, including ascorbic acid and milk proteins are often added to avoid degradation of natural oils over time. However, red discoloration is sometimes observed under ambient storage conditions. Even though, red discoloration is observed in food powders with vitamin C and milk proteins in literature, so far there are only guesses on what kind of structure causes it, but no direct proof is given for the exact structure causing this phenomenon.

### Methods

We studied the red color by spray-drying a natural oil powder containing vitamin C and milk protein, and a control powder containing only vitamin C and milk protein. UV/VIS spectroscopy was used to fingerprint absorption bands of fresh and oxidized powders. LC-IMS-MS elucidated a single structure causing the red color in both powders. A red pigment based on the MS proposal was synthesized and its similarity to the observed species in the powders was confirmed. Finally, an additional robust HPLC-UV/VIS method was developed to quantify the amount of the red pigment in powders using an external calibration approach.

### Preliminary data (results)

The red pigment observed within natural oil powders is stable in the solid state. However, upon dissolving in basic or acidic water or methanol the powder quickly loses its color, indicating that the red pigment is easily hydrolyzed. The red pigment has a very low concentration in the natural oil powder. To increase the concentration of the red pigment, we produced a control sample by formulating spray-dried ascorbic acid and milk protein which was put in a pressurized oxygen chamber to increase formation of the red pigment. The control sample yields identical UV/VIS absorbance bands compared to the natural oil powder and LC-IMS-MS data show that the color originates from a single component in all samples. The molecular formula of the proposed structure is  $C_{12}H_{12}NO_{10}$  and contains a murexide-type chromophore that is likely formed through a reaction of an oxidized ascorbic acid with scorbamic acid (intermediate amino reductone of a Strecker degradation). The MS-proposed structure was retro-synthesized and compared to the original-colored powders. Identical analytical data (UV/VIS, MS/MS, HRMS, RT,  $1/K_0$  values) are obtained. Based on the proposed structure we developed an additional robust HPLC-UV/VIS method to quantify the red colored species within the powders. A worst-case scenario indicates a content of up to 50 ppm of the red pigment, while food powders start to appear reddish at concentration levels of 20 ppm.

### Please explain why your abstract is innovative for mass spectrometry?

Hyphenated mass-spectrometry approaches are used to deduce a very low abundance component that may cause a red coloring in dietary supplements and functional food powders.

### Co-authors:

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Poster number: FP-PB-057

## CLASSIFICATION AND AUTHENTICATION OF SPANISH BLOSSOM- AND HONEYDEW-HONEYS BY OFF-LINE SPE HPLC-LRMS POLYPHENOLIC PROFILING AND CHEMOMETRIC TECHNIQUES

Abstract ID: 840

**Presenting author:** Oscar Nuñez, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. E-08028, Barcelona, Spain. , Research Institute in Food Nutrition and Food Safety, University of Barcelona, E-08921, Santa Coloma de Gramanet, Spain.

### Introduction

Honey is a natural product produced by bees from nectar and other non-floral secretions. Honey is classified as multifloral or monofloral depending on the pollen content. The honey is considered monofloral if more than 45% of the pollen belongs to the same botanical species. Honeys are also classified as blossom-honeys if they are produced from the nectar of flowers, while honeydew-honeys are produced from plant secretions or sugar-rich materials that plant-sucking insects excrete. Honeydew-honeys tend to be darker and with higher total polyphenol content (leading to higher antioxidant properties). Honey is susceptible to adulteration because of the variable composition of this product regarding different conditions (botanical origin, region of production, etc.), and the similarity between many adulterants (syrup-based products) and the natural components of honey.

### Methods

136 honeys of different botanical varieties were analyzed by off-line solid-phase extraction (SPE) using HLB (60mg) cartridges to isolate and preconcentrate polyphenolic compounds. The obtained extracts were then analyzed by liquid chromatography coupled to low-resolution mass spectrometry (HPLC-LRMS). Polyphenolic profiling was accomplished in a Kinetex C18 column (100 x 4.6 mm I.D., 2.6  $\mu$ m) under gradient elution using 0.1% formic acid aqueous solution and acetonitrile as mobile phase components (0.8  $\mu$ L/min). Full-scan acquisition ( $m/z$  100-550) in a quadrupole-linear ion trap instrument with ESI(-) was employed to obtain the off-line SPE HPLC-LRMS polyphenolic profiles used as honey chemical descriptors.

### Preliminary data (results)

Off-line SPE HPLC-LRMS polyphenolic profiles obtained in negative ESI mode have proven to be excellent sample chemical descriptors to address the characterization and classification of Spanish blossom- and honeydew-honeys by partial least squares-discriminant analysis (PLS-DA) according to the different botanical varieties. Perfect discrimination between the different blossom-honeys (orange/lemon blossom, rosemary, eucalyptus, thyme, and heather) and honeydew-honeys (holm oak, mountain, and forest) was accomplished. Validation of paired PLS-DA models between the different botanical origins showed, in general, 100% classification rates, demonstrating the good capacity of the proposed methodology to assess honey authentication issues.

The proposed methodology was also able to characterize and discriminate the analyzed honey samples based on Spanish geographical regions related to climatic conditions: Cantabrian Sea region (North of Spain), Mediterranean Sea region (east of Spain), and Continental region (landlocked inland regions).

The proposed HPLC-LRMS methodology used in multiple-reaction monitoring (MRM) acquisition mode was also employed for the tentative identification of polyphenols in the analyzed chemical samples. Results showed that, in general, honeydew-honeys and heather blossom-honeys are richer in overall polyphenolic content, in agreement with their higher antioxidant capacity as described in the literature. In contrast, flavonoids predominate in blossom-honeys, with differences in the nature of the polyphenolic flavonoids depending on the botanical variety. The identified polyphenolic compounds will be studied in the future as possible honey markers for targeted authentication methodologies.

### Please explain why your abstract is innovative for mass spectrometry?

Off-line SPE HPLC-LRMS polyphenolic profiles were proposed to authenticate Spanish blossom- and honeydew-honeys, showing good classification capabilities based on both botanical varieties and geographical production region.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



**Co-authors:**

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Poster number: **FP-PB-058**

## **TRUE MOBILE MASS SPECTROMETRY FOR ON-SITE ANALYSIS**

Abstract ID: **892**

**Presenting author: Marco Blokland, WUR-WFSR**

### **Introduction**

Nowadays, we use optical sensors in our smartphones and smartwatches to measure all kinds of mostly health-related parameters. Unfortunately, their application is limited. Imagine the possibilities if you could take high-end laboratory equipment to the field. Current mass spectrometers are, at best transportable, not truly portable. What if you could fit an MS in a small backpack or carry it in your hand? In recent years, we have invested in research, leading to effective on-site MS.

### **Methods**

After evaluating the commercially available portable MS instruments, one was purchased by WFSR. This MS is fully self-sustaining in the field and consists of a battery, small gas cylinder, computer, GPS, WiFi, and gas chromatograph, and it is connected to WFSR via the cloud. Different sample inlet options are available, e.g. membrane inlet MS (MIMS), thermal desorption, and split/splitless injection.

### **Preliminary data (results)**

This truly portable MS is currently evaluated for various food applications. For example, the detection of illegal drug waste dumping (volatiles) in crop fields, manure pits, or other environmental-related questions. Also, confirmatory analysis of formulations is possible. For sample introduction, results of using a portable laser will be presented. The results of the evaluation of this MS system and related on-site sample preparation techniques for food safety-related applications will be presented.

### **Please explain why your abstract is innovative for mass spectrometry?**

On-site use of mobile mass spectrometry is rare. This study will present the applicability of a true mobile mass spectrometer and innovative sample introduction using a portable laser.

### **Co-authors:**

*Joshua Jager, WUR-WFSR*  
*Ane Arrizabalaga-Larrañaga, WUR-WFSR*  
*Paul Zoontjes, WUR-WFSR*  
*Saskia Sterk, WUR-WFSR*

Poster number: FP-PB-059

## FOOD PROFILING: IDENTIFICATION OF TRUFFLE SPECIES USING LC-ESI-IM-QTOF-MS BASED LIPIDOMICS FINGERPRINTING

Abstract ID: 914

**Presenting author: Marina Creydt, Hamburg School of Food Science - Institute of Food Chemistry, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany**

### Introduction

Truffles are one of the most expensive food in the world. The various varieties differ in their aroma and thus in their culinary value. These differences in quality are also reflected in the price, which is why it is particularly worthwhile to re-declare inferior truffle varieties to more expensive varieties. However, morphological distinction between the various species is difficult even for experts. In order to close this analytical gap or to be able to reliably detect cases of fraud, objective instrumental approaches are required. Therefore, the aim of this study was to develop a non-targeted lipidomics approach to distinguish between the white truffle species *Tuber magnatum* and *T. borchii* as well as the black truffle species *T. melanosporum*, *T. aestivum* and *T. indicum*.

### Methods

A UHPLC-ESI-IM-QTOF platform was used for non-targeted lipidomics classification of truffle species. A total of 78 black and white truffle samples were analyzed. The truffles were freeze-dried and extracted with a two-phase method containing water, methanol and chloroform. The non-polar phase was measured in the positive ionization mode. Subsequently, data were analyzed with different multivariate methods in order to extract the most relevant marker compounds suitable for classification of the truffle species in this study. In addition to the MS/MS fragment spectra, the CCS values of the compounds were also used as additional identification parameters.

### Preliminary data (results)

The non-targeted lipidomics analysis enabled the detection of more than 1,000 signals in the truffle samples. Of these, more than 400 were found to be suitable to distinguish the different truffle species. These included, for example, glycerol derivatives in addition to various phospholipids and ceramides. Among them, numerous compounds were identified whose CCS values have not yet been published and can be used as a reference by other researchers in the future. Just a few marker substances would be sufficient to distinguish the different truffle species with 100% accuracy. These results can be used for the development of simple targeted methods or rapid tests, which in the best case allow a truffle analysis directly on site.

Reference: M. Creydt and M. Fischer, Food authentication: truffle species classification by non-targeted lipidomics analyses using mass spectrometry assisted by ion mobility separation, *Molecular Omics*, **2022**, in press.

### Please explain why your abstract is innovative for mass spectrometry?

- Development of a non-targeted lipidomics approach study to detect different truffle species
- Some compounds were detected whose CCS values have not yet been published

### Co-authors:

Markus Fischer, Hamburg School of Food Science - Institute of Food Chemistry, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany

Poster number: FP-PB-060

## COMPREHENSIVE PHYTOCHEMICAL SCREENING OF COFFEE SILVERSKIN EXTRACTS BY ULTRAHIGH-RESOLUTION MASS SPECTROMETRY

Abstract ID: 982

Presenting author: Janne Jänis, University of Eastern Finland

### Introduction

Coffee silverskin (CS) is a primary coffee roasting side product with an estimated annual production of 200,000 tons worldwide. CS is rich in phytochemicals which could be exploited in various pharmaceutical, cosmetics, and food-related applications. However, it is currently weakly utilized and mainly discarded as solid waste. In this work, a comprehensive phytochemical screening of different solvent extracts of CS was performed with an ultrahigh-resolution Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry, which represents an unparalleled analytical method for non-targeted chemical fingerprinting of complex organic mixtures. Two different ionization methods, namely, electrospray ionization (ESI) and atmospheric-pressure photoionization (APPI), were used to target both polar and semi-/non-polar sample constituents.

### Methods

Coffee silverskin for this study was kindly provided by Meira roasting factory (Helsinki, Finland). A continuous solvent Soxhlett extraction was performed with the pelleted CS using the following solvents polar and non-polar solvents: methanol, ethanol, acetone, acetonitrile, water, hexane, toluene, dichloromethane, and chloroform. All the extracts were analyzed on a 12-T FT-ICR mass spectrometer (Bruker solariX-XR), using either negative-ion ESI or positive-ion APPI. Bruker DataAnalysis 5.0 software was used for the data post-processing and molecular formula assignments. Further structure annotations and statistical analysis were accomplished by using Bruker MetaboScape 5.0 software.

### Preliminary data (results)

Thousands of plant secondary metabolites, including lipids, alkaloids, and phenolic compounds, were detected and identified from the CS extracts with high-resolution, direct-infusion FT-ICR MS. The main compounds observed with negative-ion ESI included a variety of chlorogenic acids, fatty acids, monosaccharides, and their derivatives, while less polar compounds, like terpenoids, sterols, and nitrogen heterocycles (including caffeine), were observed with positive-ion APPI. Thus, ESI and APPI provided complementary compositional information for these samples. While non-polar solvents targeted mainly lipophilic compounds, polar solvents resulted in the enrichment of hydrophilic compounds, especially organic acids and nitrogen alkaloids. Therefore, by choosing an appropriate solvent, specific types of compounds can be recovered from coffee silverskin for possible further applications.

### Please explain why your abstract is innovative for mass spectrometry?

Use of two complementary ionization techniques with FT-ICR MS for a comprehensive chemical fingerprinting of complex organic samples.

Poster number: FP-PB-061

## **COFFEE CLASSIFICATION, CHARACTERIZATION AND AUTHENTICATION BASED ON PRODUCTION REGION BY TARGETED AND NON-TARGETED HPLC-HRMS METHODS AND CHEMOMETRICS. APPLICATION TO ADULTERATED COFFEE SAMPLES.**

Abstract ID: 985

**Presenting author: Nerea Núñez, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. E-08028, Barcelona, Spain.**

### **Introduction**

The quality of food products is an issue of great interest in our society. Considering the complexity of the food chain, the adulteration of food is increasing, causing food fraud cases, so it is important to ensure that all food products are healthy for human consumption. In that thematic, drinks are food products easily adulterated. This work will focus on coffee fraud detection.

Coffee has a beneficial antioxidant activity in humans given by its great number of bioactive compounds (being polyphenols the most prominent). Polyphenolic content is related to features such as the coffee variety, production region or climate, among other parameters. Hence, bioactive compound fingerprinting can be used as a source of analytical data to authenticate the origin of coffee.

### **Methods**

In the present work, high-performance liquid chromatography (HPLC) coupled to high-resolution mass spectrometry (HRMS) detection has been applied to characterize and classify coffee samples from different production regions. Non-targeted and targeted methods were proposed to obtain metabolomic and polyphenolic information from the analyzed coffee samples, respectively. A simple sample treatment procedure consisting of just brewing the coffees with water was employed. In addition, coffees of very nearby regions were adulterated in percentages of adulteration between 15% and 85%, to test the feasibility of the proposed methods to detect and quantify adulteration levels.

### **Preliminary data (results)**

The obtained data corresponding to targeted and non-targeted HPLC-HRMS proposed methods were considered as a source of potential descriptors to be exploited for the characterization and classification of the analyzed coffee samples using chemometric methods.

The plot of scores obtained after chemometric analysis by Partial Least Squares-Discriminant Analysis (PLS-DA) using HPLC-HRMS fingerprints revealed patterns that were perfectly correlated to the production regions of coffee samples. Moreover, sample classification and discrimination tend to be related to polyphenolic content.

In addition, coffee adulteration studies with partial least squares regression (PLSR) demonstrated the good capability of the proposed methodology for the detection and quantitation of the adulterant levels in coffee beverages down to 15% adulteration, with errors down to 10%.

The proposed PLS-DA methods were validated for the authentication of the production region with the aim of detecting consumer frauds in the future, showing good classification rates (higher than 90%).

### **Please explain why your abstract is innovative for mass spectrometry?**

Feasible targeted (polyphenolic profiling) and non-targeted (fingerprinting) HPLC-HRMS methods were developed, being able to authenticate coffees, and detect and quantify adulterations in coffee beverages.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

**Co-authors:**

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Poster number: FP-PB-062

## TARGET AND SUSPECT SCREENING OF CONTAMINANTS IN FISH FEEDS BY GAS AND LIQUID CHROMATOGRAPHY COUPLED TO ION MOBILITY-HIGH RESOLUTION MASS SPECTROMETRY USING ATMOSPHERIC PRESSURE IONIZATION

Abstract ID: 1010

Presenting author: Juan Vicente Sancho Llopis, University Jaume I

### Introduction

The size of the global population has experienced a steep increase and there is a necessity of ensuring an adequate and sustainable fish food supply to the population. However, an important percentage of the fish stocks are overexploited, depleted or recovering, partly due to overfishing or increasing pollution of the aquatic environment arising from human activity. Aquaculture appears to be the solution to meet the high fish demand of the population. However, aquaculture faces the challenge of sustainable development due to its dependence on meals and oils of marine origin. This situation promotes the need to search for new nutritional strategies for the production of a more profitable and sustainable diet, based on the replacement of these conventional aquafeeds with plant-based or other alternative ones.

### Methods

The introduction of alternative ingredients in feed formulations can modify the contaminants profile. First, screening of contaminants and their metabolites in final feed samples is required. In addition, analysis of the different raw materials is needed to obtain information about the pollution source. Due to the complexity of the matrices and the number of potential contaminants a comprehensive target and suspect screening strategy was developed based on LC and GC separation coupled to ion mobility-QTOF using ESI and APCI, respectively. Generic sample preparation (QuEChERS) was performed for both GC and LC determinations.

### Preliminary data (results)

18 raw ingredients and 8 feed formulations were analyzed by GC and LC-API-IMS-QTOFMS. Target screening was performed for 250 GC-amenable contaminants and around 500 LC-amenable contaminants. Moreover, in the case of LC, suspect screening was also performed with a list of 900 additional pesticides and pharmaceuticals. Several positives were found, and the benefits of the additional ion mobility separation as well as the CCS value obtained improved the confidence in the identification of the detected contaminants. CCS values can be matched against available databases or even predicted for suspects thanks to CCS prediction approaches.

### Please explain why your abstract is innovative for mass spectrometry?

Ion mobility adds an extra separation dimension to reduce the DIA mass spectra complexity improving annotation and rendering CCS values, that can be matched against CCS databases or predicted ones.

### Co-authors:

*Tania Portoles, University Jaume I*

*Maria Ibáñez, University Jaume I*

*Jaume Pérez-Sánchez, Institute of Aquaculture Torre de la Sal - CSIC*

*Jaime Nacher-Mestre, Institute of Aquaculture Torre de la Sal - CSIC*

*David Izquierdo-Sandoval, University Jaume I*

## Theme: Instrumentation and methods

### Session: Alternative dissociation methods

Poster number: IM-PB-001

## TIME-RESOLVED PHOTODISSOCIATION OF SIZE- AND CHARGE-STATE SELECTED POLYANIONIC TIN CLUSTERS

Abstract ID: 525

Presenting author: Alexander Jankowski, Institute of Physics, University of Greifswald

### Introduction

In contrast to other metals, small tin clusters ( $\text{Sn}_n^-$  of sizes  $n \leq 50$ ) are known to consist of building blocks of  $\text{Sn}_7$ ,  $\text{Sn}_{10}$  [1] and, in the case of anionic clusters,  $\text{Sn}_{15}$  [2]. These three species lead to corresponding fragmentation patterns when larger species are probed, e.g. CID [3]. These dissociation pathways have been confirmed and further investigated [4] at the ClusterTrap setup [5]. Previous findings suggested di-anionic tin clusters to fission into two mono-anionic fragments – in analogy to the case of lead di-anions [6], where fission was deduced from the fragment patterns of the clusters of different charge states. However, in the case of tin, the fragmentation patterns are not sufficient for this approach ([4] and Fig 1).

### Methods

A tin cluster ensemble produced by a laser ablation source is stored in a Penning trap. The clusters are subjected to several selection steps, isolating a single mono-anionic cluster species in the trap. This species is exposed to an electron beam, leading to electron attachment and the production of poly-anions [7]. Produced di-anionic tin clusters are excited with a nanosecond-laser pulse, causing dissociation. After a variable delay time, the new cluster ensemble consisting of precursor and reaction products is analysed by time-of-flight mass spectrometry.

### Preliminary data (results)

In continuation of previous experiments [8], time-resolved photoexcitation has recently been extended to size and charge-state selected poly-anionic tin clusters. The decay processes have been traced on a timescale ranging from a few tens of microseconds to one second. The time-resolved data allows for a reconstruction of the decay pathways of the excited clusters, confirming the presence of fission processes.

[1] C. Majumder et al., *Phys. Rev. B* **64**, 233405 (2001); H. Li et al., *J. Phys. Chem. C* **116**, 231 (2011)

[2] A. Lechtken et al., *J. Chem. Phys.* **132**, 211102 (2010)

[3] E. Oger et al., *J. Chem. Phys.* **130**, 124305 (2009); A. Wiesel et al., *Phys. Chem. Chem. Phys.* **14**, 234 (2012)

[4] S. König et al., *Eur. Phys. J. D* **72**, 153 (2018); M. Wolfram et al., *Eur. Phys. J. D* **74**, 135 (2020)

[5] F. Martinez et al., *Int. J. Mass Spectrom.* **266**, 365 (2014)

[6] S. König et al., *Phys. Rev. Lett.* **120**, 163001 (2018)

[7] S. König et al., *Eur. Phys. J. D* **72**, 153 (2018)

[8] M. Wolfram et al., *Eur. Phys. J. D* **74**, 135 (2020)

Please explain why your abstract is innovative for mass spectrometry?

Time-resolved photoexcitation has been used to characterize and disentangle the fission process of di-anionic tin clusters, highlighting the non-metal behavior of the post-transition metal as small cluster.

**Co-authors:**

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*Lutz Schweikhard, Institute of Physics, University of Greifswald*

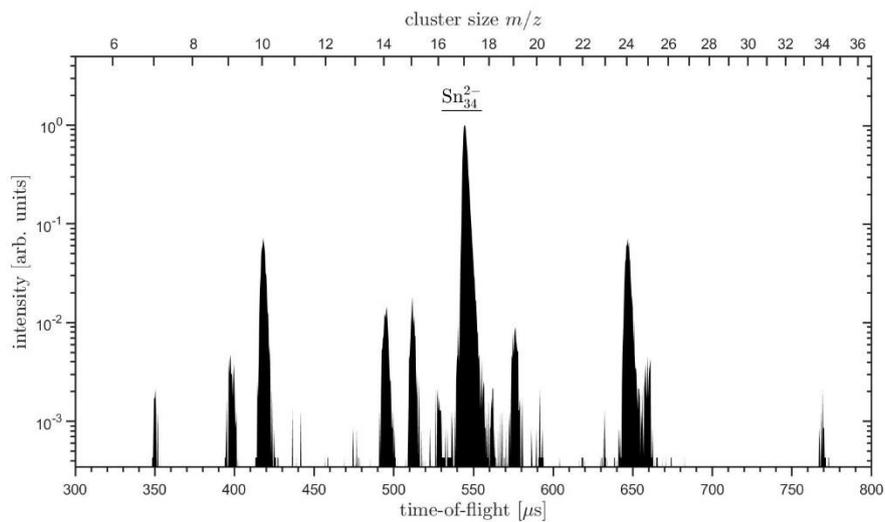


Fig. 1: Time-of-Flight spectrum of  $\text{Sn}_{34}^{2-}$  after photoexcitation.

Poster number: **IM-PB-002**

## REVEALING C-TERMINAL PEPTIDE AMIDATION BY THE USE OF THE SURVIVAL YIELD TECHNIQUE

Abstract ID: **532**

**Presenting author: Elodie Logerot, Institut des Biomolécules Max Mousseron**

### Introduction

Since the majority of C-terminal amidated peptides are bioactive, there is hence a great interest to identify and characterize them from biological matrices and natural extracts. From a mass spectrometry point of view, they are difficult to pinpoint owing to the only 1 Da mass difference between the amidated and the corresponding native carboxylated forms producing overlapping isotopic contributions of both molecular ions. To circumvent this analytical difficulty, usage of energy-resolved tandem mass spectrometry experiments and of the survival yield technique was investigated. Pair of peptides were thus dissociated in positive and negative mode according to the SY technique, in MS<sup>2</sup> and MS<sup>3</sup> experiments, in order to separate them giving a reliable MS/MS methodology to detect such post-translationally modified sequence in natural extracts.

### Methods

Energy-resolved mass spectrometry (ERMS) allows the study of gas phase ion fragmentation. By modifying the CID acceleration voltage, the energy transferred to ions and thereafter their dissociation output can be controlled leading to the opening/closing of different fragmentation paths. Collision with an inert gas (CID) is preferred since it permits a better control of transferred energy and accurate mass-selection of ions of interest, and thus a better fragmentation monitoring. For each applied voltage, a survival yield is calculated. This value corresponds to the precursor ions peak intensity divided by the intensity of all peaks present on the MS<sup>2</sup> spectrum.

### Preliminary data (results)

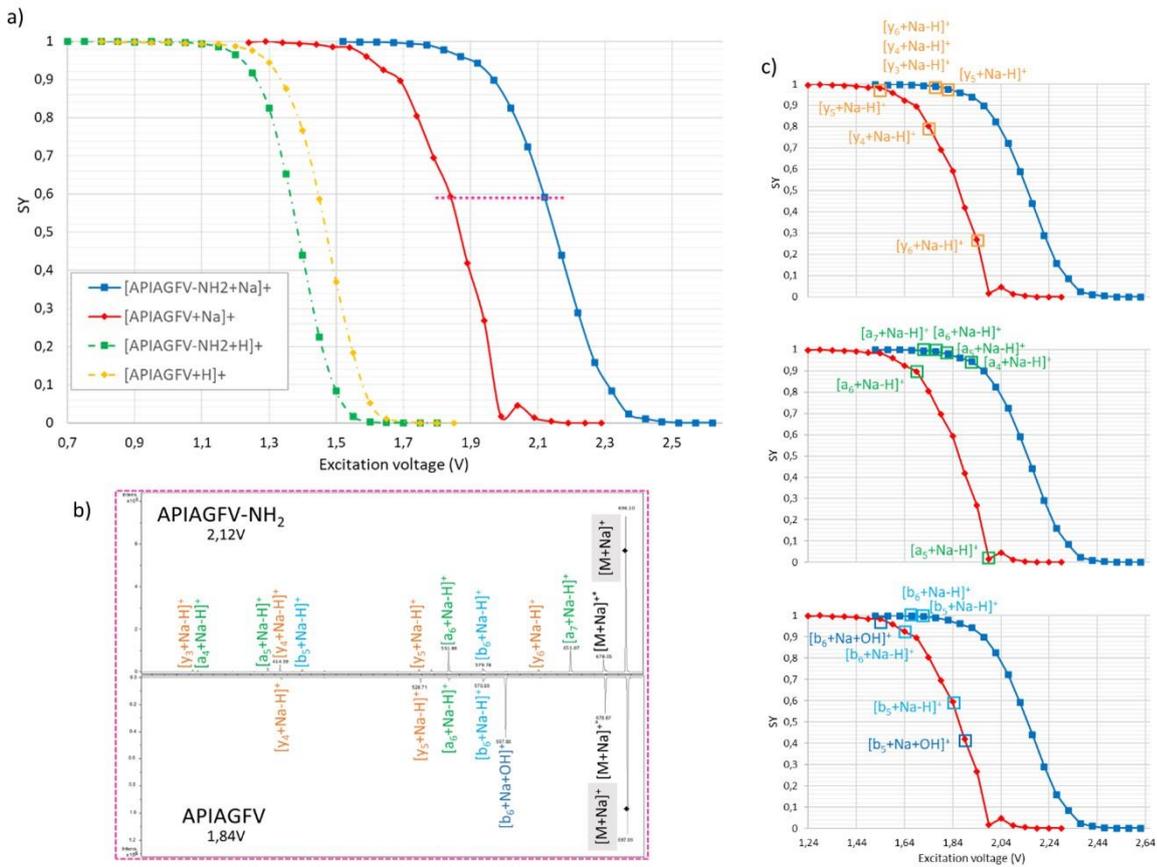
Fragmentation outputs of protonated amidated and carboxylated peptides do not differ greatly and their separation by the survival yield (SY) technique was not achievable. Thus, according to previous results (<https://doi.org/10.1021/jasms.0c00269>), the studied sequences were first cationized with sodium to further proceed to MS<sup>2</sup> experiments in order to obtain many distinct fragmentation pathways. Indeed, sodium cationization causes different fragmentation behaviors between amidated and native peptides that were found efficient enough to induce a viable separation of both SY curves. For peptide pairs in which dissociation of sodiated precursor ion MS<sup>2</sup> spectra did not separate distinctly (about 29% of all studied peptides), MS<sup>3</sup> experiments were conducted. The nature of chosen fragment ions to make these curves from MS<sup>2</sup> spectra was found very significant. Indeed, ions informing N-terminal part of peptides did not permit to differentiate the two analyzed biomolecules. Therefore, it was compulsory to select fragment ions related to the peptide C-terminal part, namely  $\gamma$ -type ions. Moreover, if the succession of these two methods do not give results, it is possible to work in negative mode by anionizing the peptides with halogens (Cl, Br, I) providing the expected SY curves separation. In that case, it is worth noticing that a shift to higher collision energies for iodine experiences was observed. The sole cases where SY curves were not separated thanks to previous experiments in the negative mode were related

### Please explain why your abstract is innovative for mass spectrometry?

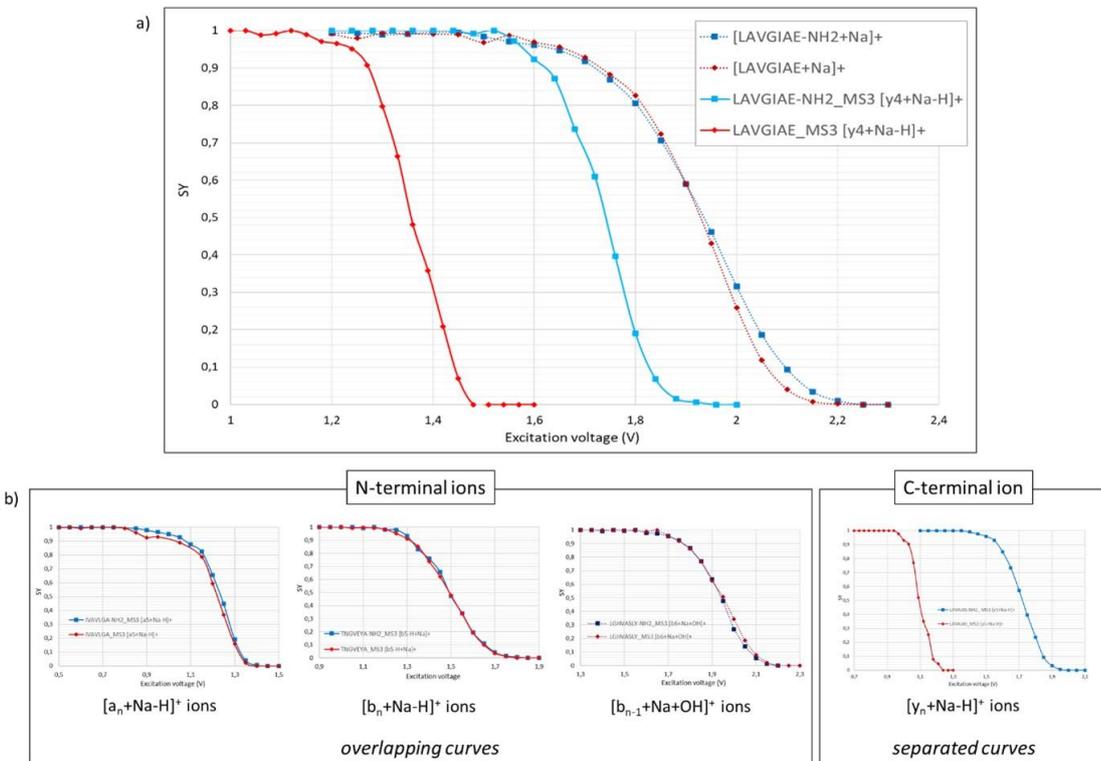
SY technique, applied from MS<sup>2</sup>/MS<sup>3</sup> positive and negative, spectra allowed reliable C-terminal amidated peptide detection which represent a post-translational modification particularly subtle to pinpoint in *De Novo* sequencing

### Co-authors:

*Guillaume Cazals, Institut des Biomolécules Max Mousseron*  
*Antony Memboeuf, CNRS-Université Bretagne Occidentale*  
*Christine Enjalbal, Institut des Biomolécules Max Mousseron*



Principle of survival yield technique applied to sodiated peptides.



Recourse to MS<sup>3</sup> experiments on different sodiated ion types.

Poster number: **IM-PB-003**

## MULTIMODAL GENERATION AND FRAGMENTATION OF PROTONATED AND RADICAL CATION PRECURSORS IONS UTILIZING DOPANT-ASSISTED MICRO-LIQUID CHROMATOGRAPHY ATMOSPHERIC PRESSURE PHOTOIONIZATION

Abstract ID: **608****Presenting author: Patrick Mueller, University of Geneva, Life Sciences Mass Spectrometry, Geneva Switzerland**

### Introduction

While dopant-assisted atmospheric pressure photoionization (APPI) has been well studied and developed for the generation of protonated ions, the potential of radical cation formation and fragmentation has been neglected and in most cases studied incidentally. As the formation of protonated ions is hypothesized to be a subsequent reaction of radical cation formation, altering the kinetic factors of corresponding gas phase reactions allows to tailor the stability of radical cations. Competing reactions strongly depend on the ionization potential and proton affinity of solvents, analytes and dopants. This can lead to a continuous decline of ionization efficiency along typical LC-gradients. Despite that, radical cation collision induced dissociation has been demonstrated to yield orthogonal fragmentation spectra but has never been studied in detail.

### Methods

43 standard compounds were analyzed by APPI using flow injection analysis or ESI using direct infusion. MS and MS/MS spectra were acquired on a QqTOF (6600 TripleTOF, Sciex, Concord, ON, Canada). Collision energy ramp spectra from 10-115V of protonated ions formed by ESI or APPI with toluene as dopant were compared to the fragmentation of radical cations formed by APPI with chlorobenzene as dopant. APPI has been coupled with  $\mu$ LC and post-column addition of methanol for information dependent acquisition (IDA) of radical cations. Acquired IDA spectra were used for NIST EI library searches and compared to  $\mu$ LC-ESI IDA spectra.

### Preliminary data (results)

Within this study we demonstrate, that the formation radical cations and protonated species can be favored by using either chlorobenzene or toluene for a variety of polar and non-polar analytes relevant for environmental, food and pharmaceutical sciences. Moreover, collision induced dissociation of radical cations leads to the formation of electron impact like spectra. Solvent-based radical cation suppression is diminished by partially decoupling ionization and chromatographic separation on a micro column by post-column addition of methanol. The molecule specific response factor for radical cations is for 24 % of the analytes better than for protonated ions formed by  $\mu$ LC-ESI. To this effect,  $\mu$ LC-APPI allows to perform information-dependent acquisition of radical cations as well as protonated ions in a sequential manner. Due to the high fragmentation efficiency of radical cations, more informative and less precursor dominated IDA spectra are formed. The orthogonality and electron-impact like fragmentation of radical cation precursor IDA spectra allows to perform additional EI library searches for increased confidence in molecular identification. Accordingly, 84 % of the analytes were identified as the most probable hit using EI library searches. The high similarity between radical cation IDA and EI spectra allows to differentiate isomeric compounds where conventional IDA spectra of protonated species fail. Despite the potential use for untargeted analysis and EI library searches, the orthogonal radical cation fragmentation fosters targeted analysis. Altogether, this allows to conclude that  $\mu$ LC-APPI-MS with post column-addition of methanol serves as an orthogonal alternative in terms of ionization and fragmentation for  $\mu$ LC-ESI with yet unseen potential.

### Please explain why your abstract is innovative for mass spectrometry?

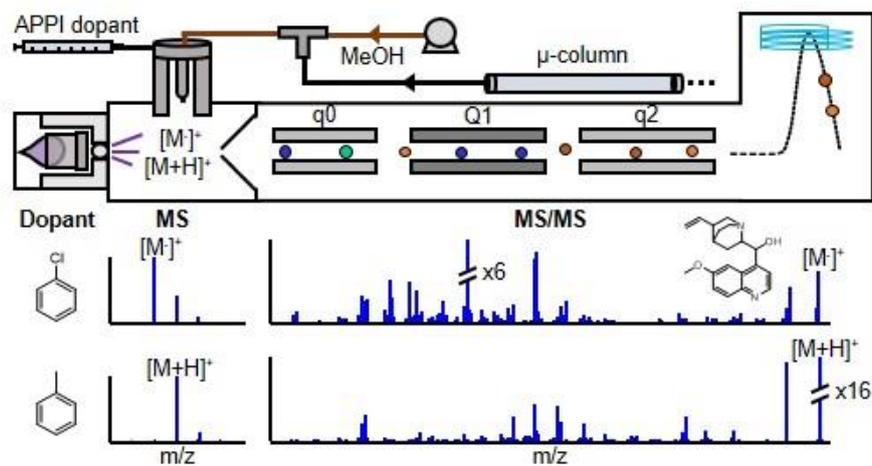
The multimodal generation of radical cation and protonated precursor ions with APPI-MS enables NIST MS/MS and EI library searches with increased confidence in molecular identification on a LC timescale.

### Co-authors:

*Gérard Hopfgartner, University of Geneva, Life Sciences Mass Spectrometry, Geneva Switzerland*  
*Ron Bonner, Ron Bonner Consulting, Newmarket, ON, Canada*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Sequential IDA fragmentation of quinidine radical cations and protonated ions

Poster number: **IM-PB-004**

## **STRUCTURAL INTERROGATION OF MYRIAD CHEMICAL SPECIES USING CYCLIC ION MOBILITY COUPLED WITH AN EXTENDED RANGE OF DISSOCIATION CAPABILITIES**

Abstract ID: **818****Presenting author: Dale Cooper-Shepherd, Waters Corporation**

### **Introduction**

Most modern mass spectrometers are equipped with the capability to perform tandem mass spectrometry analysis to probe ion structure through the generation of sub-molecular fragments. Ion mobility spectrometry-mass spectrometry (IMS-MS) has also become established in the analysis of structure as well as being utilised as a separation device. Here we describe an instrument platform that combines cyclic IMS-MS with collision-induced dissociation and IMS<sup>n</sup>, surface-induced dissociation, and electron capture dissociation. We discuss the benefits of these technologies for a range of applications.

### **Methods**

All data were acquired on a cyclic IMS-enabled quadrupole time-of-flight mass spectrometry system. The small molecules tramadol and desvenlafaxine were first separated by UHPLC before mass spectrometry analysis. The isomeric trisaccharides melezitose, raffinose, isomaltotriose and maltotriose were introduced into the instrument by direct infusion after dissolution in 70 % acetonitrile. Bovine ubiquitin and the two phosphopeptide standards RSpYpSRSR and RYpSpSRSR (Merck-Sigma) were introduced by direct infusion in a solution of 0.1 % formic acid. Streptavidin (Pierce) was buffer exchanged into 200 mM ammonium acetate for SID analysis by static nanoflow infusion.

### **Preliminary data (results)**

The cyclic IMS-enabled Q-ToF is equipped as standard with the capability to perform collision-induced dissociation (CID) both before and after the IMS device, allowing separation of either precursor or product ions. The isomers tramadol and desvenlafaxine were subjected to pre-IMS CID to probe the structures of their isomeric product ions. Clear differences were observed between the product ion mobilities, allowing discrimination of the compounds. In addition, the design of the cyclic IMS region enables users to perform sequential rounds of mobility separation or IMS<sup>n</sup>. The isomeric trisaccharides raffinose, melezitose, maltotriose and isomaltotriose were all separated by multipass cyclic IM and selected in turn for IMS<sup>2</sup>. Interrogating the mobilities of the resulting product ions revealed structural differences between the isomeric species. Furthermore, the instrument was equipped with a surface-induced dissociation (SID) device after the quadrupole, enabling structural studies of protein complexes. The pre-IMS positioning of the SID cell allowed similar m/z product ions to be separated, facilitating assignment. Finally, an optional ExD cell was installed in either the pre- or post-IMS position. Separation of pre-IMS generated ECD product ions enabled complex spectra to be assigned for topdown analysis of ubiquitin. When installed post-IMS, high resolution IMS was performed on isomeric phosphopeptides followed by ECD, enabling phosphosite localisation.

### **Please explain why your abstract is innovative for mass spectrometry?**

The mass spectrometry system has a broad suite of fragmentation options allowing detailed analysis of a wide range of species

Poster number: **IM-PB-005**

## **ULTRAVIOLET PHOTODISSOCIATION (UVPD) FOR NATURAL PRODUCT STRUCTURE CHARACTERIZATION USING ORBITRAP IQ-X TRIBRID MASS SPECTROMETER**

Abstract ID: **835**

**Presenting author: Kate Comstock, Thermo Fisher Scientific**

### **Introduction**

Mass spectrometry is one of the major techniques for natural product structure characterization. High resolution accurate mass (HRAM) allows unequivocal molecular formula determination, and higher-energy collisional dissociation (HCD) and collision-induced dissociation (CID) with multi-stage fragment ( $MS^n$ ) provide the critical fragment information for structure elucidation.

Ultraviolet photodissociation (UVPD) with high-energy photons allows access to new dissociation pathways, which can provide valuable structure insight and enhance structure characterization. UVPD provides complementary additional information for in-depth natural product and small molecule structure analysis.

This study demonstrates that UVPD spectra unique fragment ions for the selected natural product facilitated structure characterization. This feature can also be used for targeted compound screening, for example, screening toxins in clinical settings.

### **Methods**

Commercially available natural product standards were used.

The LCMS were performed on Vanquish Horizon binary UHPLC and Orbitrap IQ-X MS with UVPD source. Chromatographic separation was conducted on Accucore C18 column (2.1X50 mm, 2.6  $\mu$ m) with gradient elution: H<sub>2</sub>O/0.1% FA (A), ACN/0.1% FA /5 mM NH<sub>4</sub>FA.

MS analyses were performed using ESI source in positive mode. HRAM full scan and  $MS^n$  fragments using multiple dissociation CID, HCD, and UVPD were collected with EASY IC internal calibration. UVPD source used 213 nm from a solid-state laser.

Thermo software Freestyle and Mass Frontier were used for data processing.

### **Preliminary data (results)**

In this study, three dissociation techniques were employed to obtain fragmentation information. The following parameters were used: CID, fixed collision energy (%) 30, HCD, normalized stepped energy: 15, 30, 45, 60, and UVPD, 213 nm laser source with activation times: 25 ms, 50 ms, and 70 ms.

The data show that tribrid MS with multiple dissociations and multi-stage fragments capabilities provided a comprehensive tool set for natural product structure characterization.

The multi-stage CID fragments on a single precursor and its product ions with high mass accuracy yielded structural information and the linkage between them. The high-energy HCD and UVPD generated richer fragments. In addition, depending on the structure, the HCD and UVPD fragments were similar for some compounds, but for certain compounds, only UVPD generated meaningful fragments for structure identification.

The data revealed that for some compounds, the singly charged ions didn't fragment for either HCD or UVPD. But the doubly charged UVPD spectra yielded more fragments, and showed unique fragment(s) which are indicative of their structures.

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The study results demonstrate that UVPD fragments are complementary to HCD and CID for natural product structure characterization. For certain compounds, UVPD is the best dissociation technique to obtain structure information.

### **Please explain why your abstract is innovative for mass spectrometry?**

Ultraviolet photodissociation, as orthogonal dissociation technique, can generate unique fragment ions for selected compounds to facilitate natural product structure characterization.

### **Co-authors:**

*Yiqi Ruben Luo, School of Medicine, Stanford University*

*Brandon Bills, Thermo Fisher Scientific*

*Vlad Zabrouskov, Thermo Fisher Scientific*

Poster number: **IM-PB-006**

## **DIFFERENTIATING SUGAR PHOSPHATE ISOMERS USING HIGH RESOLUTION MASS SPECTROMETRY COUPLED WITH MS<sup>n</sup>, COLLISION INDUCED DISSOCIATION, AND ULTRAVIOLET PHOTODISSOCIATION**

Abstract ID: **941****Presenting author: Brandon Bills, Thermo Fisher Scientific**

### **Introduction**

High resolution mass spectrometry is a useful tool in differentiating molecules. With sufficient resolution, isobars and other molecules with similar molecular weights can be distinguished and characterized. However, isomers still pose an analytical challenge, especially if they cannot be resolved chromatographically. Tandem mass spectrometry offers a means to differentiate isomers that are sufficiently different in their structure by examining differences in the fragmentation spectra, but there are still isomers that are still too similar using high energy collisional dissociation (HCD) to be differentiated. In this work we use additional fragmentation options available on the Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer (MS), including MS<sup>n</sup>, collision induced dissociation (CID) and ultraviolet photodissociation (UVPD), to differentiate compounds such as sugar phosphates.

### **Methods**

An Orbitrap IQ-X Tribrid MS was run using direct infusion of standards of isomeric compounds. Data was collected up to MS<sup>4</sup> in positive and negative mode using HCD and CID fragmentations at collision energies ranging from 10 to 150 and UVPD at activation times ranging from 8 to 1000 milliseconds. Spectra were evaluated for unique fragments using Thermo Scientific™ FreeStyle™ 1.8 software and further annotated using Thermo Scientific™ Mass Frontier™ 8.0 software.

### **Preliminary data (results)**

Fragmentation data for multiple sugar phosphate isomers was collected and evaluated for unique features that could reliably differentiate each molecule. A feature was considered unique if it was found solely in the spectra for that sugar phosphate and not any of the others coeluting with it. It was found that by using MS<sup>n</sup> fragmentation in conjunction with CID and UVPD data that there were unique fragments sufficient to differentiate each of the isomers. Further work is planned to differentiate these compounds in more complex biological matrices during a LC-MS experiment. The collection of fragmentation options available on the Orbitrap IQ-X Tribrid mass spectrometer makes the instrument a powerful tool in differentiating isomeric structures.

### **Please explain why your abstract is innovative for mass spectrometry?**

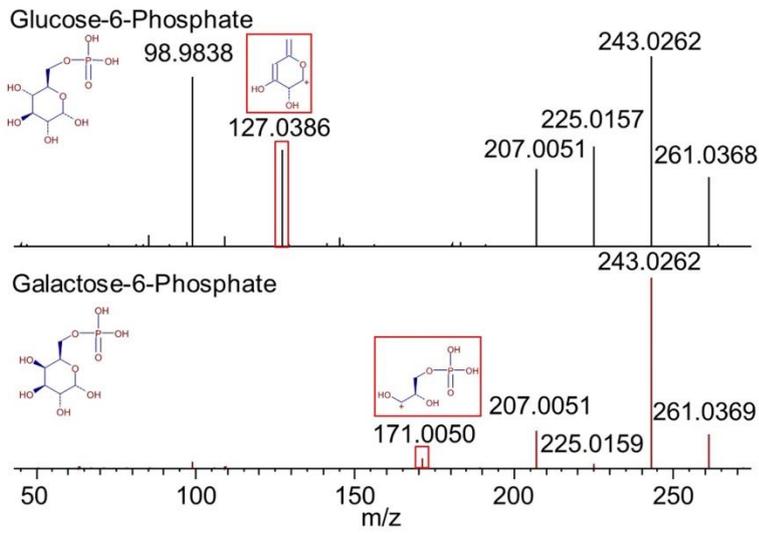
A combination of HRAM, HCD, CID, MS<sup>n</sup>, and UVPD finds unique fragments to differentiate sugar phosphate isomers.

### **Co-authors:**

*Sunandini Yedla, Thermo Fisher Scientific*  
*Rahul Deshpande, Thermo Fisher Scientific*  
*Bashar Amer, Thermo Fisher Scientific*  
*Susan Bird, Thermo Fisher Scientific*  
*Vlad Zabrouskov, Thermo Fisher Scientific*

POSTER SESSION B

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Thursday 1 September 2022 from 14:00 to 15:30 hours



UVPD fragments for glucose-6-phosphate and galactose-6-phosphate.

## **Session: Ambient technologies (and their applications)**

Poster number: **IM-PB-007**

### **BENEFITS OF A TRUE DUAL SOURCE IN DRINKING WATER ANALYSIS UTILIZING A QSIGHT® TRIPLE QUAD**

Abstract ID: **139**

**Presenting author: Derek J Mattern, PerkinElmer**

#### **Introduction**

The QSight triple quadrupole mass spectrometer from PerkinElmer has a unique feature in that it has two stand-alone ionization sources. This gives the user maximum flexibility by enabling them to switch easily between electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Moreover, there is no need to manually change anything on the instrument and no tools are required. This switch is scheduled in the MS method and the LC flow is simply diverted with a diverter valve to the chosen source. The examples presented will demonstrate two possible scenarios a user could encounter during method development when dealing with difficult compounds. These two examples are related to drinking water analysis and being able to easily test different ionization techniques will be highlighted.

#### **Methods**

The PerkinElmer LX50, ultra high-performance liquid chromatography system (UHPLC), was coupled to the QSight 400 series triple quadrupole tandem mass spectrometer were implemented for chromatographic separation and subsequent detection, respectively. For switching between ESI and APCI a Rheodyne diverter valve was used and programmed into the LC method. The instrument was controlled by the Simplicity™ 3Q software platform. All solvents and standards were sourced locally (Germany).

#### **Preliminary data (results)**

Two separate methods demonstrating the advantages of a true dual source relating to drinking water analysis are presented. The first method involves acrylamide and according to EU regulations, a level of 0.1 µg/L are required. During initial method development two separate methods were setup, one using ESI and the second APCI. The QSight allows the user to easily switch between ESI and APCI because of its true dual source design and requires no manual changes to the instrument. Figure 1 shows how APCI was the best ionization technique drastically reducing the matrix effect and with no loss in sensitivity. Moreover, a quantitation limit of 0.01 µg/L could be reached which is 10x lower than the required limit.

The second method showcasing the benefits of a true dual source switches between APCI and ESI within the same LC/MS/MS method. The example presented is shown in Figure 2, where the fungicide metabolite, N,N-dimethylsulfamide (DMS), was first measured with APCI and then the method switches to ESI to measure both chlorate and perchlorate. Moreover, the sensitivity observed was more than sufficient when comparing to EU drinking water regulations, where chlorate has a parametric value of 0.25 mg/L and DMS, an organic fungicide, is 0.1 µg/L.

#### **Please explain why your abstract is innovative for mass spectrometry?**

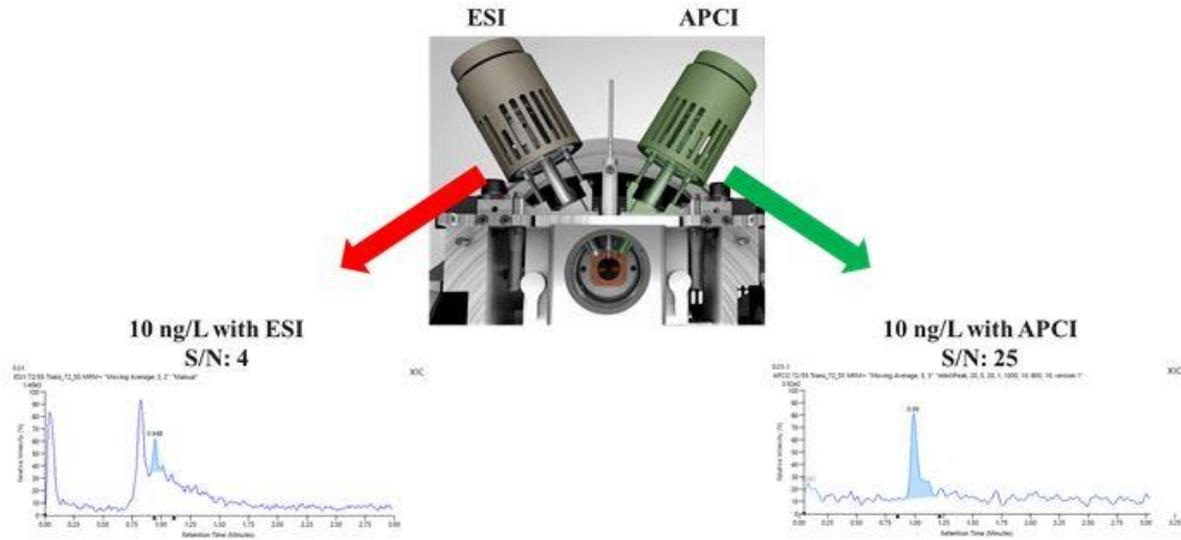
Two separate analytical methods demonstrated the benefits of a true dual source QSight LC/MS/MS. The examples were presented in the context of reaching EU limits regarding drinking water.

#### **Co-authors:**

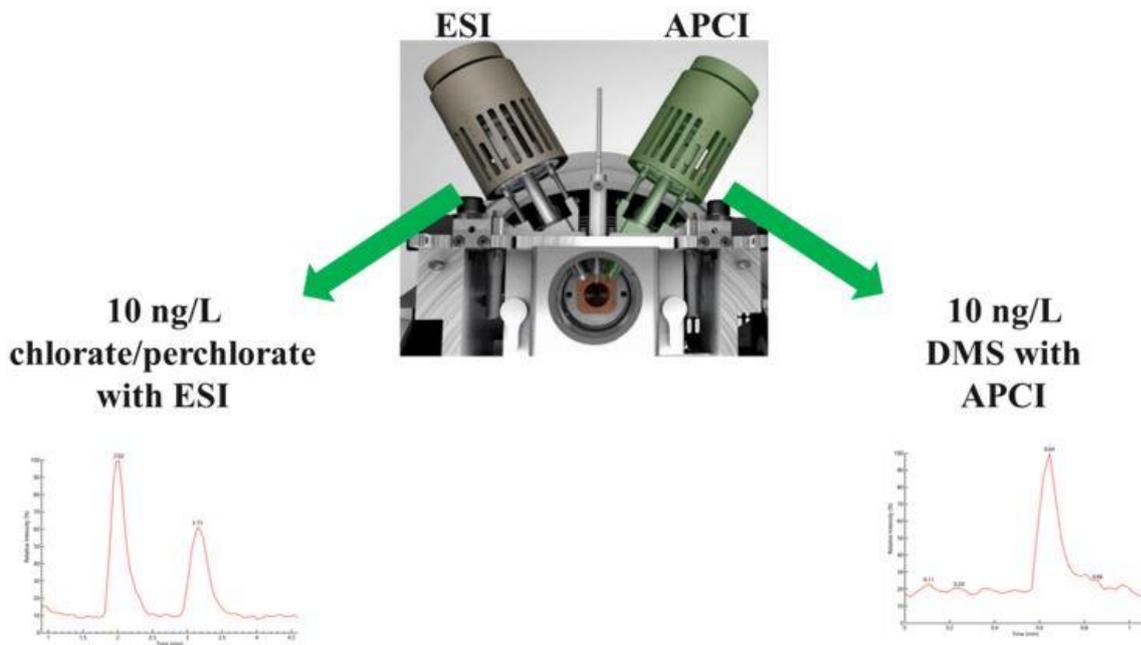
*Roberto Bozic, PerkinElmer*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Comparison of ESI vs. APCI of acrylamide at 10ng/L



Switching between APCI and ESI in the same method

Poster number: **IM-PB-008**

## **A MINIATURISED METHOD FOR CONTACTLESS, HIGH-THROUGHPUT AND QUANTITATIVE ANALYSIS OF FREE AMINO ACIDS**

Abstract ID: **308**

**Presenting author: Martin Bachman, Medicines Discovery Catapult**

### **Introduction**

Accurate and scalable methods for the analysis of free amino acids (AA) are required at various stages of drug discovery from biochemical and cellular assays to animal and human studies. In cultured cells, their levels can be used to measure the activity of metabolic enzymes or to follow the changes in growth rates after treatment with test compounds. Similarly, free AA in the blood can reveal various conditions affecting the uptake, usage or metabolism of these key building blocks around the body. We sought to overcome the throughput and cost limitations of current (LC)-MS methods, and invented a simple, fully acoustic sample preparation and analysis workflow that can be scaled to 100,000 samples per day.

### **Methods**

All work was carried out in 384-well microtitre plates. Echo liquid handler was used to transfer nL amounts of samples, standards and reagents. MultiDrop plate filler was used to dispense  $\mu$ L amounts of diluents. Flow injection ESI-MS and Acoustic Mist Ionisation MS were used to carry out direct quantitative analysis in the negative HRMS mode. Data was processed using Genedata Expressionist.

### **Preliminary data (results)**

We found that the miniaturised workflow provides reproducible and accurate quantitation of most proteinogenic and non-proteinogenic amino acids. The assay is sensitive down to low nM levels (low  $\mu$ M levels in undiluted samples) and linear across the concentration ranges typically found in cultured media and blood. We used a pool of stable isotope labelled amino acids as internal standards. Sample preparation takes less than 5 min per 384-well plate, and data acquisition can be done in as little as 0.5 s and 30 s per sample using AMI-MS and flow injection ESI-MS, respectively. We applied the workflow to a number of projects such as for a triage of biochemical hits in a cellular assay, for annotating free amino acid levels in a set of biobanked blood samples, or for determining the efficacy of a drug delivery system in a mouse model of a metabolic disorder.

### **Please explain why your abstract is innovative for mass spectrometry?**

The described generic workflow improves the throughput and cost of free amino acid analysis from biological samples by several orders of magnitude.

### **Co-authors:**

*Bruno Bellina, Medicines Discovery Catapult*  
*Ian Sinclair, AstraZeneca*

Poster number: **IM-PB-009**

## CONDENSED-PHASE ELECTRICAL DISCHARGE FOR ON-SLIDE DIGESTION OF PROTEINS AND PEPTIDES

Abstract ID: **344**

Presenting author: **Dan McGill, Rosalind Franklin Institute**

### Introduction

Proteins are immensely important to medical research; their presence, absence, or misregulation can act as biomarkers of disease, diet, lifestyle, or genetics – for example, in diagnosis of cancers, bacterial identification, or assessment of therapeutic responses (a key component of personalised medicine). While techniques like MALDI are capable of imaging whole proteins, sensitivity is typically greater for smaller molecules - however, the generation of small molecules from proteins (e.g by enzymatic digestion) requires significant sample preparation, and can cause delocalisation of peptides in the sample, blurring images. Here we introduce an ambient method utilising the electrical discharge generated from the potential difference between a needle and a conductive slide to digest proteins and peptides for subsequent analysis using ambient methods, producing replicable, unique, and spatially resolved fragments.

### Methods

Protein and peptide standards are applied to an electrically conductive slide; an in-house manufactured ion source with annular needle attached to a DC generator is used to create a visible corona discharge, which the standards are exposed to. The resulting digested standards are then either washed off and examined by LC-MS, or are directly analysed by Desorption Electrospray Ionisation (DESI).

### Preliminary data (results)

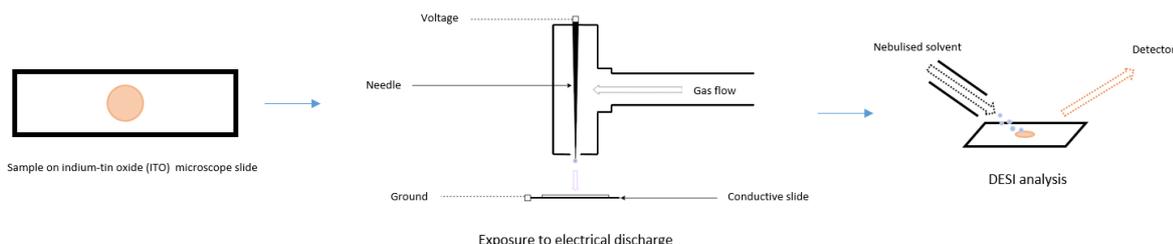
The method has been applied to common mass spectrometry standards triglycine, trialanine, leucine enkephalin, and bradykinin - in each instance, after analysis by both LC-MS and DESI, peaks were generated which were unique, replicable, and spatially resolved, with longer exposures to the corona discharge resulting in differences in peak intensity. This applies both when standards are applied 'neat', as well as when they are applied as mixtures - in the latter instance, peaks associated with digestion for both compounds are visible.

### Please explain why your abstract is innovative for mass spectrometry?

Condensed-phase minimal preparation digestion of proteins and peptides using electrical discharge

### Co-authors:

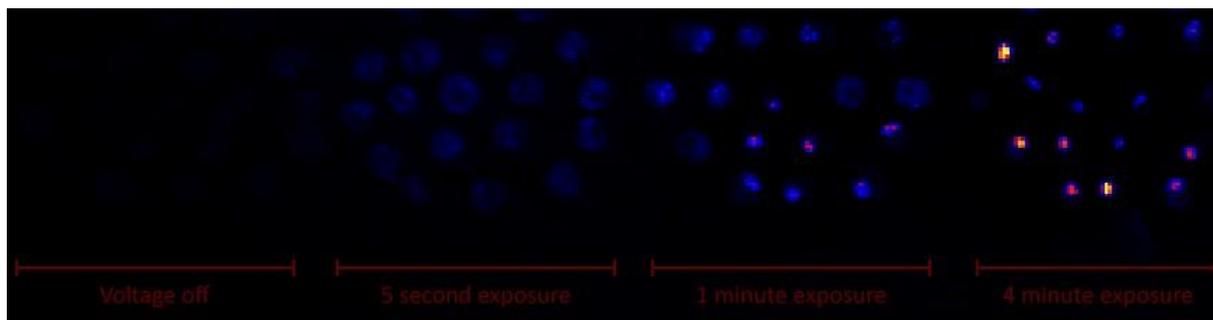
*Daniel Simon, Rosalind Franklin Institute, Imperial College London*  
*Josephine Bunch, Rosalind Franklin Institute, National Physical Laboratory*  
*Zoltan Takats, Rosalind Franklin Institute, Imperial College London*



Condensed-phase digestion workflow

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Heatmap of m/z 293.21 from leucine enkephalin after exposure

Poster number: **IM-PB-010**

## QUANTITATIVE METABOLOMICS WORKFLOWS ENABLED BY ISOTOPICALLY ENRICHED BIOMASS

Abstract ID: **584****Presenting author: Veronika Fitz, Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria**

### Introduction

Best-practice quantification in LC-MS based metabolomics relies on normalization with stable isotope-labeled standards [AnalChem,2021,93,1,519-545]. <sup>13</sup>C-labeling is preferred due to minimal isotope effects in the biological system and the measurement platform [MassSpectromRev,36,1,58-85]. A cost-effective solution to obtain a high number of labeled standards with high labeling degree (> 99%) is the implementation of biomass from lower organisms fed with fully labeled nutrients. Spiking the same amount of internal standard to samples and external calibrators (reversed isotope dilution), it is not necessary to know the exact metabolite concentrations in the spiking material. A classical isotope dilution strategy carried out as one-point calibration would simplify the quantification procedure and expense for (external) standards but relies on known concentrations and stability of the labeled spiking material.

### Methods

We use uniformly isotope-labeled cell extracts from yeast (*Pichia pastoris*) as internal standard with a <sup>13</sup>C enrichment degree of > 99% [JPharmBiomedAnal,2018,155,329-334] to study isotope dilution strategies based on external calibration with internal standardization, where the concentrations of the spike material are not known, versus one-point calibration with standards of characterized concentration and uncertainty. We are further planning to extend our quantification strategies to non-fully labeled biomass, where isotopologue patterns have to be considered and not just single isotopologue ratios.

### Preliminary data (results)

A fully controlled fermentation and extraction procedure has been established to produce cell extracts of universally <sup>13</sup>C-labeled *P.pastoris*. Implementing these extracts as internal standard (ISTD) for metabolomics experiments has improved accuracy and precision in multi-point calibration LC-MS quantification following a reversed isotope dilution strategy [JPharmBiomedAnal,2018,155,329-334, Analyst,2018,144,1,220-229] and in direct infusion experiments with just-in-time quantification of the ISTD [Analyst,2021,146,8,2591-2599]. A panel of 70 metabolites is sufficiently stable to provide reliable quantitative values over a period of six months [AnalBioanalChem,2021,https://doi.org/10.1007/s00216-021-03694-w]. The feasibility of one-point calibration was preliminarily assessed by retrospective analysis of HILIC-HRMS data obtained from three separate experiments employing the same analytical procedure. All data sets comprised an external calibration curve and repeated injections of a standard mixture as quality control samples. All samples and standards were spiked with <sup>13</sup>C-labeled yeast extract. The ISTD was quantified for each data set separately using the corresponding calibration curve. Accuracy was expressed as concentration bias of the quality control standard. The data demonstrate that one-point-calibration with labeled biomass is feasible. They also stress the need to adapt yeast extract dilution to the sample of interest to match metabolite abundances between the two biological extracts as closely as possible while maintaining good signal intensity and considering the linear range of the analytical platform. We will repeat quantification of the yeast extract to confirm that accuracy and precision are not biased by improper ISTD quantification and challenge the quantification procedure with real, traceable samples by quantifying certified reference materials.

### Please explain why your abstract is innovative for mass spectrometry?

We discuss workflows for metabolomics enabled by isotopically enriched biomass, scrutinizing the impact of labeling degree and addressing the capabilities for non-fully labeled biomass in targeted quantification.

### Co-authors:

*Mate Rusz, Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria*

*Gunda Koellensperger, Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria, Vienna Metabolomics Center (VIME), University of Vienna, Althanstr. 14, 1090 Vienna, Austria*

Poster number: **IM-PB-011**

## HIGH-THROUGHPUT LASER-ASSISTED RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (LA-REIMS) MICROBIAL IDENTIFICATION PLATFORM FOR PHARMACEUTICAL MICROBIOLOGY

Abstract ID: **627**Presenting author: **Toma Ramonaite, Imperial College London**

### Introduction

The development of rapid, sensitive, and precise technologies for identifying various micro-organisms has generated a lot of interest in the pharmaceutical industry in recent years. Traditional microbiological detection procedures are time-consuming and can take up to several days to produce results. MALDI-ToF has drastically reduced identification times, although it still requires the introduction of a matrix to aid ionisation. Furthermore, additional extraction processes are required for some microbial groups, such as yeasts, to get precise species-level identifications. LA-REIMS is a rapid microbial detection technology that can provide high-accuracy results in as little as a few minutes after initial incubation. Using a single MS technique, a wide range of bacteria, yeast, and fungus can be identified at the species, genus, and family levels.

### Methods

LA-REIMS works by applying a CO<sub>2</sub> laser beam to the microorganism, which causes rapid heating of microbial biomass, resulting in the formation of aerosol containing gas phase ions of metabolites and structural lipids, which are aspirated into to a mass spectrometer (Xevo G2-XS QToF) for MS analysis. Aerosol is co-aspirated, at a flow rate of 0.2 mL/s, with 2-propanol (HPLC Chromasolv grade, Sigma-Aldrich), containing Leuvenkephalin at a concentration of 10 ng/μL. Data were acquired in negative mode from three repeats per sample and using repeat laser pulsing with super pulse and 2 W power.

### Preliminary data (results)

The LA-REIMS platform is designed to analyse samples from any size culture plates, as well as 96 well plates, 24 well plates or tubes with no preparative steps. Here we present work on, environmental monitoring, water analysis, and testing of absence and presence of microbial contaminants. The trained model, which includes 25 isolates per class, was developed by combining 15 of the most common pharmaceutical bacteria and yeast species identified in water and environmental testing, as well as required species in pharmacopoeia for absence and presence tests. Speciation accuracy was tested using initial incubation plates as well as refrigerated plates for 24 hours, 48 hours, and 72 hours at 4°C fridge before being analysed.

An in-house data processing workflow with peak detection and biomarker detection functionalities was used, with 748 peaks identified across the spectral range and 141 ultimately selected as unique features from the Random Forest model. The accuracy of the model was first tested using leave-one-out cross-validation (CV), which yielded an accuracy of 98.93%. An independent, validation set was also produced and contained the same 15 species and 10 isolates for each class. The accuracy obtained at the species level for these 150 isolates was 98.66%, although most isolates that were incorrectly identified were still placed in the right Genus group. Finally, this accuracy was retained after 24, 48 and 72 hours in the 4°C fridge, with their classification accuracies respectively at 98.00%, 100% and 98.00%.

### Please explain why your abstract is innovative for mass spectrometry?

Laser-assisted REIMS improves analytical throughput and sensitivity with full automation, maximizing the impact for industrial microbiology and direct-from-sample isolate detection.

### Co-authors:

*Yuchen Xiang, Imperial College London*  
*Miriam Guest, AstraZeneca*  
*Andrew Ray, AstraZeneca*  
*Zoltan Takats, Imperial College London*

Poster number: **IM-PB-012**

## OPTIMIZATION OF NEGATIVE ION PAPER SPRAY MASS SPECTROMETRY FOR THE ANALYSIS OF THE ENVIRONMENTAL EXPOSURE CHEMICALS

Abstract ID: **721****Presenting author: Sangwon Cha, Dongguk University**

### Introduction

Human exposure to environmental chemicals has been rapidly growing over the past decades. Scientific evidence suggests that many types of chemicals that we use or are exposed to on a regular basis are toxic in nature causing many chronic diseases. Liquid chromatography or gas chromatography-mass spectrometry (MS) with a proper ionization method has been commonly utilized for exploring the environmental exposure chemicals. However, this approach relies on laborious, time-consuming procedures. To overcome these shortcomings, ambient desorption/ionization (ADI) MS techniques have been employed for this purpose. Here, we tried to optimize paper spray ionization (PSI), one of the simplest forms of liquid extraction based ADIs, for simultaneous detection of various classes of environmental exposure chemicals in the negative ion mode.

### Methods

Standards for representative exposure chemicals such as bisphenols, parabens, and phthalates were obtained from Sigma-Aldrich. Some representative exposure chemicals investigated in this study are displayed in the figure. A home-built PSI set up was interfaced either with a linear ion trap mass spectrometer (Thermo LTQ XL) or with a triple quadrupole mass spectrometer (Thermo TSQ Quantum) for performing PSI MS experiments.

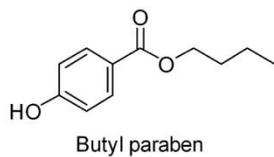
### Preliminary data (results)

In the negative ionization mode, electrospray ionization and its related ionization methods such as PSI have suffered from the instability of the ionization due to the corona discharge. To overcome this issue, the introduction of an electron scavenger gas, a halogenated solvent, and an acidic, neutral, or basic additive has been suggested. In this study, we also tested various organic solvents and aqueous/organic mixture solvents as a PSI spraying solvent for detecting exposure chemicals in the negative ion mode. The preliminary results suggest that a pure protic organic solvent generally performed better than a pure aprotic solvent or a water/organic solvent mixture in terms of various analytical performance measures such as signal intensity, limit of detection, linearity, and recovery. Moreover, we found that the addition of a proper amount of a halogenated solvent such as carbon tetrachloride (CTC) improved the PSI performance significantly against these exposure chemicals. After optimizing the solvent composition, we evaluated the effects of various salt additives in the negative PSI MS. We found that one of the ammonium salts tested showed the much better sensitivity and linearity than other ammonium salt, acid, and base additives.

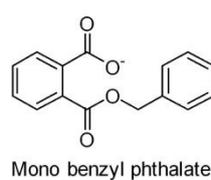
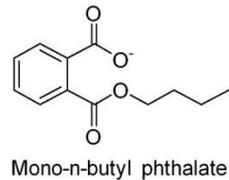
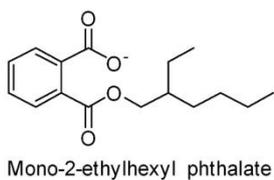
### Please explain why your abstract is innovative for mass spectrometry?

Intensive systematic evaluation and optimization of paper spray ionization in the negative ion mode for the simultaneous analysis of various classes of environmental exposure chemicals

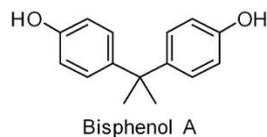
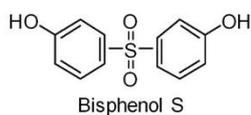
(a) Parabens



(b) Phthalates



(c) Bisphenols



Representative environmental exposure chemicals investigated in this study

Poster number: **IM-PB-013**

## REACTIVE-DESI TO RETRIEVE ANTICOUNTERFEITING TAGGANTS FROM POLYMERIC GEL SURFACES

Abstract ID: 777

Presenting author: **Isaure Carvin-Sergent, Aix Marseille Univeristé - CNRS, Institut de Chimie Radicalaire**

### Introduction

The defined sequence of two co-monomers in sequence-controlled oligomers can be used to store binary information which is further decoded by MS/MS sequencing. From their early development, such species have become popular for anti-counterfeiting purposes. However, in analytical workflows reported in this field, the rate-limiting step is the procedure required to extract the coded molecule from the tagged material. Because it combines fast surface extraction with analyte ionization in ambient conditions, desorption electrospray ionization (DESI) was advantageously employed to retrieve and decode various oligomers from different surfaces. Yet, in order to prevent tag loss during sample manipulation or transportation, covalent attachment of the coded oligomer to the surface would be an advantageous alternative.

### Methods

Sequence-coded polyurethanes (PU) containing a disulfide linker and a terminal methacrylamide moiety were synthesized by stepwise solid-phase synthesis. These methacrylamide macromonomers were afterwards copolymerized with acrylamide and bisacrylamide in order to achieve crosslinked hydrogels containing PU taggants covalently-bound via a disulfide bond. Mass spectrometry experiments were performed with a Waters Synapt G2 instrument equipped with a DESI source. A methanolic solution of  $\text{NH}_4\text{OH}$  (0.1%) and dithiothreitol (DTT, 4 mM) was used for reactive-DESI analyses.

### Preliminary data (results)

Disulfide groups were selected here to anchor coded oligomers to the gels because they survive mild condition radical polymerization while they can be readily cleaved upon reaction with reducing agents such as DTT. Small pieces of each gel were first analyzed in standard DESI conditions to check for the absence of residual non-covalent attached coded oligomers. Then, adding DTT to the methanolic solution sprayed onto gel samples allowed the desired ion/molecule reaction to occur at the gel surface, as evidenced by MS detection of the targeted taggants as deprotonated molecules. It should however be acknowledged that ion abundance highly varied when analyzing multiple pieces of the same gel sample, due to inhomogeneous distribution of the taggants within a given sample combined with difficulties to obtain flat surfaces when cutting the hydrogels. Nevertheless, in most cases, the amount of released taggants was sufficient to recover their sequence by MS/MS because the selected PUs dissociate *via* one bond cleavage per each repeating unit, which prevents extensive signal dilution over multiple fragment series.

### Please explain why your abstract is innovative for mass spectrometry?

Implementation of disulfide bond cleavage in reactive DESI to release coded oligomers from hydrogels.

### Co-authors:

*Itab Youssef, Université de Strasbourg - CNRS, Institut Charles Sadron*  
*Jean-Arthur Amalian, Aix Marseille Univeristé - CNRS, Institut de Chimie Radicalaire*  
*Salomé Poyer, Aix Marseille Univeristé - CNRS, Institut de Chimie Radicalaire*  
*Jean-François Lutz, Université de Strasbourg - CNRS, Institut Charles Sadron*  
*Laurence Charles, Aix Marseille Univeristé - CNRS, Institut de Chimie Radicalaire*

Poster number: **IM-PB-014**

## LC-MS/MS-IMS METHOD FOR THE IDENTIFICATION OF UNKNOWN METABOLITES

Abstract ID: 882

**Presenting author: Yuandi Zhao, Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, the Netherlands**

### Introduction

Cytochrome P450 monooxygenases (CYPs) are members of a superfamily of mono-oxygenases, which play an essential role in metabolizing a wide range of xenobiotics. Among CYPs family, CYP BM3 from *Bacillus megaterium* is a very interesting candidate, which is a highly stable enzyme and possesses the highest activity.

A liquid chromatograph-tandem mass spectrometry-ion mobility spectrometry (LC-MS/MS-IMS) method has been developed to screen biocatalytic activity of the BM3 mutants, besides the structural identification of the metabolites formed (Fig. 1). In this paper, we discussed the application of different analytical strategies using LC-MS/MS-IMS for the tentative structural identification of unknown small molecules.

### Methods

Selected compounds were incubated with 5000 BM3 mutants and injected to an LC-MS system. In the LC method 10 mM ammonium acetate in water was used as A phase, and a solution of methanol: A phase: isopropanol = 85:10:5 was used as B phase. A BEH C18 (130 Å, 1.7 µm, 2.1x50 mm) column was used for the separation at the flowrate of 0.4 mL/min. SYNAPT G2-Si Mass Spectrometer (Waters) was used for the identification in both positive and negative ion mode. For MS/MS and IMS analyse, a 20 eV collision energy was applied.

### Preliminary data (results)

With this method, the metabolites could be separated. 5000 mutants were detected for their bioactivity, and the mutants that had bioactivity were selected as heat plates for further research. In addition, MS/MS was expected to generate structural information of the unknown metabolites based on the fragments formed after collisional activation. However, in the case, the mass-to-charge ratios ( $m/z$ ) of the metabolites and their fragments remained identical, since they could not be distinguished. Herein, an IMS method was added to the separation, with which the isomers could be separated and identified especially. The unknown metabolites could be differentiated based on their shapes, which led to a possibility of several peaks observed in the mobilogram. Therefore, LC-MS/MS-IMS can be used as a novel method not only for the screening of bioactivity metabolism, but also can be used for the structure identification of unknown metabolites.

### Please explain why your abstract is innovative for mass spectrometry?

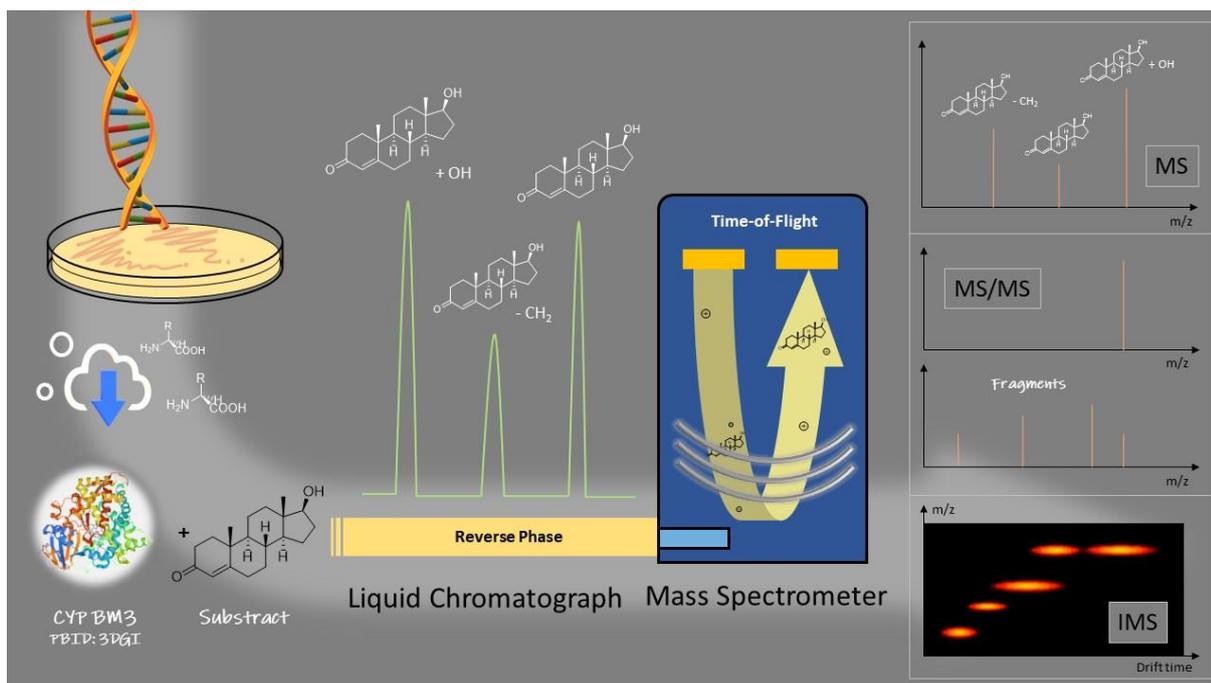
With the application of IMS, more demissions can be added to traditional LC-MS method, and can be used for structure identification during drug discovery, especially for the isomers identification.

### Co-authors:

*Maarten Honing, Maastricht Multimodal Molecular Imaging Institute (M4i), University of Maastricht, the Netherlands*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



The scheme of LC-MS/MS-IMS method for structure identification.

## **Session: Instrumentation development: Mass Analyzers**

Poster number: **IM-PB-016**

### **APPLYING TRAPPED ION MOBILITY SPECTROMETRY (TIMS) FOR VITAMIN D EPIMER DIFFERENTIATION**

Abstract ID: **331**

**Presenting author: Viola Jeck, Bruker Daltonics GmbH & Co. KG**

#### **Introduction**

Vitamin D deficiency is common in children and adults. It is associated with growth retardation, skeletal deformation, and osteomalacia and is presumed to play a role in chronic illnesses, such as common cancer, autoimmune diseases, and cardiovascular diseases. Hence, the interest in the analysis of vitamin D metabolites has seen a remarkable increase. However, difficulties arise from interfering epimers.

Vitamin D<sub>3</sub> is photosynthesized in animals and enzymatically converted to 25-hydroxyvitamin D<sub>3</sub> - one of the most abundant vitamin D metabolites. However, the common clinical target can erroneously be confused with its C<sub>3</sub>-epimer.

In this study, the separation power of the Trapped Ion Mobility Spectrometry (TIMS) is explored for the vitamin D epimers 25-hydroxyvitamin D<sub>3</sub> and 3-epi-25-hydroxyvitamin D<sub>3</sub>.

#### **Methods**

The Vitamin D standards 25-hydroxyvitamin D<sub>3</sub>, 3-epi-25-hydroxyvitamin D<sub>3</sub>, and its mixture were diluted in acetonitrile/water (50:50) and investigated via direct-infusion experiments. Data acquisition was performed on a Bruker timsTOF Pro 2 instrument in TIMS mode. The instrument was equipped with an ESI-source and operated in the positive ionization mode. Data processing was conducted in DataAnalysis 5.3 software (Bruker).

#### **Preliminary data (results)**

First, the TIMS data was collected for standard solutions of 25-hydroxyvitamin D<sub>3</sub> and 3-epi-25-hydroxyvitamin D<sub>3</sub>. Both full mass spectra revealed the sodiated monomer [M+Na]<sup>+</sup> as major ion identified. The separation power of the TIMS device revealed distinctive mobility peaks for the sodiated monomer of 25-hydroxyvitamin D<sub>3</sub> and 3-epi-25-hydroxyvitamin D<sub>3</sub> which only differ in their C<sub>3</sub> hydroxyl group. Moreover, in the investigation of the mixture the mobility dimension enabled a separation of both epimers by means of the high resolving power.

#### **Please explain why your abstract is innovative for mass spectrometry?**

In summary, the Trapped Ion Mobility Spectrometry technique presents a high resolving power, capable to resolve epimers. The CCS of vitamin D epimers measured agreed well with published values.

#### **Co-authors:**

*Lucy Woods, Bruker Daltonics GmbH & Co. KG*

Poster number: **IM-PB-017**

## **ION PRE-ACCUMULATION FOR HIGH SPEED ORBITRAP EXPLORIS OPERATION**

Abstract ID: **446**

**Presenting author: Hamish Stewart, Thermo Fisher Scientific**

### **Introduction**

Orbitrap instrument operation is normally limited to maximum acquisition rates <50Hz, primarily due to time constraints imposed by the operation of the Orbitrap's ion preparation device, the C-Trap, and its conjoined collision cell, the Ion Routing Multipole (IRM). This fixed operation time runs in series with ion injection, thus faster operation cuts into the allowed injection time and induces a substantial loss of duty cycle.

A modified operation has been implemented on a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer, whereby ions are first pre-accumulated and stored upstream of the C-Trap, in the Bent Flatapole, before being transferred to the C-Trap/IRM upon its availability. This runs in parallel with C-Trap/IRM ion processing and injection, allowing improved sensitivity at faster scan rates.

### **Methods**

Sensitivity comparisons were made between standard and pre-accumulation mode via measurements of infused Pierce™ FlexMix™ calibration solution. For high-throughput performance evaluation, HeLa digest was resuspended in 5% ACN / 0.1% FA, injected via autosampler onto a trapping column and separated on a 15cm PepMap™ Reversed Phase (RP) column using a Vanquish™ Neo UHPLC or EASY-nLC™ system. Various gradient lengths were used to separate samples before introduction to the mass spectrometer. The instrument was operated in a Data Dependent Acquisition (DDA) mode and acquired raw data files were processed with Proteome Discoverer™ software.

### **Preliminary data (results)**

At the normal maximum 20ms cycle time, signal intensity of FlexMix ions was observed to double upon implementation of pre-accumulation, matching the understanding that the normal maximum inject time for this 20ms cycle time is 10ms, a 50% duty cycle, boosted to 100% via pre-accumulation.

Comparison of 1-hour DDA gradients using 200ng HeLa digest showed roughly 5- 15% improvements in terms of detected peptides and protein groups, which represents a reasonable result for a duty cycle improvement alone.

To reach >70Hz operation, the Orbitrap transient was lowered from 16 to 8ms, corresponding to a resolution of 3750, and the maximum in-series fill time dropped to 3ms. 80Hz operation was recorded by cutting the allowed ion transfer time of the IRM into the C-Trap. It is thought that potential exists for substantial further optimization. The improvement in duty cycle / sensitivity granted by pre-accumulation was observed to explode at these higher repetition rates, to >4x greater at 70Hz.

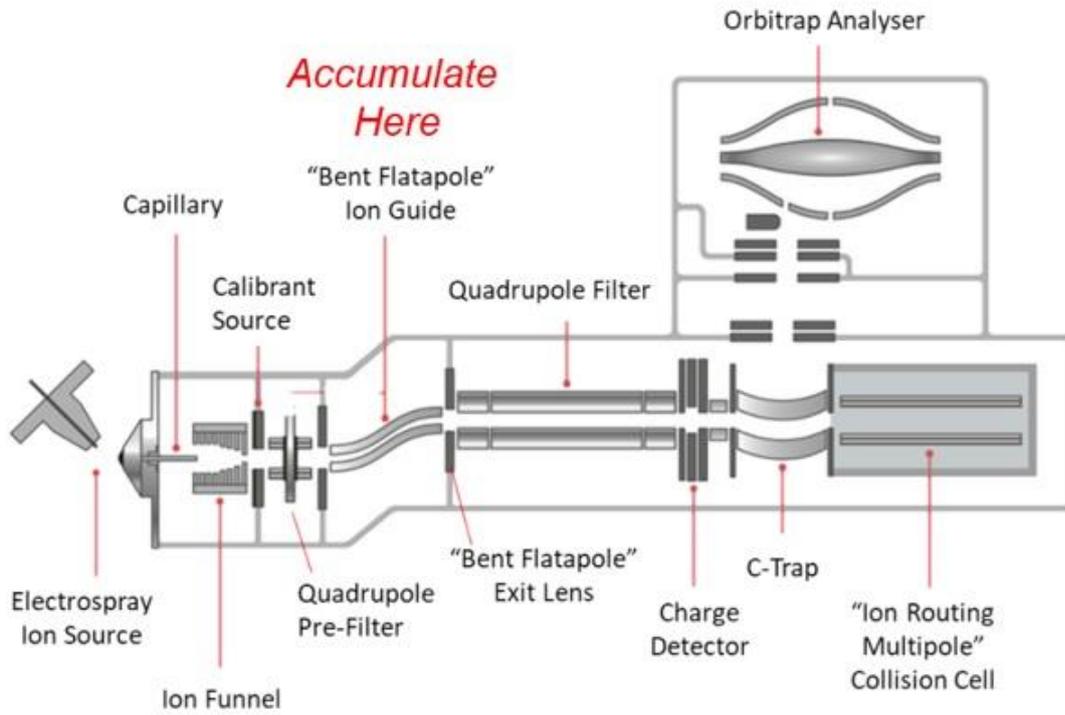
For 200ng HeLa DDA LC-MS experiments, the 70Hz pre-accumulation mode was compared to the standard 40Hz mode. The first results showed improvement of more than 20 percent in the number of peptides and proteins identified and quantified. For the concentration used, the boost to number of peptides and proteins identified and quantified decreased with gradient length. This is because the longer the gradient gets, the lower the benefit of high repetition rates, whilst the shorter transient imposes an additional sensitivity penalty.

### **Please explain why your abstract is innovative for mass spectrometry?**

Novel pre-accumulation process for Orbitrap instruments unlocks far higher repetition rates without sacrifice of duty cycle, improving performance of high-throughput applications.

### **Co-authors:**

Tabiwang Arrey, Thermo Fisher Scientific  
Alexander Harder, Thermo Fisher Scientific



Thermo Scientific Orbitrap Exploris™ 480 Layout and Pre-Accumulator Position

Poster number: **IM-PB-018**

## **IN SILICO DEVELOPMENT OF NEW STYLES OF 2DMS IN A WIDER SELECTION OF INSTRUMENTATION**

Abstract ID: **467**

**Presenting author: Callan Littlejohn, University of Warwick**

### **Introduction**

Two-dimensional mass spectrometry (2DMS) is a truly data independent tandem MS (MS/MS) technique, which allows for the analysis of complex mixtures without the need for any electric, chemical or size based isolation prior to analysis. This technique is currently unique to FT-ICR and LIT based instruments. 2DMS typically works by modulating ions in and out of a fragmentation zone between progressive scans to build a signal of ion intensity which, when the Fourier Transform is applied; elutes the  $m/z$  of the precursor mass, because the ion intensity of the fragment is, by definition, inversely linked to the intensity of the precursor.

Through in-silico work using SIMION, new Pulse sequences has made 2DMS possible in a variety of other MS techniques.

### **Methods**

All experiments were performed in silico using SIMION ion trajectory simulations. A variety of systems including TOF and Magnetic sector based instruments were investigated for their suitability for this novel 2DMS approach. The implementation was tested by measuring the intensity of a fragmentation zone at the ion location and deriving the frequency of the modulation of fragmentation intensity. This was then plotted to prove that ions of different  $m/z$  will fragment at different frequencies using this approach, and therefore 2DMS is possible.

### **Preliminary data (results)**

A number of MS techniques have been sampled for their suitability for 2DMS and it has been found that it is possible in all cases. The Time of Flight application is the most promising, showing reliable and consistent periodicity of fragmentation corresponding to derived calibration equations. It has been shown that minimal modification to a regular TOF instrument would be needed in order to perform 2DMS, without having any negative effects on standard TOF-MS studies.

By using a magnetic sector, implementation of 2DMS was also achieved. Similarly, using Simion ion trajectory calculations, simulation has shown that the fragmentation intensity modulates at a periodicity correlated to its  $m/z$  through a fundamentally derived calibration equation, indicating that 2DMS was possible in this implementation, and has shown the possibility to couple this to an orthogonal TOF-MS device for higher resolution or a further 2DMS experiment, allowing for the possibility of 2DMS/2DMS experiments which previously have only been theoretically possible by either coupling LIT to FTICR or double excitations; which are both time consuming and costly.

A variety of other systems have also been simulated and optimised for 2DMS. While current work is in silico, the possibility of physical implementations of these simulations would allow for 2DMS to be performed not only on more affordable and widespread MS platforms but also with significantly improved speed compared to the current FTICR implementations, which would potentially lead to applications in clinical and diagnostic work, in addition to high-throughput applications.

### **Please explain why your abstract is innovative for mass spectrometry?**

A novel technique for the implementation of 2DMS on a wide variety of MS platforms.

### **Co-authors:**

*Meng Li, University of Warwick*  
*Peter O'Connor, University of Warwick*

Poster number: **IM-PB-019**

## **A DIGITAL ION FILTER AND TRAP FOR THE MS SPIDOC PROTOTYPE**

Abstract ID: **533**

**Presenting author: Florian Simke, Universität of Greifswald, Institut of Physics**

### **Introduction**

The MS SPIDOC (Mass Spectrometry for Single Particle Imaging of Dipole Oriented protein Complexes) prototype [1] will deliver mass- and conformation-separated samples of protein-based biomolecules for single-particle imaging analysis at the European X-Ray Free-Electron Laser Facility (XFEL) [2]. Here, the design [3], commissioning, and first experimental results of one of its key modules are presented.

[1] C. Uetrecht et al., J SYNCHROTRON RADIAT (3) (2019) 653-659

[2] Europe turns on bright x-ray source, NAT PHOTONICS 11 (2017) 609{609}09

[3] F. Simke et al., INT J MASS SPECTROM 473 (2022) 116779

### **Methods**

The module consists of a linear-quadrupole filter assembly and a linear-quadrupole ion trap, both operated with digital radio frequencies. The restriction to keep investigated biomolecules as native as possible leads to a unique design, separating ion filtering and trapping. The ion trap is utilized to collect and bunch incoming ions from a continuous source and eject them with a narrow temporal width for downstream analysis and X-ray interaction. Pin electrodes located between the quadrupole rods are used to alter the axial trapping and ejection potential to tune the characteristics of the outgoing ion bunch.

### **Preliminary data (results)**

Ion-optical simulations with SIMION 8.1 show that the mass resolving power of the quadrupole filter is around 100 for transmission efficiencies close to 100 % and can be increased to approximately 700 at efficiencies on the order of 15 %. In the digital ion trap, a buffer-gas pressure on the order of a few  $10^{-3}$  hPa is sufficient to capture and accumulate nearly all incoming ions up to a mass-to-charge ratio of around 12000 Th, which corresponds to GroEL proteins in the 62 charge state. They thermalize within a few milliseconds and can be ejected as a condensed bunch with a phase space smaller than that of the incoming ions and a temporal spread on the order of one microsecond. Thus, the module matches the requirements for the preparation of heavy biomolecules.

The commissioned module is characterized with CsI ions produced from an electrospray ionization (ESI) source. Experimental results are consistent with the simulations. Additionally, native GroEL could be trapped, bunched, and ejected successfully.

### **Please explain why your abstract is innovative for mass spectrometry?**

The usage of a digital waveform opens up the possibility to change the mass range of the system from single atoms to heavy biomolecules within short timeframes.

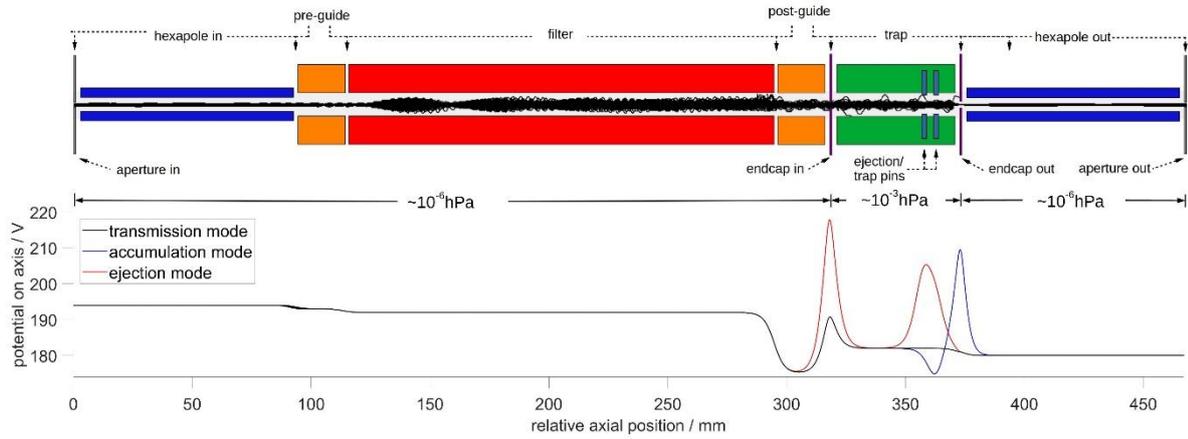
### **Co-authors:**

*Paul Fischer, University of Greifswald, Institut of Physics*

*Lutz Schweikhard, University of Greifswald, Institut of Physics*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Schematic of the simulated module, axis-potential for three operation modes

Poster number: **IM-PB-020**

## REAL-TIME MONITORING OF DYNAMIC ISOMER POPULATIONS WITH CI-SLIM IMS-MS

Abstract ID: **586****Presenting author: Luca Cappellin, Università degli Studi di Padova, Tofwerk AG**

### Introduction

SLIM IMS-MS has become popular in the world of ion mobility as this technology enables very long ion mobility path lengths in compact spaces. In this work, we interface SLIM IMS with a chemical ionization (CI) source, which allows one to sample from the gas phase directly. As such, one can monitor dynamic isomer populations in ambient air, headspace, and other gas-phase samples in real time without prior sample preparation. This opens the door to new possibilities in applications such as atmospheric chemistry, fragrances, and food & beverage where isomer activity is known to play a key role, which has been difficult to characterize due to the dynamic nature of the relevant chemical processes.

### Methods

We performed controlled laboratory experiments to demonstrate the performance of a prototype CI-IMS-TOF instrument. This instrument produces ions via a two-step chemical ionization process, which involves producing reagent ions, and ionizing neutral analyte molecules via the reagent ions through adduct formation. Once generated, these secondary ions travel into the SLIM IMS region, where a series of DC- and AC-electrodes on printed circuit boards create a traveling wave driving force. As the ions travel through the helium buffer gas, they separate based on their rotationally averaged collision cross sections. The ions exit the IMS region and enter a time-of-flight mass spectrometer.

### Preliminary data (results)

We report results from experiments that demonstrate the capabilities of our CI-IMS-TOF instrument. We begin with a model system of isomer standards that includes methyl salicylate and methylparaben. Using this system, we demonstrate how isomeric molecules in air can be ionized and separated using SLIM technology. By adjusting the concentrations of the neutral analytes on the second timescale, we demonstrate how the isomer populations can be characterized in real time. Such real-time analysis is a clear advantage when one wants to monitor dynamic chemical processes where MS alone or GC-MS would only be able to provide a partial picture of the chemical progression.

Secondly, we present IMS separations for several atmospherically relevant isomeric molecules. These molecules have diverse functionalities including ketones, aldehydes, alcohols, and carboxylic acids. Ion mobility helps differentiate such compounds, which play important roles in the formation and growth of particles in the Earth's atmosphere. Further, we recreate atmospheric conditions in the lab to study the ozonolysis of atmospherically relevant compounds using an atmospheric flow reactor. We monitored the time evolution of the oxygenated products. We emphasize that many such compounds are very difficult if not impossible to transmit through a standard GC-MS instrument. Hence, the combination of chemical ionization with ion mobility allows one to differentiate such species and understand chemical processes at an unprecedented level.

The SLIM-based instrument will ultimately be deployable into the field to monitor dynamic isomer populations in real-time at the source of various chemical processes.

### Please explain why your abstract is innovative for mass spectrometry?

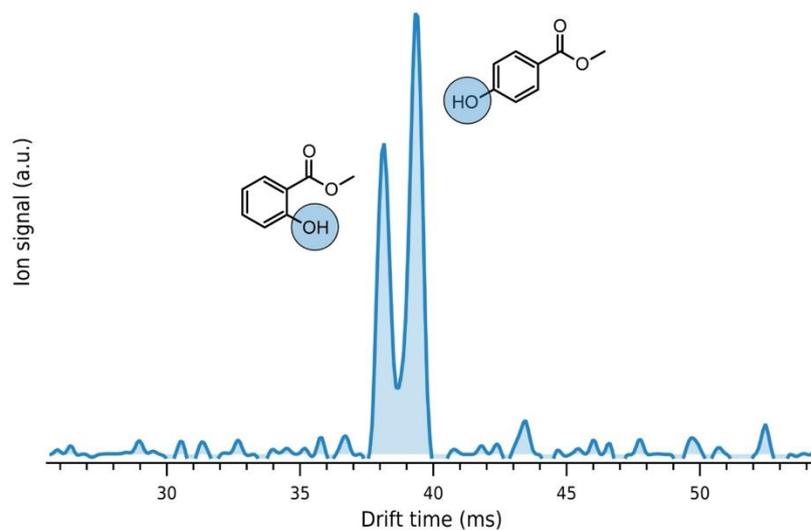
This work reports for the first time real-time monitoring of dynamic isomer populations using CI-IMS-MS technology.

### Co-authors:

*Michael Kamrath, Tofwerk AG*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Real-time isomer separation by CI-IMS-MS allowing real-time isomer monitoring.

Poster number: **IM-PB-021**

## **ACTIVE & ITERATIVE DATA-DEPENDENT REINJECTION LOGIC FOR MAINTAINING THROUGHPUT, UPTIME, AND CONSISTENCY IN TRIPLE QUADRUPOLE LC/MS ANALYSIS**

Abstract ID: **778**Presenting author: **Carola Damen, Agilent Technologies**

### **Introduction**

Triple quadrupole LC/MS measurements are often associated with targeted, quantitative, large batch sample analysis with an emphasis on non-stop continuous operation. Such use cases are in the continuous processing of QA/QC samples for contaminants in pharmaceuticals, pesticides and veterinary drug detection in foods, or measurements of biological analytes from a sizeable population. Regardless of application, consistent results, high sample throughput, and avoidance of sample reprocessing is highly desired. Herein, we present a technique utilizing an active and immediate data processing algorithm that evaluates and reinjects samples in a data-dependent manner based on the following scenarios: (1) detection of previous sample carryover, (2) detection of a sample outside of the calibration range, and (3) fast analyte screening.

### **Methods**

Measurements were carried out using a novel triple quadrupole LC/MS hardware and MassHunter software system coupled to an Infinity II 1290 HPLC system. Ions are monitored. A worklist sample queue is created to stress test and demonstrate the data-dependent logic. Analyte signal thresholds are set according to the desired workflow being carried out. Reinjection commands are defined as logical conditions based on the current abundance measurement for the Sample or Blank. If the logical commands are activated, a new line in the sample queue is appended or inserted to iterate on until a pass condition is met.

### **Preliminary data (results)**

Data-dependent outcomes were highly reliant on the selected workflow and were presented as separate experiments to demonstrate use case.

(1) **Detection of Sample Carryover** – an abundance threshold for target analytes was previously set for an analyte. A worklist containing Sample 1, Blank, Sample 2 was created; where sample 1 was intentionally spiked at a higher concentration to induce carryover. During the blank injection, the carryover abundance threshold was met, so sequential blank injections were iteratively added until the carryover threshold was sufficiently low enough to proceed to the next sample.

(2) **Sample is outside the set calibration range** – the abundances for the highest calibration standards in the analytical method was set as the trigger threshold. In a worklist containing Calibration standards L1-L5 and a Sample, the sample was intentionally spiked to be greater in abundance than L5. In this case, the following injection of the Sample was introduced with a reduced sample volume such that the signal abundance is below L5.

(3) **Fast analyte screening** – two methods were created and defined as the Screening Method and Analytical Method using the same dMRM transitions adjusted for retention time. The Screening method uses a sharp LC gradient to determine the presence of target analytes. When a spiked sample was detected, the data-dependent trigger appended a new line in the sample queue to evaluate the sample with the Analytical Method

### **Please explain why your abstract is innovative for mass spectrometry?**

Automated appending and inserting of samples or blanks requiring reinjection, following predetermined logical conditions based on target abundance.

Poster number: **IM-PB-022**

## **AN END-TO-END SOFTWARE ALGORITHM FOR LC/MS/MS METHOD DEVELOPMENT, OPTIMIZATION, AND QA/QC DEPLOYMENT**

Abstract ID: **779****Presenting author: Carola Damen, Agilent Technologies**

### **Introduction**

The act of developing targeted triple quadrupole LC/MS (LC/TQ) methods from start to finish is a complex and time-consuming, multi-step workflow. Method development becomes even more challenging if former mass spectrometry parameters for each analyte were not established – especially in the case of novel compounds. Using a modular end-to-end workflow approach, users may input chemical formula information which will result in (1) optimized MRM transitions for each compound and (2) optimal ion source parameters for the overall method. A unique feature of this algorithm is the ability to take advantage of the LC/TQ's speed to allow the simultaneous optimization of multiple compounds on a per-method basis, such that pre-production method development time is dramatically reduced compared to compound-by-compound approaches.

### **Methods**

Neutral chemical formulas of the analytes found in the LCMS 7-Analyte Sys Suitability Standard were entered into the method development interface to automatically calculate the potential [M+H]<sup>+</sup> or [M-H]<sup>-</sup> precursor ions. Optimization was done unattended in two major phases. First, MRM optimization: MS1 fragmentor voltage, Product Ion selection, and MRM collision energy voltage. Then, ion source optimization: capillary voltage, nozzle voltage, nebulizer pressure, drying gas pressure, sheath gas heater, then drying gas heater. Optimization results done “by-hand” were compared to the results of the algorithm to determine parameter accuracy.

### **Preliminary data (results)**

Upon entry of chemical formulas for analytes found in each sample, possible precursor ions were computed then detected in SIM mode. Following observation of the target precursor, the algorithm proceeded to optimize the MS1 fragmentor voltage to produce abundance-rich product ion mass spectra. As product ions were generated for each analyte, algorithmic determination of the most abundant product ions were selected for an appropriate Qualifier and Quantifier MRM transition. Once MRM transitions were corroborated by overlapping retention times, collision energy ramping and construction of each analyte's breakdown curve was carried out simultaneously – taking advantage of the fast electronic settling times and specificity afforded by the LC/TQ's dual quadrupole filtering. Final algorithmically produced results show the retention times, product ion spectra, and breakdown curves of each analyte.

Following MRM optimization and finalization, the algorithm automatically proceeds to optimize “global” ion source parameters, a significant challenge due to the confounding and inter-related nature of 7 parameters. Voltage, pressure, and temperature parameters are ramped one variable at a time, however, the order of parameter ramping was originally determined in a previous experiment using a design of experiments approach. Analyte MRM signals were measured and automatically evaluated giving priority to the weakest responders.

Major differentiators from existing method optimization algorithms today is (1) method-by-method approach and (2) abundance weighting. The result of this new algorithmic approach is unattended optimization that produces a fine-tuned and well-balanced method to be pushed forward for validation and verification.

### **Please explain why your abstract is innovative for mass spectrometry?**

A method-by-method MRM and ion source optimization algorithm workflow that will hasten the development and deployment of targeted methods.

Poster number: **IM-PB-023**

## **HIGH FIDELITY LEGACY-TO-MODERN METHOD TRANSFER ON A NOVEL TRIPLE QUADRUPOLE LC/MS PLATFORM FOR LARGE OUTPUT PRODUCTION LABS**

Abstract ID: **780**

**Presenting author: Carola Damen, Agilent Technologies**

### **Introduction**

Large output production labs often resist changes in physical hardware or software revision despite improved performance or enhanced features due to the time consuming nature of re-verification, re-validation, or reconstruction of the analytical method. Often, the method needs to be “rebuilt”, using the former method as a template – possibly leading to transcription errors or unexpected performance differences. Here we present a demonstration and metadata framework to handle the direct method transfer from a “legacy” triple quadrupole LC/MS instrument to a novel triple quadrupole LC/MS hardware and software platform with minimal adjustments and similar performance for accelerated incorporation into the production environment.

### **Methods**

Representative dMRM methods used for Environmental and Food screening lab was developed and validated using a 6470 triple quadrupole LC/MS containing optimal MRM transitions, collision energies, fragmentor voltages, and source parameters. The method was imported into a novel triple quadrupole LC/MS with no input of chemical data. Verification of analytes were compared between the existing and novel LC/MS systems to ensure MRM targets are properly observed and parameters require minimal fine tuning. If the signal was not within the expected range, automated collision energy and fragmentor optimization was carried out to ensure that signals were recovered.

### **Preliminary data (results)**

Migration and import occurred by successfully transferring the HPLC chromatography flowrates and setpoints, MRM transitions, and ion source parameters directly. The XML metadata framework allowed parameters to be carried forward without manual intervention to transcription errors.

Due to fundamental differences between the legacy system and novel triple quadrupole LC/MS system electronics and hardware, identical method transcription is not possible due to various physical mechanics (ion optics timing, electronics settling times, software overhead, etc...). Upon import of the existing method, back-end adjustments are made to automatically adjust and recalculate dMRM dwell times, ion optic voltage settling time, and detector gain voltages that it appears to be invisible to the user.

The resulting chemical verification demonstrates that analyte signal observed on the novel triple quadrupole LC/MS system is within  $\pm 20\%$  tolerance of the legacy system, otherwise within the tolerance of instrument-to-instrument variation. In the case that the signal was not within the stated tolerance, an automated collision energy and fragmentor voltage fine-tuning was carried out manually allowing us to recover signal without having to do a full revalidation of the analyte.

### **Please explain why your abstract is innovative for mass spectrometry?**

Direct method migration from legacy hardware and software platform using a unique XML framework to a novel triple quadrupole LC/MS platform.

Poster number: **IM-PB-024**

## **ACCELERATED LIFETIME TESTING WITH REAL-TIME EARLY MAINTENANCE FEEDBACK (EMF) DIAGNOSTIC MONITORING ON A NOVEL LC/MS/MS PLATFORM**

Abstract ID: **781**

**Presenting author: Carola Damen, Agilent Technologies**

### **Introduction**

Triple quadrupole LC/MS systems have become widely accepted as a platform for targeted, large-batch, sample analysis on a day-to-day basis. A primary concern for routine/targeted analysis is signal stability; which can drift over time due to the soiling of crucial ion optics components. While incorporating an internal standard and measuring abundance ratio may help alleviate signal drift from a data-analysis standpoint, it does not give key indications to the quality of the ion's path. Here we present an accelerated lifetime test on a novel triple quadrupole LC/MS system with an advanced on-board computation that provides real-time monitoring of instrument health for early maintenance feedback (EMF) to ensure instrument performance for at least 10,000 sample injections.

### **Methods**

Synthetic urine diluted 1:1 in acetonitrile/water, spiked at 1ppb with the LCMS 7-Analyte Sys Suitability Standard. Samples were injected using an Infinity II 1290 HPLC with dual injector setup in overlapped injection mode with isocratic flow of 50:50 acetonitrile/water. Real time monitoring of the instrument health was incorporated into the hardware firmware to monitor for crucial events such as ion injector blockage, precipitation on the nebulizer, and detector lifetime while reporting on its estimated lifetime. The MRM signals from 10,000 injections (1  $\mu$ L) were plotted to evaluate stability and then continued until point of failure.

### **Preliminary data (results)**

This study was designed to be representative of a typical production lab analyzing a challenging "dilute & shoot" biological sample. Due to endogenous components found in urine, significant precipitation and accumulation of salts and organic material were observed throughout the ion source region. Precipitation poses a severe threat of disrupting the ion flow path, however - positive real-time monitoring of rough vacuum pressure, high-vac manifold pressure, ion current, and detector voltage provided an acceptable readout of instrument status and health as data acquisition proceeded. Similarly, real time monitoring of parameters in the ion source region ensured that nebulizer blockage and charge accumulation did not occur. Due to the overall positive readouts obtained by through the EMF display the study was able to proceed without user intervention until completion.

It is important to note that EMF monitoring was implemented in the LC/MS hardware's computer firmware and occurred outside of data acquisition system. Tolerances and limits for trigger events were obtained in a past study through various multifactorial statistical analysis tools (design of experiments and machine learning) to evaluate the possible cross interactions from various components along the ion optics rail. This ensured that EMF trigger events are truly representative of the issue's root cause to be resolved.

### **Please explain why your abstract is innovative for mass spectrometry?**

Demonstration of active, real-time maintenance feedback logic to help maintain instrument stability on a novel triple quadrupole LC/MS system.

Poster number: **IM-PB-025**

## **A NON-DESTRUCTIVE DETECTOR FOR MEGADALTON MOLECULES WITH LOW CHARGE**

Abstract ID: **790**

**Presenting author: Adrien Poindron, Aix-Marseille Université, CNRS, PIIM**

### **Introduction**

Thanks to the recent advances in biology and instrumentation in mass spectrometry, it has been demonstrated that MegaDalton viruses such as MS2 bacteriophage or Tobacco Mosaic Virus could be manipulated without altering their structure and later viability. Nevertheless, the bibliography emphasizes the lack of a non-destructive detection method able to detect such MegaDalton molecules, especially in a low charge state ( $Q < 6$ ) (Fig. 1) as it can be found in its native environment.

### **Methods**

The detection principle we propose is intended to fill this gap by providing a non-destructive detection method for MegaDalton molecules in low charge state. The detection is based on a laser-cooled and trapped ensemble of ions perturbed by the analyte (Fig. 2). When the injected analyte interacts with the ensemble of ions via the coulomb interaction, it perturbs its motion, induces a heating, which in turn induces a significant change in the fluorescence level of the ensemble.

### **Preliminary data (results)**

A numerical study reproducing the interaction between an individual 1 MDa, singly-charged molecular ion, and an ensemble of 1024  $\text{Ca}^+$  ions, provides a proof-of-concept for the detection principle we propose. In those simulations, the measured change in the fluorescence level is induced by the temperature increase of the ion ensemble by six orders of magnitude. This heating is triggered by the coulomb interaction between the molecular ion and the target ions of the detector, then is amplified by the radio-frequency heating, which is a phenomenon inherent to ensembles of ions in radio-frequency traps. Those simulations allow to explore the realistic set of parameters for which the detector provides an efficient detection.

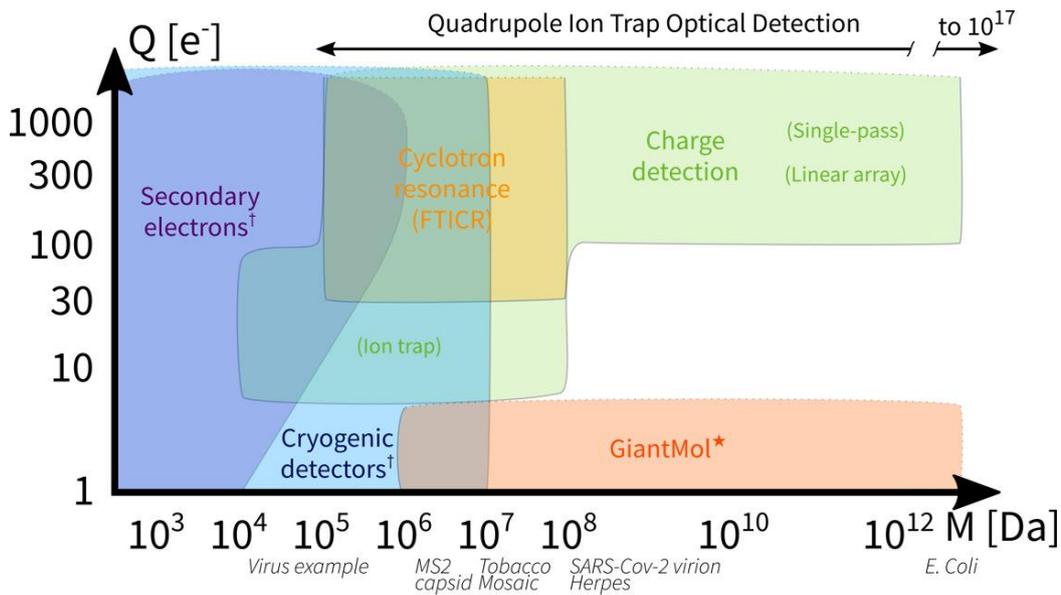
The experimental setup intended to test this detector uses an electrospray to produce the molecular ions in gas phase.

### **Please explain why your abstract is innovative for mass spectrometry?**

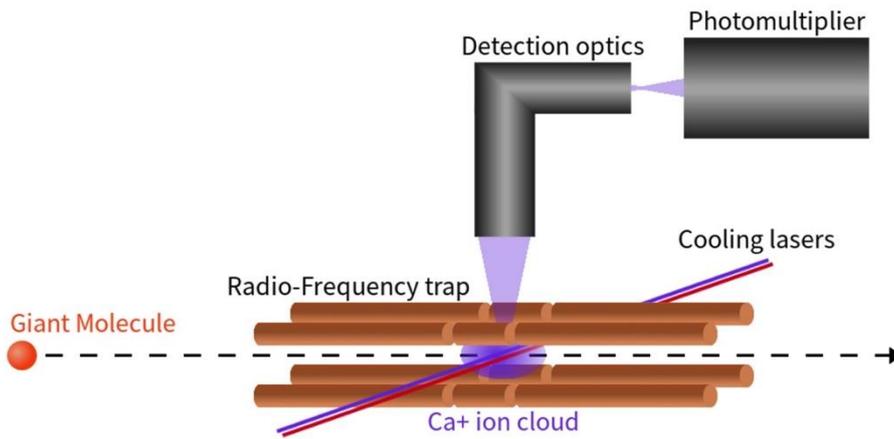
The invention of Trapped Ion Cloud Fluorescence (TICF) detector is patented and a prototype is currently under development.

### **Co-authors:**

*Jofre Pedregosa-Gutierrez, Aix-Marseille Université, CNRS, PIIM*  
*Aurika Janulyte, Aix-Marseille Université, CNRS, PIIM*  
*Christophe Jouvét, Aix-Marseille Université, CNRS, PIIM*  
*Martina Knoop, Aix-Marseille Université, CNRS, PIIM*  
*Caroline Champenois, Aix-Marseille Université, CNRS, PIIM*



Comparative performances of single-molecule mass spectrometry techniques (ours is GiantMol).



Detection principle : perturbation of an ion ensemble by the analyte.

Poster number: **IM-PB-026**

## KAPPA/LAMBDA LIGHT-CHAIN TYPING IN ALZHEIMER'S DISEASE

Abstract ID: **836****Presenting author: Zelal Zuhail Kaya, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University**

### Introduction

Alzheimer's disease is a progressive neurodegenerative disorder characterized by memory loss and cognitive impairment. The diagnosis of Alzheimer's disease according to symptomatic events is still a puzzling task. Developing a biomarker-based, low-cost, and high-throughput test, readily applicable in clinical laboratories, dramatically impacts the rapid and reliable detection of the disease. This study aimed to develop an accurate, sensitive, and reliable screening tool for diagnosing Alzheimer's disease, which can significantly reduce the cost and time of existing methods

### Methods

We have employed a MALDI-TOF-MS-based methodology combined with a microaffinity chromatography enrichment approach using affinity capture resins to determine serum kappa ( $\kappa$ ) and lambda ( $\lambda$ ) light chain levels in control and patients with AD.

### Preliminary data (results)

We observed a statistically significant difference in the kappa light chain over lambda light chain ( $\kappa$ LC/ $\lambda$ LC) ratios between patients with AD and controls (mean difference -0.409; % 95 CI:- 0.547 to -0.269;  $p < 0.001$ ). Our method demonstrated higher sensitivity (100.00%) and specificity (71.43%) for discrimination between AD and controls. We have developed a high-throughput screening test with a novel sample enrichment method for determining  $\kappa$ LC/ $\lambda$ LC ratios associated with AD diagnosis. Following further validation, we believe our test has the potential for clinical laboratories.

### Please explain why your abstract is innovative for mass spectrometry?

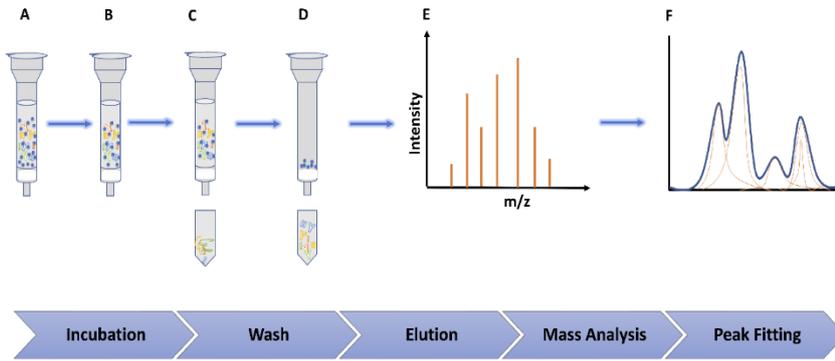
- a sensitive, cost-effective, and highly analytical performance MALDI-TOF MS-based method that includes a high selective resin enriched spin
- biomarker-based low-cost and high-throughput method

### Co-authors:

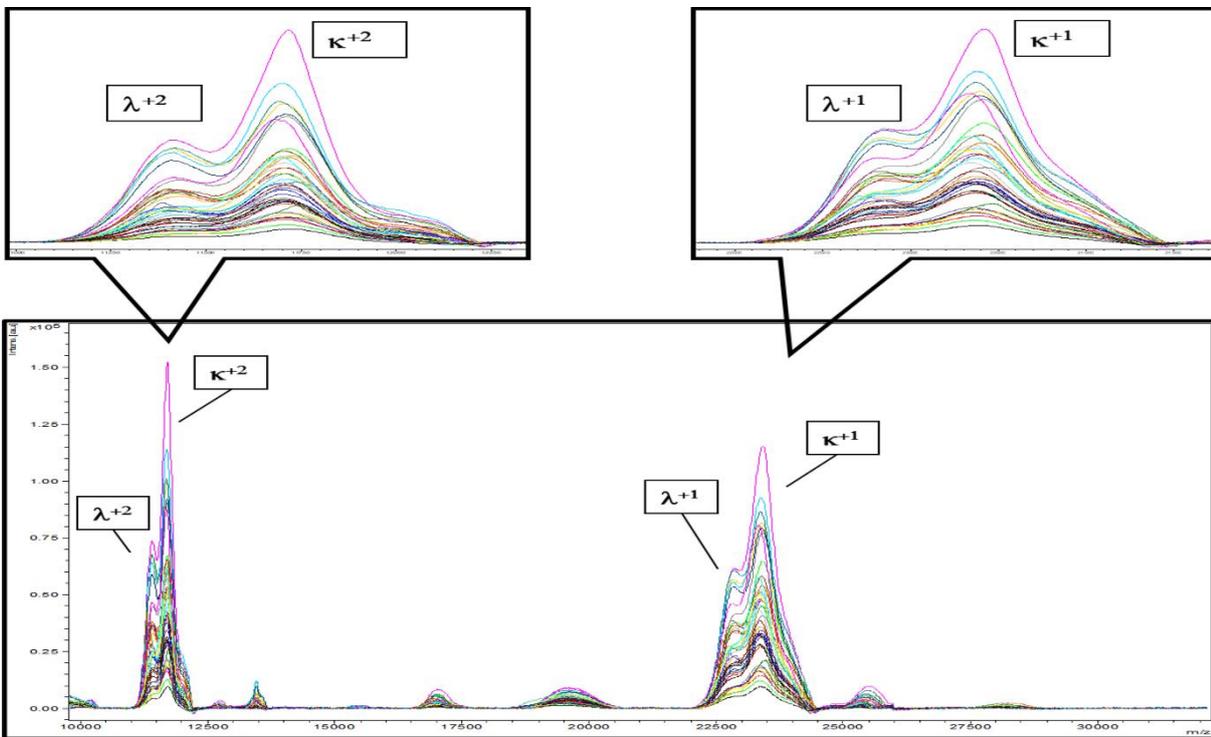
*Mete Bora Tuzuner, Acibadem Labmed Clinical Laboratories**Betul Sahin, Acibadem Labmed Clinical Laboratories**Emel Akgun, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, Acibadem Labmed Clinical Laboratories**Fehime Aksungar, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, Acibadem Labmed Clinical Laboratories**Sebile Koca, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University**Muhittin Serdar, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University**Sevki Sahin, Department of Neurology, School of Medicine, Maltepe University**Nilgun Cinar, Department of Neurology, School of Medicine, Maltepe University**Sibel Karsidag, Department of Neurology, School of Medicine, Maltepe University**Hasmet Ayhan Hanagasi, Department of Neurology, Istanbul Medical Faculty, Istanbul University**Meltem Kilercik, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, Acibadem Labmed Clinical Laboratories**Mustafa Serteser, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, Acibadem Labmed Clinical Laboratories**Ahmet Tarik Baykal, Department of Medical Biochemistry, Faculty of Medicine, Acibadem Mehmet Ali Aydinlar University, Acibadem Labmed Clinical Laboratories*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Study design and workflow of this study



Representative superimposed spectra of control and patient samples

Poster number: **IM-PB-027**

## **SIMPLIFYING CANNABIS & HEMP MASS SPECTROMETRY WORKFLOWS WITH LABORATORY AUTOMATION TO MEET ISO/GMP STANDARDS AND REDUCE EXPERIMENTAL ERROR**

Abstract ID: **846**

**Presenting author: Derek Mattern,**

### **Introduction**

With regulated cannabis markets continuing to open globally, compliance labs are increasingly required to test over 1000 samples a month, and managing sample and data workflows can be challenging and time-consuming. With dedicated fully automated mass spectrometry workflows, designed specifically for pesticide and mycotoxin testing, automation enables a cannabis lab to minimize errors, improve data quality, reduce hands-on time, and increase throughput and reproducibility. This presentation will present new data and material to show the time savings, the benefit of adding hardware automation to reduce labor requirements, and adding cloud-based software solutions to integrate data across the ISO or GMP lab environment. References will show that having a verifiable method for the cannabis testing industry is key to ensuring the safest products reach the consumer.

### **Methods**

High-Performance Liquid Mass Spectrometry, Triple Quad, Sample Prep Automation

### **Preliminary data (results)**

LC/MSMS ESI and APCI methods will be presented to meet US and Canadian Regulatory Testing Requirements

### **Please explain why your abstract is innovative for mass spectrometry?**

Full sample preparation automation, no human intervention required. Suitable for compliance production lab environments.

### **Co-authors:**

*Toby Astill, PerkinElmer*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



PerkinElmer's QSight 420 LC/MSMS



PerkinElmer Janus G3 420 fully automates sample preparation

Poster number: **IM-PB-028**

## **DETERMINATION OF 1,4-DIOXAN IN COSMETICS PRODUCT, SHAMPOO AND TOOTHPASTE BY GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)**

Abstract ID: **862**

**Presenting author: Abdullah Bawazir, Researcher at Saudi Food and Drug Authority**

### **Introduction**

1,4-Dioxane is one of the chemicals encountered in various cosmetic products, this chemical may be formed during the storage and packaging, by regulations, 1,4-dioxane is considered one of the restricted chemical, and it may be allowed at maximum of 10 parts per million (ppm) according to the Gulf Standard for Cosmetics. In this study, we present the development of a method for analysing cosmetic products to detect 1,4-dioxane in shampoos and toothpastes. The performance of the developed method was evaluated by analysing many brands in the local Saudi market. The developed method utilizes the power of GC-MS and the method demonstrated high sensitivity and reliability results

### **Methods**

Basically, the most common compounds including 1,4- Dioxane were analysed by using Agilent 5975 Gas Chromatography Mass Spectrometry GC-MS. Separation was carried out on a DB-624MS capillary column obtained from Agilent J&W GC columns, In addition. The Internal Standard was used to minimize the interferences effects from other peaks on the matrix. The samples were prepared by weighting 0.2 g in 5 mL acetonitrile, then 1,4-dioxane was extracted by water bath assisted to facilitate the dissolution at 40°C for fifteen minutes and then the clear solution was injected in GC-MS.

### **Preliminary data (results)**

Validation parameters namely; linearity, accuracy, precisions, matrix effect, and measurement of uncertainty showed satisfactory results. The linearity was  $\geq 0.995$  and  $\geq 0.993$  in solvent and matrix, respectively. The accuracy ranged from 95 – 120 % in both solvent and matrix. Precision for 1,4-dioxane in terms of relative standard deviation (RSD) was below 10 % and the matrix effect of 1,4-dioxane was below 20 %. During the validation, 30 different samples from shampoos and kinds of toothpaste were purchased from the local markets, in order to identify and quantify 1,4-dioxane. The extraction of the developed method illustrated satisfactory inter-day and intra-day precisions, along with an acceptable range of standard deviation (SD) and RSD.

### **Please explain why your abstract is innovative for mass spectrometry?**

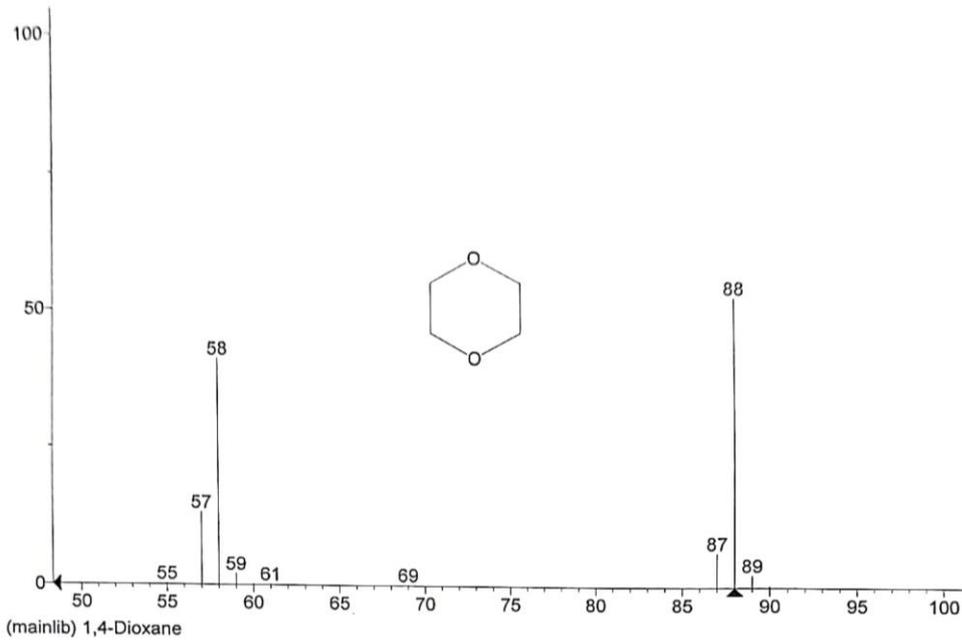
The method was developed to gas chromatography mass spectrometry, which is more accurate in its results, selectivity, accuracy and least in bias.

### **Co-authors:**

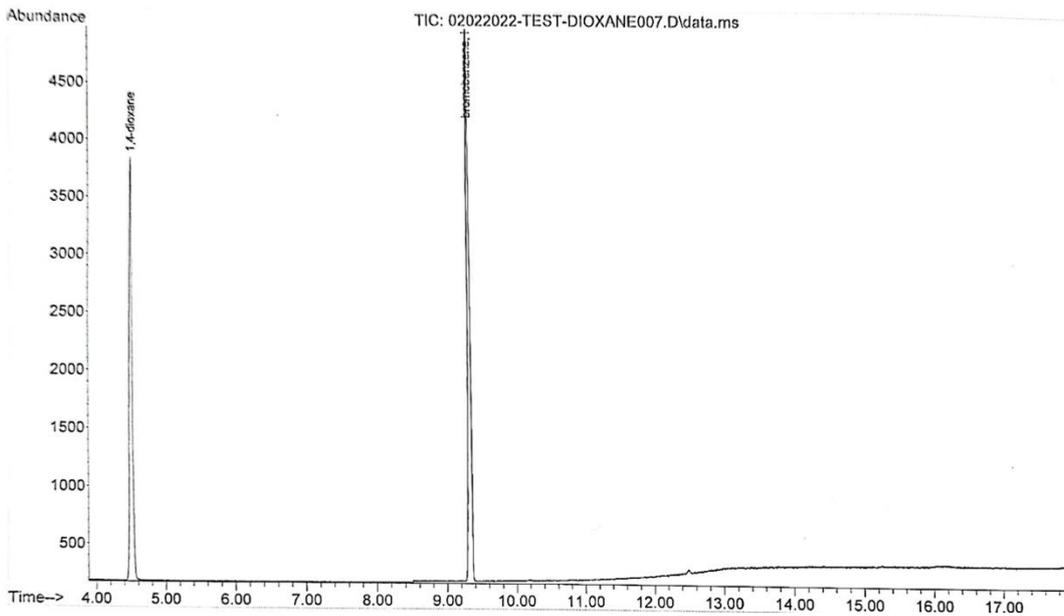
*Adnan AL-Mussallam, Head of Department at Saudi Food and Drug Authority*  
*Rawan Al-Shethri, Researcher at Saudi Food and Drug Authority*  
*Fahad Al-Dawsari, Director Manager at Saudi Food and Drug Authority*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Gas chromatography instrument Library Identified The 1,4-Dioxane Mass Spectrum.



Peaks of the Standard (1,4-Dioxane) and the IS (Bromobenzen).

Poster number: **IM-PB-029**

## **VALIDATION AND QUANTIFICATION OF RHODAMINE B IN COSMETICS CONSUMER PRODUCTS**

Abstract ID: **869**

**Presenting author: Adnan Al-Mussallam, Head of Cosmetic product Department "Saudi Food & Drug Authority"**

### **Introduction**

Rhodamine B is a basic red cationic dye which is highly soluble in water, methanol and ethanol. Rhodamine B is harmful if swallowed by humans and animals. Moreover, it is a carcinogen colourant and can cause irritation to the skin, eyes and respiratory tract. The analytical method for rhodamine B was employed by using infinity high-performance liquid chromatograph (HPLC) comprising a G1315D UV detector, as a result of its multiple effects on the human health, rhodamine B classified as illegal dye in cosmetics. Therefore, analytical methods for quantitative analysis of rhodamine B is crucial to be developed to ensure cosmetic products safety.

### **Methods**

. Separation was carried out on a Zorbax column . The gradient mobile phase was (A) 100mM ammonium formate buffer pH 5.4 (B) methanol– acetonitrile, delivered at flow rate of 1.00 ml/min, using column temperature at 40 °C, and the injection volume was 10.00 µl. The detection wavelength was set at 520-570 nm. The standard of rhodamine B was purchased from Sigma Aldrich as well as ammonium formate methanol and acetonitrile. The standard was prepared in water while the preparation of samples was as 0.2 g in 5 mL of water, utilizing solid liquid extraction by using Ultrasound extraction assisted.

### **Preliminary data (results)**

The optimized HPLC condition was validated by assessing several performance characteristics including selectivity, linearity, and sensitivity expressed by the limit of detection and limit of quantification, precision, and accuracy. The validation study demonstrated that the method was able to achieve satisfactory linearity in both solvent and samples with  $r^2$  more than 0.995. Recoveries were in a range of 85-115 % in both solutions and samples. The repeatability and intermediate precision in both solution and samples were less than 5% in terms of relative standard deviation. The developed method was applicable to achieve accurately repeatable results during inter and intraday analysis. Importantly, during the validation, 20 different lip-care products were purchased from the local market in Riyadh, Saudi Arabia, and have been used to identify and quantify the presence of rhodamine b in these products.

### **Please explain why your abstract is innovative for mass spectrometry?**

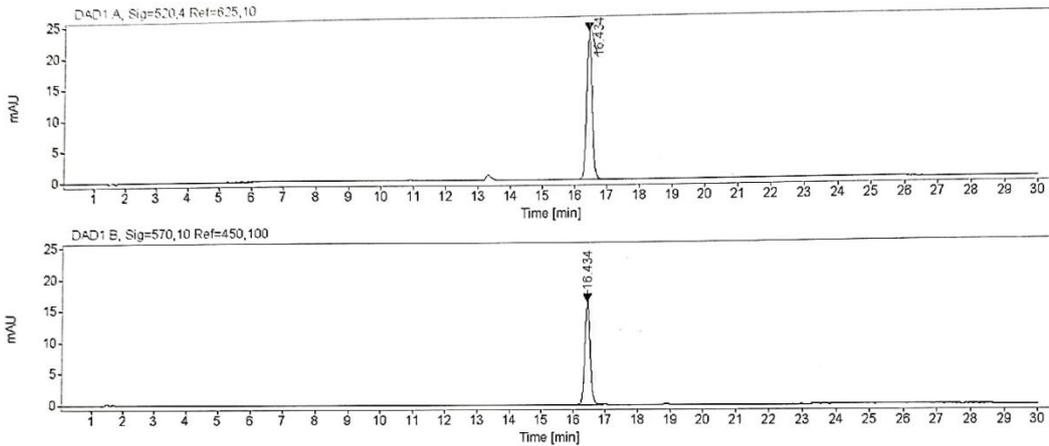
Quantification of rhodamine B in cosmetics consumer products demonstrated acceptable statistical criteria, rendering this method suitable for routine analysis. Finally, performance of the developed method was assessed using real samples.

### **Co-authors:**

*Rawan Al-Shathri, Lab Researcher "Saudi Food & Drug Authority"*  
*Fahad Al-Dawsari, Director Manager of Reference Lab "Saudi Food & Drug Authority"*  
*Abdullah Bawazir, Lab Expert Researcher "Saudi Food & Drug Authority"*

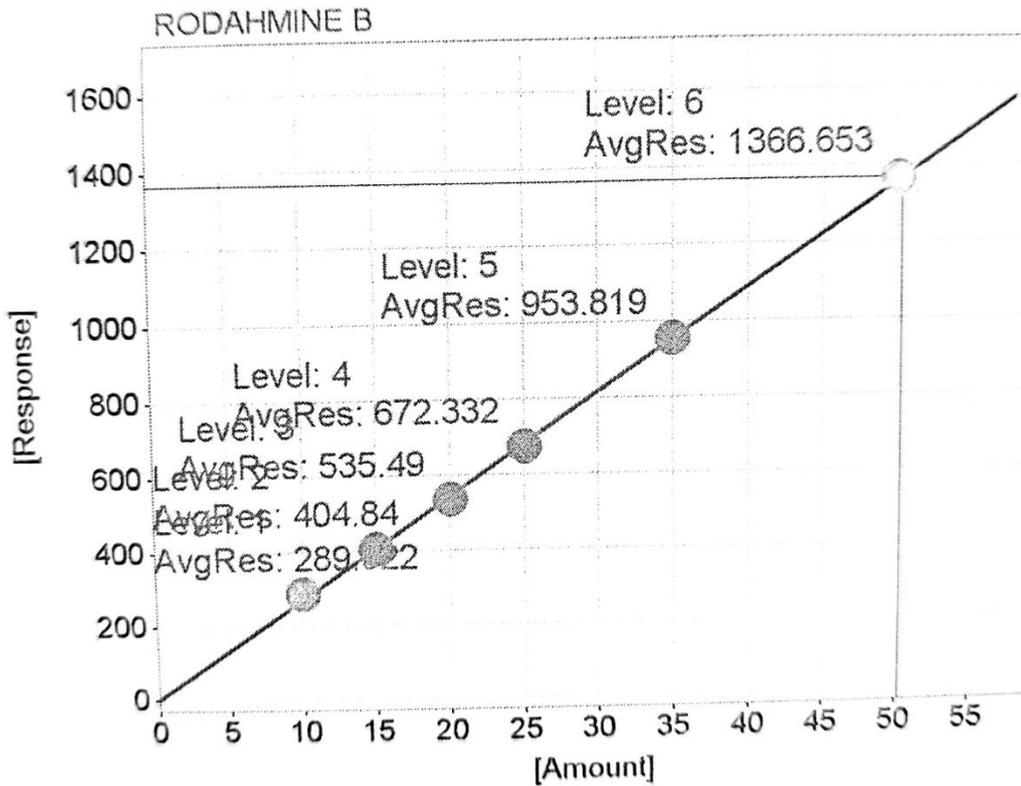
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Thursday 1 September 2022 from 14:00 to 15:30 hours



Name : RHODAMINE B

Standard Chromatogram of Rhodamine B



Calibration Curve of Rhodamine B

Poster number: **IM-PB-030**

## **A REAL-TIME GAS MONITORING SYSTEM BASED ON ION MOBILITY SPECTROMETRY FOR WORKPLACE ENVIRONMENTAL MEASUREMENTS**

Abstract ID: **873**

**Presenting author: Kazunari Takaya, National Institute of Occupational Safety and Health, JAPAN**

### **Introduction**

We have developed a volatile organic compounds (VOC) gas monitor by using an ion mobility spectrometer (IMS). The aims of this study are first to realize real-time measurement of the concentration of chemical substances known to have a health impact on workers, and second to examine in detail the short-term exposure to chemical substances that is difficult to achieve with conventional GC/MS analysis. In this study, the identification capability, response time, and quantitative accuracy of the VOC gas monitor based on IMS were assessed for practical use in actual work sites.

### **Methods**

The device was evaluated based on three aspects: identification capability, response time, and quantitative accuracy. For identification capability, methyl ethyl ketone, acetone, and ethanol, each of which has been widely used in many factories, were selected as sample vapors. The mobility was obtained from the arrival time of each substance. For response time, the time taken to detect the spectral shift after sample introduction / elimination was evaluated. For quantitative accuracy, we obtained a calibration curve derived from the shift of the nominal arrival time spectrum of methyl ethyl ketone overlapped with that of water clusters.

### **Preliminary data (results)**

The arrival time spectra of three sample gases were measured under the identical conditions of ambient temperature, humidity, and device setup. The peak position was observed to change depending on the chemical substance. Since the maximum measurement error of this device is very small (approximately 1%), quantitative analysis can be performed by using VOC gas monitor based on IMS.

For response time, the peak has completely shifted 10 s after the introduction of methyl ethyl ketone. By only 10 s after elimination, the peak position has completely returned to the background RIP (reactant ion peak) position. This means that methyl ethyl ketone can be analyzed in about every 10 s using this device. Therefore, the real-time monitoring difficulties with GC/MS in a work environment can be overcome by using this device.

For quantitative accuracy, we obtained a calibration curve of methyl ethyl ketone. Because saturation of methyl ethyl ketone has already occurred near the 300 ppm, the concentration cannot be estimated based on the ion intensity of the spectrum. Therefore, the concentration of methyl ethyl ketone was estimated from the peak shift amount, and the calibration curve with high accuracy was obtained. This means that the error is small in the range from 100 to 500 ppm and accurate measurements can be performed. It was confirmed that methyl ethyl ketone could be adequately monitored in real-time in a work environment by using this device.

### **Please explain why your abstract is innovative for mass spectrometry?**

We proposed a quantitative analysis technique using the calibration curve obtained from the nominal arrival time shift of the spectrum of a chemical substance overlapped with that of water clusters.

### **Co-authors:**

*Masayoshi Hagiwara, National Institute of Occupational Safety and Health, JAPAN*  
*Shiro Matoba, High Energy Accelerator Research Organization, KEK*  
*Mitsutoshi Takaya, National Institute of Occupational Safety and Health, JAPAN*  
*Nobuyuki Shibata, National Institute of Occupational Safety and Health, JAPAN*

Poster number: **IM-PB-031**

## **ENHANCED PERFORMANCE OF A TRIPLE STAGE QUADRUPOLE MASS SPECTROMETER WITH A NOVEL AXIAL FIELD COLLISION CELL AND FAST SWITCHING HIGH VOLTAGE POWER SUPPLIES**

Abstract ID: **907****Presenting author: Harald Oser, Thermo Fisher Scientific**

### **Introduction**

Performance of a collision cell is a significant determinant of product ions production by collision-induced dissociation (CID) for MS<sup>2</sup> analysis. Both parent and product ions need to maintain stable trajectories inside the cell to insure maximum transmission. We present a newly developed PCB collision cell with improved electrode profiles and overall mechanical design which enhance several aspects of performance including sensitivity and robustness. Practical benefits of the new ion optical system and speed improvements due to newly introduced fast switching high voltage power supplies have been verified using regulated method for the analysis of Haloacetic acids(HAA). HAAs are among the disinfection byproducts produced (DBP) during chlorination of water containing natural organic matter and bromide.

### **Methods**

New electrode profiles have been optimized using ion simulations to achieve a wide m/z transmission. Balancing both RF field responsible for containing ions and DC field penetration from DC drag vanes has been used to achieve the best SRM performance. The new collision cell and power supplies have been evaluated in TSQ MS systems using a wide variety of compounds. Emphasis was placed on finding optimum RF amplitude settings that balance precursor and product transmission. We executed quantitation of HAAs, Bromate and Dalapon in water with IC-MS/MS using Thermo Scientific™ ICS6000 and TSQ Fortis™ MS based prototype system.

### **Preliminary data (results)**

Tests confirmed improvements in the width of m/z transmission. This benefits transitions where there is a large difference between parent and product masses. The observed speed enhancement is attributed to better penetration of axial DC gradient into the ion path. The robustness of the new design is likely to provide more uniform performance with less need for optics tuning.

The HAAs, bromate and dalapon has been analyzed on a TSQ Fortis Plus and a TSQ Fortis system. A 100 µL sample was injected onto a 2 x 250 mm Dionex™ IonPac AS31 analytical column, which is specifically designed to separate method analytes from the common anions in water. The LLOQ was defined as the lowest concentration at which the back calculated calibrator concentration on a linear calibration curve was within 20% of theoretical, and replicate injections have a %RSD of less than 15%. HAA workflow having low mass fragment ions, shows promising improvement in the lower limit of quantitation (LLOQ) with the TSQ Fortis Plus system compared to the TSQ Fortis MS. Eight out of eleven analytes had two-fold improvement in LLOQ, while the remaining three analytes had the same LOQ but with a response of >30% higher in Fortis-based prototype system. Negative ion transitions for TBAA, DCAA, MBAA and MCAA respectively showed >50% improvement in the prototype system.

An acquisition method based on a subset of system-suitability-test assay was used to evaluate polarity switching time for low and high m/z ions with signal stabilization under 5 ms.

### **Please explain why your abstract is innovative for mass spectrometry?**

A novel PCB based collision cell and fast switching high voltage power supplies have been developed and implemented in a triple stage quadrupole mass spectrometer (TSQ MS).

### **Co-authors:**

*Oleg Silivra, Thermo Fisher Scientific*  
*Hans Schweingruber, Thermo Fisher Scientific*  
*Michael Ugarov, Thermo Fisher Scientific*  
*Neloni Wijeratne, Thermo Fisher Scientific*  
*Claudia Martins, Thermo Fisher Scientific*

Poster number: **IM-PB-032**

## **HYPHENATION OF ION MOBILITY MASS SPECTROMETRY AND ACTION SPECTROSCOPY TO PROBE STRUCTURE AND KINETICS OF PEPTIDE AGGREGATION**

Abstract ID: **953**

**Presenting author: Sjors Bakels, Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HV, Amsterdam**

### **Introduction**

A key physical process in the human body is the aggregation of peptides and proteins, the transition from soluble functioning proteins into insoluble amyloid aggregates. This unavoidable build-up of aggregates is directly linked to age-related, neurodegenerative diseases, including Alzheimer's and Parkinson's disease. Gaining full understanding of the early, neurotoxic steps of the aggregation process is essential as this can lead to its control and prevention. However, this knowledge is obscured by a cascade of events occurring at various time and energy scales, producing complex and heterogenous mixtures of aggregates. Therefore, we are developing a novel, multidimensional spectroscopy- and mass spectrometry-based method, that allows us to probe the structure and kinetics of the initial steps of the aggregation process in a single measurement, see Figure 1.

### **Methods**

To develop the Photo-Synapt, a Waters Synapt G2 ion-mobility time-of-flight mass spectrometer was modified in collaboration with MSVision providing optical access and to allow for trapping of ions (see Figure 2 for a photo of the instrument). This was done by adding two hexapoles coupled with pin traps in differentially pumped stages after the mobility stage. Mass- and ion mobility selected ions can therefore be stored. Optical access ports were added to allow irradiation by UV or IR photons. To precisely control the gas pressure in the irradiation cell additional gas inlets were attached.

### **Preliminary data (results)**

The addition of the hexapoles to the instrument changed the characteristics of the instrument in terms of required voltages, timings, and pressures. The first task at hand was therefore to benchmark the system by comparing it to unmodified instruments. We did this for some well-studied molecules such as bradykinin and cytochrome c. Our results show that we can very well reproduce mass selected ion-mobility measurements.

The abovementioned complex mixtures consist of peptides of different masses, but also of differently folded peptides with the same  $m/z$ . To be able to investigate these different conformers we need to be able to slice a particular part of the ion mobility spectrum of a mass selected peptide, thereby only letting the ions of interest enter the hexapole pin traps.

After the slicing, the trapping conditions were optimized, and sequence files were created to control the voltages in the hexapoles. These were designed to allow for optimal storage of the ions and subsequent release into the time-of-flight mass spectrometer to detect the fragment ions. Some design considerations will be touched upon in the poster.

The final step is to perform action spectroscopy on the mass- and shape selected ions in the trap. We present UV photo-dissociation experiments on peptides and proteins where good sequence coverage is obtained. IR multi-photon dissociation experiments are currently on their way.

### **Please explain why your abstract is innovative for mass spectrometry?**

The development of a novel, multidimensional ion mobility mass spectrometry approach to probe the structure of peptide aggregation in a single experiment.

### **Co-authors:**

*Steven Daly, MS Vision, Televisieweg 40, 1322 AM Almere, Netherlands*

*Agathe Depraz Depland, Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life*

## POSTER SESSION B

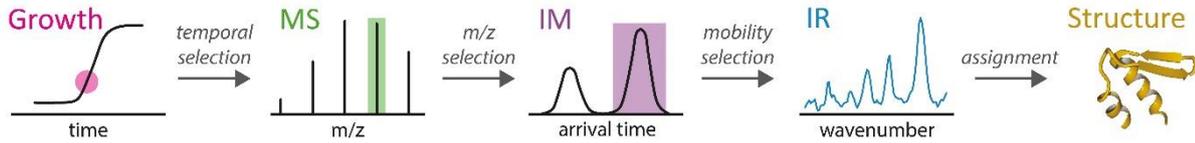
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Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HV, Amsterdam

Iuliia Stroganova, Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HV, Amsterdam

Jan Commandeur, MS Vision, Televisieweg 40, 1322 AM Almere, Netherlands

Anouk M. Rijs, Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HV, Amsterdam



Schematic workflow of the newly built instrument.



Photo of the MSLaserLab with the Photo-Synapt in the center.

Poster number: **IM-PB-033**

## COMPACT, HIGH-RESOLUTION TIME OF FLIGHT MASS SPECTROMETER BASED ON AN ELECTROSTATIC ANALYSER.

Abstract ID: **1008**Presenting author: **Steven Daly, MS Vision, Televisieweg 40, 1322AM**

### Introduction

Time-of-flight (TOF) mass spectrometry is one of the cornerstones of mass spectrometry, combining a rapid analysis speed with high resolution and an unlimited mass range. The resolution of a TOF is determined by the flight time of the ions. This means that the highest resolution TOFs will have flight tubes several metres long. The use of a reflectron can increase the flight time without significantly increasing the footprint. This means that high resolution, compact TOF mass spectrometers based on a reflectron is not feasible. Here, we present the design and theoretical performance of a TOF mass spectrometer based on an electrostatic analyser (ESA). This instrument was conceived to provide a resolution of greater than 10,000 with a footprint of 450x190x180 mm (*lxhxw*).

### Methods

The conceptual design was converted into a 3D CAD drawing which can be mounted into the SPIDOC chamber to be used as a diagnostic TOF. The design of the instrument was evaluated by ion optics simulations using a combination of the SIMION and SIMAX programs. The model was first tested for ultimate performance using different idealised ion populations. Subsequently, realistic ion populations expected from the SPIDOC experiment were modelled to indicate expected real work performance.

### Preliminary data (results)

The design was conceived to provide a compact, high resolution diagnostic TOF within the SPIDOC project. The footprint available was incompatible with a TOF with high enough resolution. The design, based on that of Poteshin et al.[1], using orthogonal acceleration coupled to an ESA. By choosing the correct geometry of pusher, flight tube and ESA allows to perform second order time focusing, providing the high resolution required. This led to the TOF design shown in Figure 1.

The design was validated using SIMION and SIMAX simulations. The model was validated by calculating the expected dependences of flight time and resolution on  $m/z$ . The performance of the TOF was then explored. The dependence of the resolution on the initial energy spread of the ions in all 3 dimensions was explored, finding that resolutions as high as 40,000 was found for idealised ion distributions. The sensitivity of initial ion position was also explored with similar results. These provided a baseline for ideal performance. Real world performance as tested by simulating ions entering the TOF from the interaction region of the SPIDOC experiment, providing a stringent test as ion focusing into the pusher is difficult, giving values between 5,000 and 9,000. These values will be higher in a design with proper pusher optics, with simulations suggesting resolutions as high as 20,000 can be achieved.

[1] Poteshin, S.S., Chernyshev D.M., Sysoev, A.A., Sysoev, A.A., *Physics Procedia*, 72, 2015, 266-273. DOI: 10.1016/j.phpro.2015.09.084

### Please explain why your abstract is innovative for mass spectrometry?

The development of a high resolution TOF with a very small footprint will allow to design a compact tabletop high resolution TOF mass spectrometer.

### Co-authors:

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Figure 1. CAD model of proposed TOF design.

## Session: Nico Nibbering session on ion chemistry

Poster number: **IM-PB-034**

### **STUDY OF POLLEN PHENOLAMIDES BY TANDEM MASS SPECTROMETRY**

Abstract ID: 76

**Presenting author: Irène Semay, Organic Synthesis and Mass Spectrometry Laboratory (S<sup>2</sup>MOs), University of Mons**

#### **Introduction**

Phenolamides (PA) constitute one of the major classes of phenylpropanoid metabolites found in nature. They are composed of the association of at least one hydroxycinnamic acid derivative linked through an amide bond to an aromatic monoamine or an aliphatic polyamine (Figure 1). Those specialized metabolites play crucial roles in plant protection and development, and are especially present in pollen, therefore impacting bee nutrition. However, their structural characterization is still a challenge as natural extracts often contain a large number of congeners presenting only subtle structural differences. Moreover, being sensitive to UV irradiation, phenolamide derivatives undergo efficient *trans-cis* isomerization (Figure 1). In this study, we developed mass spectrometry techniques, including CID and IMS, to thoroughly establish the PA structures, including regio- and stereoisomers.

#### **Methods**

*Crataegus monogyna* Jacq. bee pollen (Figure 2) was used as a natural PA source, through a methanolic extraction. Additionally, reference PA were synthesized by reacting aliphatic di- or triamines with different hydroxycinnamic acids, including ferulic acid and caffeic acid (Figure 2). The extracted and synthesized PA were analyzed by LC-MS, in both the positive and negative ionization modes. Collision-induced dissociation experiments were performed to identify key fragmentations. Ion mobility mass spectrometry experiments were also carried out to discriminate regio- and stereoisomers. All the experiments were conducted on a Waters Synapt G2-Si mass spectrometer.

#### **Preliminary data (results)**

CID mass spectra on the negatively charged ions  $[M-H]^-$  are observed to be more structurally informative than the CID spectra on the cations  $[M+H]^+$ . As for a typical example, when facing the typical case of a triamine backbone, such as spermidine, with different hydroxycinnamic acid derivatives on the three nitrogen atoms, it is possible to establish whether a given substituent is positioned on the central nitrogen atom or on the two distal nitrogen atoms, making a huge difference with the generally-used CID analysis in the positive ion mode, where transamidation reactions hinder the distinction between  $[M+H]^+$  regioisomers. We associate a mechanistic study to our experimental results to rationalize our findings.

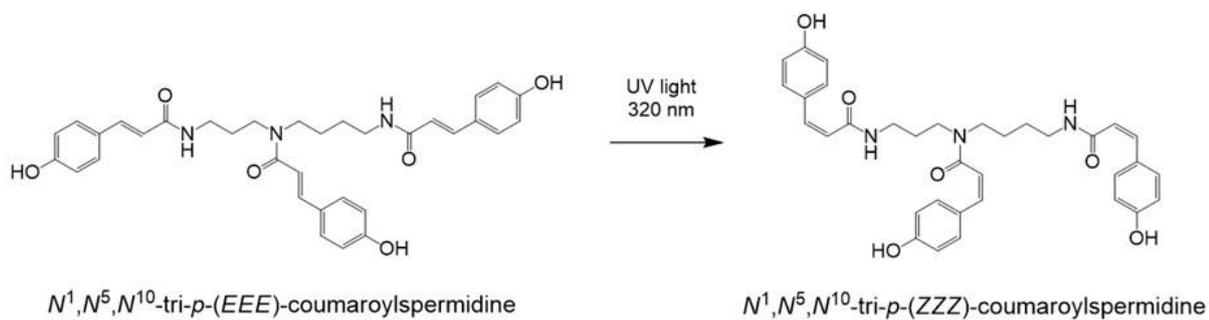
As far as the *cis* and *trans* stereoisomers are concerned, ion mobility experiments are used for the first time to successfully resolve the PA isomers, especially when considering the  $[M+Na]^+$  ions. Additionally, our experiments highlight that the shorter the amine backbone is, the better the ion mobility separation between stereoisomers.

#### **Please explain why your abstract is innovative for mass spectrometry?**

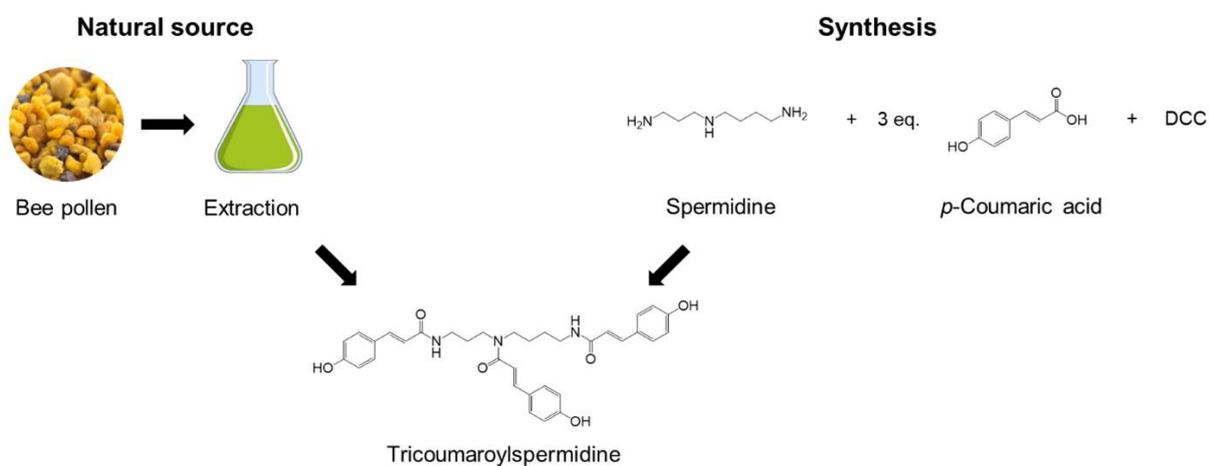
Development of CID and ion mobility experiments for the structural characterization of phenolamides from pollen, including regioisomer and stereoisomer discrimination.

#### **Co-authors:**

Maryse Vanderplanck, Functional and Evolutive Ecology Center, University of Montpellier  
Denis Michez, Zoology Laboratory, University of Mons  
Pierre Duez, Therapeutic Chemistry and Pharmacognosy Laboratory, University of Mons  
Pascal Gerbaux, Organic Synthesis and Mass Spectrometry Laboratory (S<sup>2</sup>MOs), University of Mons



Trans-cis isomerization of tricoumaroylspermidine



Natural and synthetic origin of tricoumaroylspermidine

Poster number: **IM-PB-035**

## SUPERELECTROPHILIC ANIONS: UNCONVENTIONAL BINDING PROPERTIES OF $[B_{12}X_{11}]^-$ FRAGMENT IONS

Abstract ID: 122

**Presenting author: Markus Rohdenburg, Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, D-04103, Leipzig, Germany**

### Introduction

*Closo*-dodecaborate dianions  $[B_{12}X_{12}]^{2-}$  ( $X=H$ , (pseudo-)halogen) are highly stable multiply charged anions (MCAs) with various applications in e.g., chemistry and health sciences. In recent years, extensive research was dedicated to properties and applications of  $[B_{12}X_{11}]^-$  fragment ions that can be generated in the gas phase of mass spectrometers via collision-induced dissociation (CID) out of the respective  $[B_{12}X_{12}]^{2-}$  precursors.  $[B_{12}X_{11}]^-$  ions have been characterized as superelectrophilic anions: Although possessing an overall negative charge, they exhibit a reaction behavior that can typically be observed for highly electrophilic cations, i.e., binding of even weak nucleophiles. The vacant boron atom that results from cleavage of one  $X^-$  substituent is substantially positively charged and thus acts as nucleophilic center of the ion.

### Methods

We employed CID in ion traps of mass spectrometers to allow for fragmentation of  $[B_{12}X_{12}]^{2-}$  ions yielding  $[B_{12}X_{11}]^-$  monoanions. We then investigated the binding of various nucleophilic species to the vacant boron atom by introducing respective gases (including noble gases, carbon monoxide, dinitrogen and water) to the ion trap. In addition, we employed infrared photodissociation (IRPD) spectroscopy to study the vibrational spectra of the gas phase adduct ions. Complementing these experiments, we explored the unexpected electrophilicity of the  $[B_{12}X_{11}]^-$  anions by high-level DFT calculations and subsequent analyses of obtained electron densities.

### Preliminary data (results)

DFT calculations predict that the vacant boron atom of  $[B_{12}X_{11}]^-$  constitutes a positive binding site in an overall negatively charged anion.  $[B_{12}Cl_{11}]^-$  binds the noble gases Kr and Xe at room temperature – a reaction previously never observed for anions.<sup>[1]</sup> The highest atomic charge on the vacant boron atom is predicted for  $X=CN$ . In CID experiments,  $[B_{12}(CN)_{11}]^-$  was generated and binds Ar (!) at room temperature. We can thus present a bonding analysis of the first B-Ar bond in an anion. Electrostatics and dispersion play a significant role to explain the Ar binding strength of the anion, as indicated by EDA.<sup>[2]</sup> Going further, even Ne binding to  $[B_{12}(CN)_{11}]^-$  was observed for ion trap temperatures of up to 50 K.<sup>[3]</sup> We also studied  $N_2$  and CO binding to  $[B_{12}X_{11}]^-$  fragment ions by IRPD spectroscopy and analysis of the Natural Orbitals for Chemical Valence (NOCVs). Interestingly, the binding strength to these  $\pi$ -acceptor species is not generally governed by the pure electrophilicity of the vacant boron atom in  $[B_{12}X_{11}]^-$  but rather by a novel type of  $\pi$ -backbonding from the delocalized  $\sigma$ -electron system of the boron scaffold into antibonding orbitals of  $\pi$ -acceptor nucleophiles.<sup>[4]</sup>

[1] M. Rohdenburg et al., *Angew. Chem. Int. Ed.*, 2017, 56, 7980-7985.

[2] M. Mayer et al., *Proc. Natl. Acad. Sci. U.S.A.*, 2019, 116(17), 8167-8172.

[3] M. Mayer et al., *Chem. Commun.*, 2020, 56, 4591-4594.

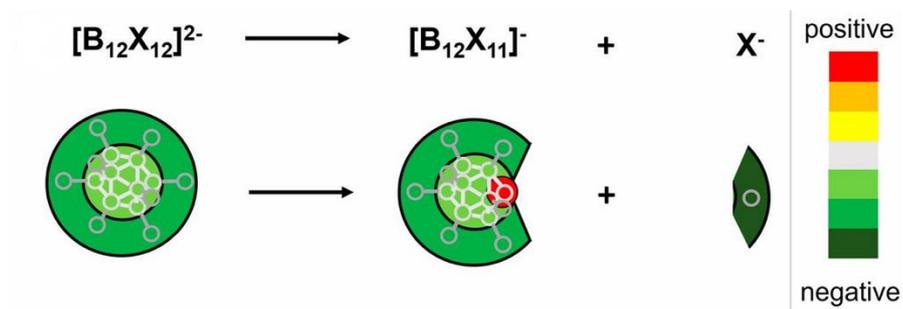
[4] M. Mayer et al., *Chem. Eur. J.*, 2021, 27, 10274.

### Please explain why your abstract is innovative for mass spectrometry?

Mass spectrometry was used to detect and analyze the first anionic Ar compound, which is stable at room temperature.

### Co-authors:

Jonas Warneke, *Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, D-04103, Leipzig, Germany, Leibniz-Institut für Oberflächenmodifizierung e.V. (IOM), Permoserstr. 15, D-04318, Leipzig, Germany*



Fragmentation of  $[B_{12}X_{12}]^{2-}$  yields the superelectrophilic fragment  $[B_{12}X_{11}]^-$ .

Poster number: IM-PB-036

## DEPOSITION AND STABILIZATION OF UNDER-COORDINATED METAL COMPLEXES VIA ION SOFT-LANDING

Abstract ID: 184

**Presenting author: Robert Schiewe, Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, 04103, Leipzig, Germany**

### Introduction

*Ion Soft-Landing* is used to deposit molecular fragment ions, generated via collision-induced dissociation (CID), on surfaces to exploit their exceptional properties in condensed material layers. Fragment ions are usually highly reactive. We explore different possibilities to control their reactivity on surfaces, e.g. by co-deposition with weakly-coordinating counter ions or by using chemically modified surfaces. This poster shows our recent results on ion soft-landing of under-coordinated metal complexes. The generated material layers might be interesting for fundamental studies in catalysis and hydrogen isotope separation.

### Methods

Ion soft-landing is a technique, which allows the deposition of mass-selected ions with controlled energy from the gas phase on conductive surfaces. Electrospray ionization is used to transfer permanently charged organometallic ions into the gas-phase. CID of the ions is initiated in a collision cell. A quadrupole mass filter is used for mass selecting the fragments of interest, which are deposited on the surface.[1] Analysis of the reaction products is performed using Liquid Extraction Surface Analysis (LESA) and different spectroscopic methods.

### Preliminary data (results)

The under-coordinated transition metal complex  $\text{Ru}(\text{bipyridine})_2^{2+}$   $[\text{Ru}(\text{bpy})_2]^{2+}$ , see Figure 1, is generated by using CID of  $[\text{Ru}(\text{bpy})_3]^{2+}$  and deposited on surfaces. The reactivity towards different reagents coated on the surface has been investigated. The under-coordinated Ru center binds efficiently anions, which are present at the surface (e.g.  $\text{Cl}^-$  and carboxylates). Also,  $\text{N}_2$  adducts  $[\text{Ru}(\text{bpy})_2(\text{N}_2)_2]^{2+}$  and  $[\text{Ru}(\text{bpy})_2\text{N}_2\text{Cl}]^+$  were identified in LESA of the deposited material. Frequently, we observed the product  $[\text{Ru}(\text{bpy})_2\text{NO}_2]^+$  in high intensity in our LESA spectra. The abundance of this product was strongly dependent on several parameters, including the use of a self-assembled monolayer on the deposition surface or the co-deposition of weakly coordinating counterions (see Figure 2). The poster leads through our investigation to unravel the formation of this ion, which has not been described in classical synthetic literature of Ru complexes so far.

[1] H. Y. Samayoa-Oviedo et al.: "Design and Performance of a Soft-Landing Instrument for Fragment Ion Deposition." *Anal. Chem.* **2021**, 93, 14489-14496.

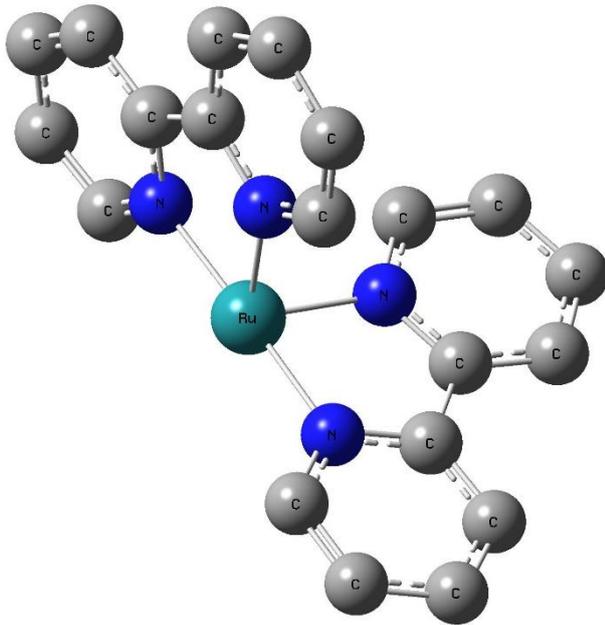
### Please explain why your abstract is innovative for mass spectrometry?

Mass-selected gaseous ions are used to generate surface layers with unique properties using ion soft-landing.

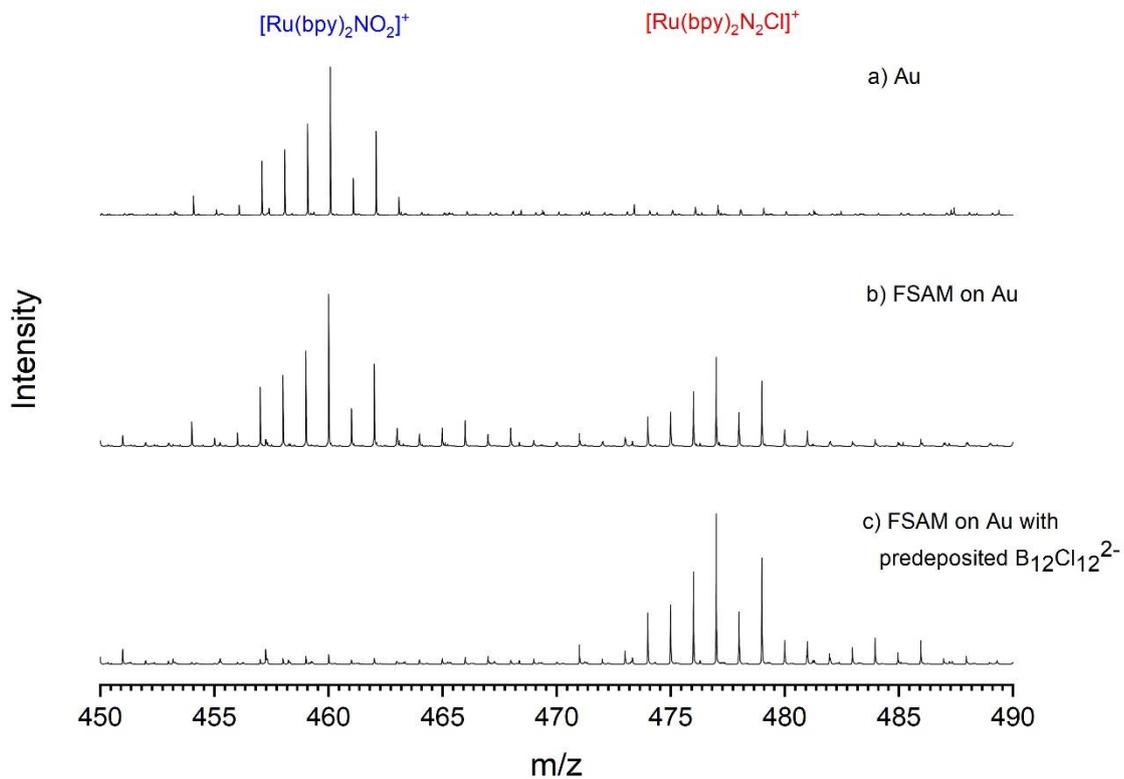
### Co-authors:

Markus Rohdenburg, Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, 04103, Leipzig, Germany

Jonas Warneke, Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, 04103, Leipzig, Germany, Leibniz-Institut für Oberflächenmodifizierung e.V. (IOM), Permoserstr. 15, D-04318, Leipzig, Germany



Structure of  $\text{Ru}(\text{bpy})_2^{2+}$  as predicted by DFT-calculations.



LESA-mass-spectra.  $\text{Ru}(\text{bipyridine})_2^{2+}$  deposited on Au and FSAM.

Poster number: IM-PB-037

## SOFT-LANDING HOST-GUEST COMPLEXES OF CYCLODEXTRINS AND POLYOXOVANADATES AS SINGLE-MOLECULE MEMORY UNITS ON SURFACES

Abstract ID: 206

**Presenting author: Fangshun Yang, Leibniz-Institut für Oberflächenmodifizierung e.V. (IOM), Permoserstr. 15, D-04318, Leipzig, Germany**

### Introduction

Next generation technologies, such as neuromorphic computing, require resistive switching memories with reversible internal electrical resistance. Therefore, metal-oxo clusters, such as Lindqvist-type polyoxovanadates (POVs) are promising for molecular memory devices. However, charge-balancing bulky counterions of POVs are also present on surfaces with solution-based precipitation methods, which leads to agglomeration problems and hinders the formation of arrays of individual switchable anionic POV units on the surface. To address these challenges, we explored the potential of preparing surfaces with mass selected cyclodextrin(CD)-POV host-guest complexes deposited from the gas phase onto surfaces. The CD host provides a structural and electronically stabilizing "shell" for the individual POV anions and this avoids aggregation of the molecular units. This approach is a promising step for evolution of molecular electronics.

### Methods

Ion Soft-Landing is a method, which allows the deposition of intact mass-selected polyatomic ions onto substrates with precisely controlled composition, charge state and kinetic energy. POV ions are transferred to the gas phase using electrospray ionization (ESI). Ions are selected using a resolving quadrupole and deposited with controlled kinetic energy onto conductive surfaces.

### Preliminary data (results)

We transferred the complex of  $\beta$ -cyclodextrin ( $\beta$ -CD) and  $[V_6O_{13}\{(OCH_2)_3CCH_2OH\}_2]^{2-}$  (abbreviated  $[V_6-(OH)_2]^{2-}$ ) into the gas phase, mass selected the ion  $[\beta\text{-CD} + [V_6-(OH)_2]^{2-}]^{2-}$  and deposited the doubly charged complex onto a gold surface with the desired kinetic energy. Using subsequent Liquid Extraction Surface Analysis (LESA), we demonstrated that  $[\beta\text{-CD} + [V_6-(OH)_2]^{2-}]^{2-}$  was deposited intact on the surface. The stability of the host-guest complex was investigated using photoelectron spectroscopy (PES) experiments in the gas phase and computational methods.

Single host-guest complexes deposited on the surface were characterized by scanning tunneling microscopy (STM) and single isolated host-guest complexes with a size of 2–3 nm were observed on the STM images. Their redox characteristics were probed via single-point scanning tunneling spectroscopy (STS) and the detected  $I$ - $V$  curves show a characteristic step-like behavior, which reproducibly indicates the change of the redox states of the individual molecular units at different tip potentials. The potential-dependent increase of the current for  $[\beta\text{-CD} + [V_6-(OH)_2]^{2-}]^{2-}$  occurs at  $E = -0.8, 0.8, 1.2, 1.6$  and  $1.9$  V. These current steps differ significantly from previous measurements of hexavanadates prepared by conventional drop casting deposition methods.

### Please explain why your abstract is innovative for mass spectrometry?

Mass selected gaseous ions are used to generate surface layers with unique properties using ion soft-landing.

### Co-authors:

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Harald Knorke, Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, 04103, Leipzig, Germany

## POSTER SESSION B

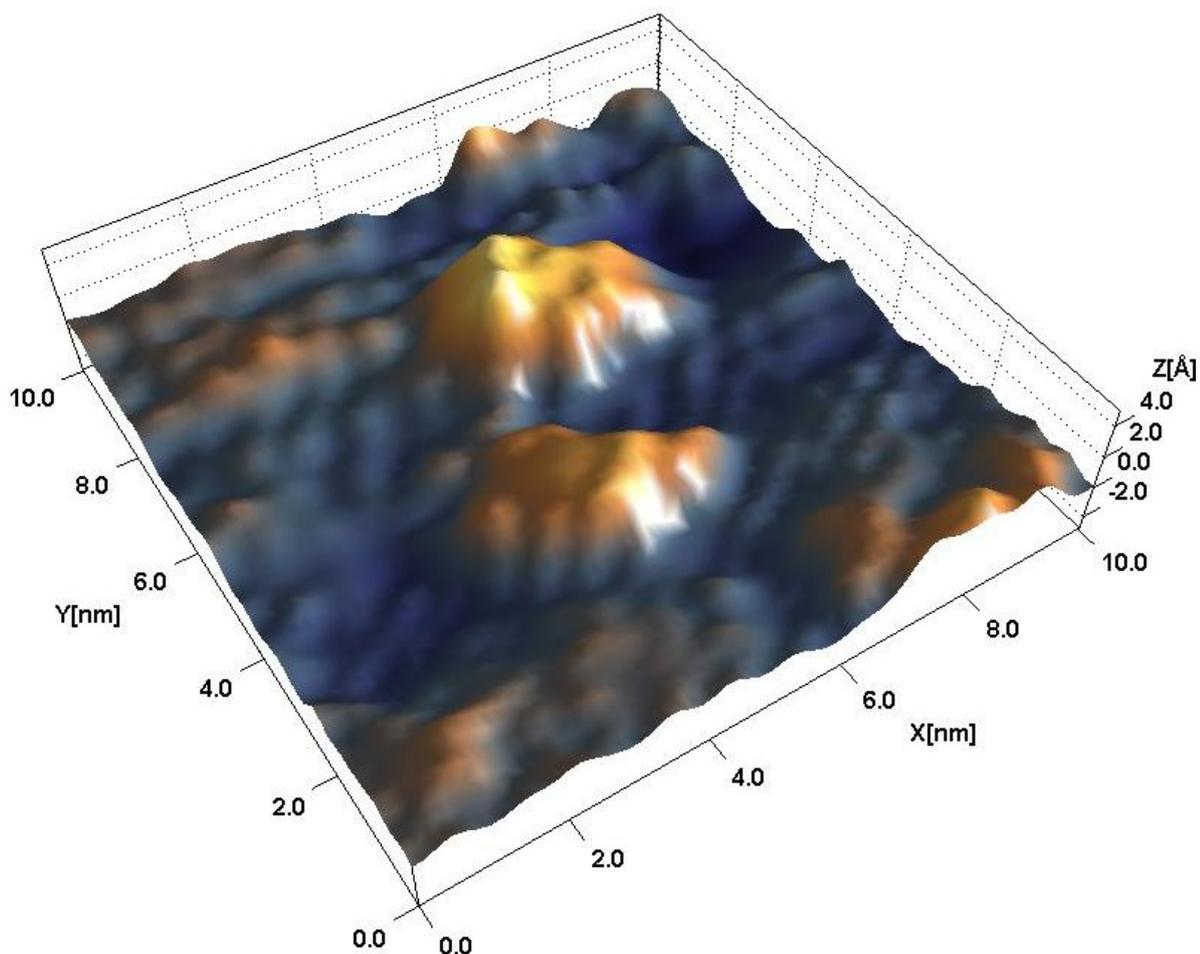
Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

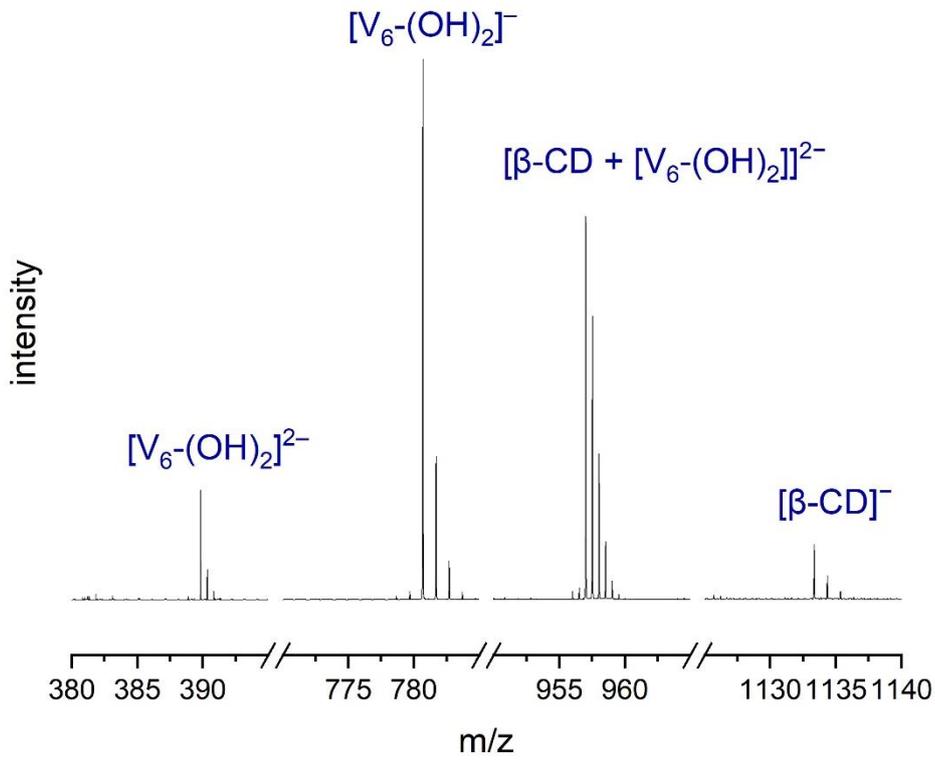
Ales Charvat, Leibniz-Institut für Oberflächenmodifizierung e.V. (IOM), Permoserstr. 15, D-04318, Leipzig, Germany,  
Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, 04103, Leipzig, Germany  
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3D STM figure of two single soft-landed polyoxovanadate-cyclodextrin anions



LESA-MS spectra of  $[\beta-CD + [V_6-(OH)_2]]^{2-}$

Poster number: **IM-PB-038**

## **REPLACING HELIUM CARRIER GAS IN SIFT-MS BY NITROGEN: CHANGES IN ION MOLECULE REACTIONS OF $\text{H}_3\text{O}^+$ , $\text{NO}^+$ AND $\text{O}_2^+$ WITH A RANGE OF VOLATILE ANALYTES**

Abstract ID: 263

**Presenting author: Patrik Španěl, J. Heyrovský Institute of Physical Chemistry**

### **Introduction**

Selected ion flow tube mass spectrometry, SIFT-MS, is used for the real time analyses of trace gases in ambient air, exhaled breath, the humid headspace of food products and biological fluids. In most previous SIFT-MS studies, helium has been used as the carrier gas because it does not undergo bimolecular reactions with any reagent cations and anions. Recently, nitrogen is becoming commonly adopted as the carrier gas. Examples of SIFT-MS research using  $\text{N}_2$  carrier include personal care products emissions, measurement of HONO in the atmosphere, hydrogen sulphide quantification, atmospheric soil emissions, and volatile compounds from archaeological artefacts, varnishes and books. Also, most newly distributed Voice 200 SIFT-MS instruments for industrial analyses routinely use  $\text{N}_2$  carrier gas.

### **Methods**

The reagent ions  $\text{H}_3\text{O}^+$ ,  $\text{NO}^+$  and  $\text{O}_2^+$  formed in a microwave discharge were mass selected and injected into a flow tube through which carrier gas flows (Figure 1). In the present studies, 1.5 mbar of helium or 0.5 mbar of nitrogen were used. The reagent ions react with analytes (often VOCs) present in sample gas forming characteristic analyte and fragment ions detected by a downstream quadrupole mass spectrometer. The differences in rate coefficients and product ion branching ratios were studied by introducing variable flows of analyte vapours diluted in clean air into He and  $\text{N}_2$  carrier gases.

### **Preliminary data (results)**

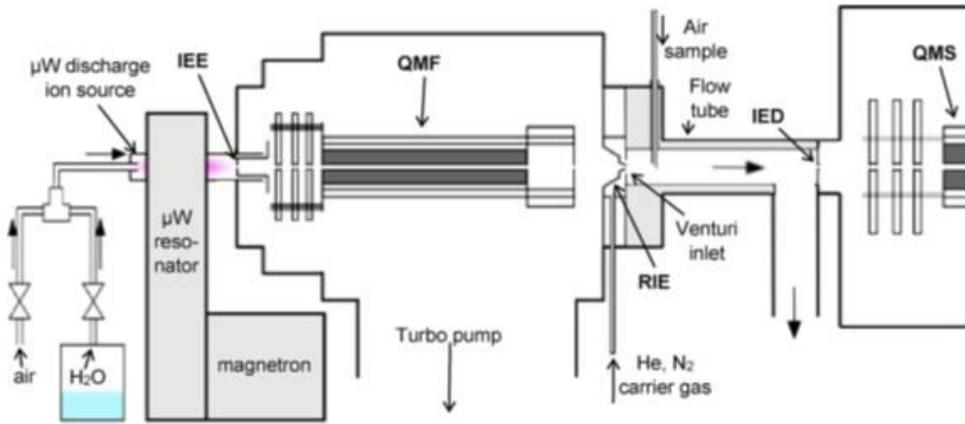
Reagent ions injected into  $\text{N}_2$  suffer collisions with centre-of-mass energies higher than those occurring in He. This results into greater fragmentation. The data are presented showing dependencies of the fragment formation on the ion injection energy.[1]

It was observed that  $\text{N}_2$  is more efficient third body than He in stabilizing adduct ions. Thus, association reactions of the reagent ions with  $\text{N}_2$ ,  $\text{O}_2$ ,  $\text{CO}_2$  and  $\text{H}_2\text{O}$  are faster ultimately enhancing formation of hydrated ions like  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_{1,2,3}$ .

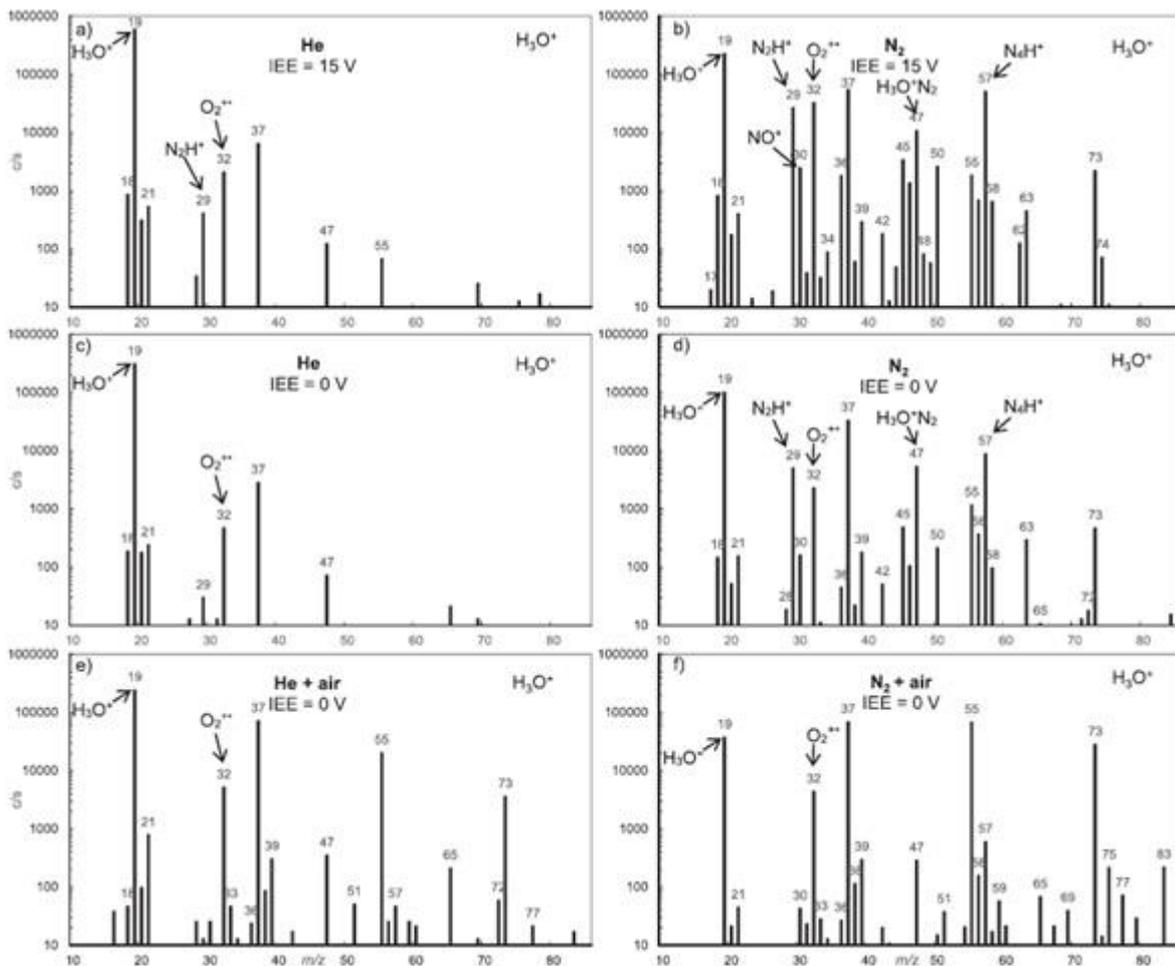
Data will be presented on comparisons of rate coefficients and product ion branching ratios for ion molecule reactions of  $\text{H}_3\text{O}^+$ ,  $\text{NO}^+$  and  $\text{O}_2^+$  with a range of volatile analytes obtained in He and  $\text{N}_2$  carrier gases. Note that for most reactions the differences are small, which is an important result supporting robust transition from He to  $\text{N}_2$ . The notable exceptions will be shown and the implications for analytical accuracy a reliability will be discussed.

### **Please explain why your abstract is innovative for mass spectrometry?**

The advantages of using much cheaper and readily available  $\text{N}_2$  carrier gas can be fully realised if its involvement in the analytical ion chemistry can be properly understood and mitigated



Profile 3 SIFT-MS instrument.



SIFT-MS spectra,  $H_3O^+$  reagent ions injected helium and nitrogen

Poster number: **IM-PB-039**

## **SENSITIVITY OF SESI-MS TO A RANGE OF VOLATILE ORGANIC COMPOUNDS**

Abstract ID: **264****Presenting author: Kseniya Dryahina, J. Heyrovský Institute of Physical Chemistry of the CAS, v. v. i.**

### **Introduction**

The secondary electrospray ionization (SESI) involves formation of highly-charged nanodroplets in a spray emitted from a capillary held at a high potential into air or nitrogen at or near atmospheric pressure. The singly-charged gas phase ions are released from the tips of transient surface protrusions on the highly-charged droplets. It is the ion chemical reactions that occur between these cluster reagent ions and trace compounds introduced into the surrounding air/nitrogen that are the basis of a very sensitive technique for gas phase VOC analyses. SESI-MS is currently only semi-quantitative. To make SESI-MS more quantitative, the mechanisms and the kinetics of the reaction processes, especially ligand switching reactions of the hydrated hydronium reagent ions,  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ , with VOC molecules, need to be understood.

### **Methods**

A ZSpray™ ESI source was adapted for SESI-MS by introducing sample gas via an inlet coaxial with the spray. The electrospray was operated in the positive ion mode using ultrapure water containing 0.1% formic acid. The ions formed in the spray react with the molecules in the gaseous sample and the resulting ion swarm was analyzed by a quadrupole mass spectrometer. 25 compounds from various classes were involved in the present study to investigate the sensitivity of SESI-MS and to understand the ion chemistry occurring between the reagent cluster ions and molecules, M. The results were compared with SuperSESI.

### **Preliminary data (results)**

Size (n) distribution was calculated of reagent cluster ions  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$  that react in ion molecule reactions with analyte VOCs, M, forming largely the protonated molecules,  $\text{MH}^+$ , and their hydrated ions  $\text{MH}^+(\text{H}_2\text{O})_{1,2,3}$ .

The efficiency of this process, which directly determines the SESI-MS sensitivity, is obtained from the slopes of the plot of total product ions signal against the analyte concentration for each of 25 VOCs. Widely variable sensitivities for different classes of VOCs were determined.

The sensitivity is observed to depend on the dipole moment, DM, and proton affinity, PA, of the analyte VOC molecule, M, as shown in the bubble plot. Sensitivities for compounds with  $\text{DM} < 1.85 \text{ D}$  (DM of  $\text{H}_2\text{O}$ ) are in general low and a correlation ( $R^2=0.87$ ) with PA is observed for  $\text{DM} > 1.85 \text{ D}$ .

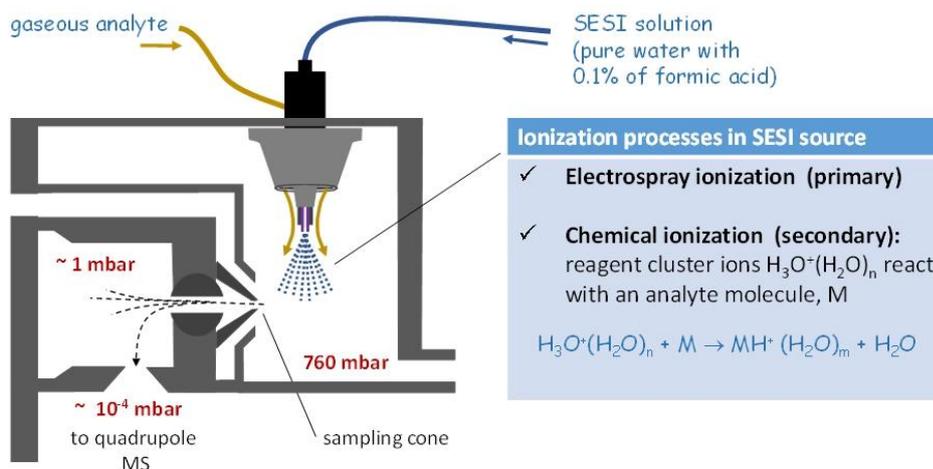
This observation indicates that the reactions occurring at atmospheric pressure proceed via ligand switching ( $\text{H}_2\text{O}$  replaced by M in the hydrated ions  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$  forming  $\text{MH}^+(\text{H}_2\text{O})_{1,2,3}$

Additionally, it is seen that the degree of hydration of  $\text{MH}^+$  ions, i.e. the percentage of  $\text{MH}^+\text{H}_2\text{O}$ , is low when the SESI sensitivity is high.

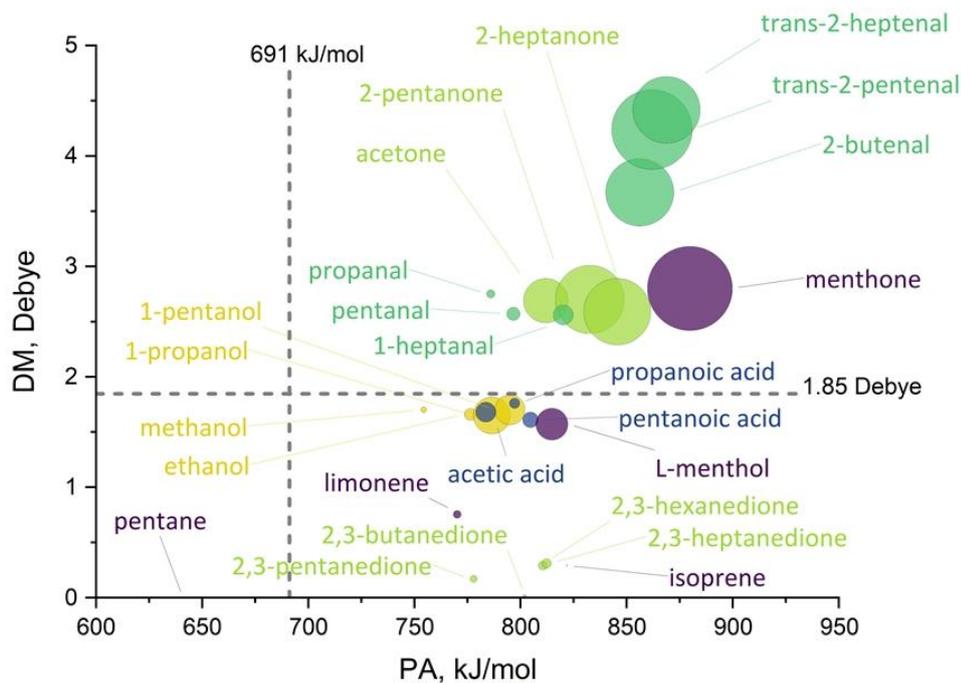
When comparing Zspray SESI sensitivity results with SuperSESI it is important to consider possible ionisation within the heated ion transfer capillary.

### **Please explain why your abstract is innovative for mass spectrometry?**

Understanding of the mechanisms and the kinetics of the gas phase ion molecule reactions, especially ligand switching is the first step to make SESI-MS more quantitative.



Schematic of the SESI ion source.



Bubble-plot of sensitivity; effect of dipole moment and proton affinity.

Poster number: **IM-PB-040**

## DESIGN AND PERFORMANCE OF A SOFT-LANDING INSTRUMENT FOR FRAGMENT ION DEPOSITION

Abstract ID: **329****Presenting author: Harald Knorke, Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, 04103 Leipzig, Germany**

### Introduction

This poster introduces a high-flux electrospray ionization-based instrument for soft landing of mass-selected fragment ions onto surfaces recently developed [1]. Collision-induced dissociation is performed in a collision cell positioned after the dual electrodynamic ion funnel assembly. High-coverage deposition of mass-selected fragment ions onto surfaces at a defined kinetic energy is enabled. This capability facilitates the investigation of the reactivity of gaseous fragment ions in the condensed phase. The reactions of deposited fragment ions are dependent on the structure of the ion and the composition of either ionic or neutral species codeposited onto a surface. The newly developed instrument provides access to high-purity ion fragments as building blocks for the preparation of unique ionic layers.

### Methods

The instrument is equipped with a high ion transmission ESI interface composed of two orthogonal-injection ESI sources, a dual ion funnel system, a collision cell, an ion guide, and an analytical quadrupole. All the electronic components are powered using a custom-designed power source system. The collision cell positioned after the dual ion funnel is a new component that distinguishes it from previously used ion soft-landing instruments. (see Figure 1).

Deposited fragment ions were analyzed by ex situ nanospray desorption electrospray ionization and liquid extraction surface analysis mass spectrometry.

### Preliminary data (results)

The ion soft-landing instrument described in this study enables the controlled preparation, isolation and deposition of reactive fragment ions for studying their reactivity on surfaces. To illustrate the concept of fragment ion soft-landing,  $\text{Ru}(\text{bpy})_2^{2+}$  and  $\text{B}_{12}I_n^-$  ( $n = 7$  to  $11$ ) ions are presented as exemplary systems.

Precursor ion  $\text{Ru}(\text{bpy})_3^{2+}$  fragments primarily by the loss of one bipyridine ligand. The collision cell was operating in pulsed mode comprising trapping and extraction events. The trapping potential was varied by the bias voltage applied to the cell, while the extraction potential was kept constant to obtain the same kinetic energy of the ions on the deposition target.

Fragment ions of the  $\text{closo-B}_{12}I_{12}^{2-}$  dianion were generated by varying the collision energy in the collision cell. The highly reactive singly charged fragment ions react with molecules present on the surface to achieve a full substitution level (12 substituents) in the stable doubly negatively charged state. A drastic change in reactivity was observed for  $[\text{B}_{12}I_7]^-$ , which possesses an opened  $\text{B}_{12}$  core. The results have been published recently. [1]

[1] H. Y. Samayoa-Oviedo et al.: "Design and Performance of a Soft-Landing Instrument for Fragment Ion Deposition." *Anal. Chem.* **2021**, 93, 14489-14496.

### Please explain why your abstract is innovative for mass spectrometry?

Development of a new soft-landing instrument for the deposition of fragment ions.

### Co-authors:

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

*Leipzig, Germany*

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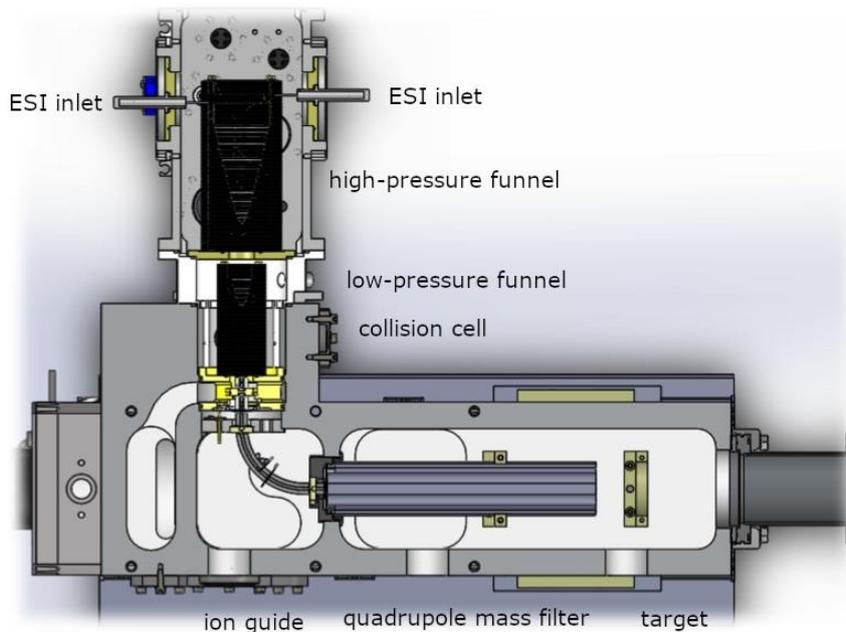
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Schematic drawing of the instrument.

Poster number: **IM-PB-041**

## UNEXPECTED STEPWISE AMMONIA LOSS FROM PROTONATED 3-HYDROXY 4-METHYLANILINE EVIDENCED BY HYDROGEN/DEUTERIUM BACK EXCHANGES IN A LINEAR ION TRAP

Abstract ID: **468**

**Presenting author: Sandra Da Sousa, Département de Recherche en sciences analytiques. L'ORÉAL Research & Innovation**

### Introduction

In the synthesis of 3-hydroxy 4-methylaniline (*HOMeA*), impurities are produced. Herein, *HOMeA* is analyzed in details to be able latter to study impurity structures. The positive electrospray mass spectrum displayed mainly the protonated *HOMeAH*<sup>+</sup> molecule. Its product ion spectrum (CID, resonant mode) unexpectedly shows abundant NH<sub>3</sub> and CH<sub>3</sub><sup>+</sup> losses. The signal related to [*HOMeAH*-NH<sub>3</sub>]<sup>+</sup> is the base peak (bp). Interestingly, methylation of the OH group into OCH<sub>3</sub> (*MeOMeA*) completely hindered the ammonia loss. In order to investigate the origin of this behavior, dissociations of compounds labeled at both stable (-CD<sub>3</sub>) and labile (-ND<sub>2</sub> and -OD) sites (*HOMe<sub>d3</sub>A* and *DOMeA<sub>d2</sub>*, respectively) were investigated. In addition, back H/D exchanges (bHDX) on labeled molecular species were explored to better describe product ion structures.

### Methods

LTQ/Orbitrap Elite and Orbitrap Fusion instruments were used. [*HOMe<sub>d3</sub>A*+H]<sup>+</sup>, [*DOMeA<sub>d2</sub>*+D]<sup>+</sup>, [*MeOMeA<sub>d2</sub>*+D]<sup>+</sup>, and [*DOMe<sub>d3</sub>A<sub>d2</sub>*+D]<sup>+</sup> were generated in positive ESI conditions. Acquisition parameters were as follows: voltage, -3.5 kV; sheath and auxiliary gas flow rates 10 and 2 a.u. (arbitrary unit), respectively; precursor isolation width, 1.2 m/z; excitation, q<sub>z</sub>=0.25, normalized collision energy (NCE), 25%; activation time (ActT), 10 ms to 3000 ms. Product ions were analyzed at 120 000 resolution. Density functional theory calculations were performed using the Gaussian09 package, at CAM-B3LYP/6-311+G(2d,2p) level.

### Preliminary data (results)

The m/z 109 (90% of bp), m/z 107 (bp), and m/z 106 (4% of bp) peaks representative for product ions of [*HOMeA*+H]<sup>+</sup> (m/z 124) submitted to resonant excitation (CID, NCE 25%) were shifted in CID spectra of the deuterated precursor ions. To rationalize these experimental results, study is focused on ammonia loss occurring from unlabeled and D-labeled species. First, the NH<sub>3</sub> and NH<sub>2</sub>D isotopologue losses are observed in a 87/13 ratio during the [*HOMe<sub>d3</sub>A*+H]<sup>+</sup> dissociation indicating that hydrogen from methyl weakly contributes to the ammonia loss. Conversely, ND<sub>3</sub> and ND<sub>2</sub>H releases occur in a 50/50 ratio from the [*DOMeA<sub>d2</sub>*+D]<sup>+</sup> dissociation, while from [*DOMe<sub>d3</sub>A<sub>d2</sub>*+D]<sup>+</sup>, this ratio increases up to 91/9. These results suggest that hydroge from the phenylic and methyl sites are involved for 9% and 13%, respectively, while the H-atom of hydroxyl involved for 28% was unexpected. To clarify the situation, bHDX from [*DOMe<sub>d3</sub>A<sub>d2</sub>*+D]<sup>+</sup> were studied. This precursor ion was stored in LTQ (during 10 ms-3000 ms with NCE=0%) to promote bHDX with the residual water in the ion trap. Only one bHDX takes place yielding m/z 130 (64% of the selected m/z 131 ion). This indicates that the corresponding site carries a deuterium (i.e., -OD) which is exchangeable with residual light water. Finally, examination of DFT calculations on the various potential competitive pathways demonstrated that ammonia loss results from highly complex and concomitant stepwise processes involving various protomers.

### Please explain why your abstract is innovative for mass spectrometry?

Stepwise loss of ammonia from di-substituted aniline, role of hydroxyl group evidenced by back H/D exchange study

### Co-authors:

Alexandre Guepet, Département de Recherche en sciences analytiques. L'ORÉAL Research & Innovation  
 Yves Gimbert, Sorbonne Université, Faculté des sciences et de l'ingénierie, Institut Parisien de Chimie Moléculaire (IPCM), Département de Chimie Moléculaire, UMR CNRS 5250, Université Grenoble Alpes  
 Ekaterina Darii, 4Génomique métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay  
 Annelaure Damont, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Nassera Lakhel, Département de Recherche en sciences analytiques. L'ORÉAL Research & Innovation*  
*Céline Ducruix, Département de Recherche en sciences analytiques. L'ORÉAL Research & Innovation*  
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Poster number: **IM-PB-042**

## INVESTIGATIONS OF PROTON/DEUTERON BACK EXCHANGE RATE CONSTANT IN A LINEAR ION TRAP: DEUTERATED POLYETHYLENEGLYCOLS

Abstract ID: **488****Presenting author: Alexandre Guepet, Département de Recherche en sciences analytiques, L'ORÉAL Research & Innovation**

### Introduction

In order to elucidate fragmentation mechanisms of a deuterated non-covalent complex submitted to collisional resonant excitation with activation times (ActT) up to 3000 ms (1), back hydrogen/deuterium exchanges (bHDX) (2) with residual water present in an ion trap were explored. This approach allowed to attribute a zwitterion structure for intermediate ions formed during stepwise dissociations of the complex. The present work introduces an improved method to precisely determine the rate constants of ion/molecule reactions resulting in bHDX and for a better structural characterization of product ions. For such an objective, a series of simple molecules such as ionized deuterated polyethylene glycols (i.e., [PEG<sub>d2</sub>+D]<sup>+</sup>) of variable sizes has been chosen as a model.

### Methods

PEGs: tetra-, penta-, hexa-polyethylene glycols (TtEG, PtEG and HxEG, respectively) and Gly-Gly as standards were purchased from Sigma. LTQ/Orbitrap Elite and Orbitrap Fusion instruments were used in the electrospray positive ion mode. Acquisition parameters were as follows: HV 3.5 kV; sheath and auxiliary gas flow rates 10 and 2 a.u. (arbitrary unit); resolution 120000 with Elite and 500000 with Fusion; Selected ion m/z width was set at 1.2 m/z; ion storage/excitation  $q_{z,exc}$  was 0.25; NCE ranged from 0% to 8%. ActT varied from 0 to 3000 ms with Elite and to 1000 ms with Fusion.

### Preliminary data (results)

The [TtEG<sub>d2</sub>+D]<sup>+</sup> ion (m/z 198) was selected and stored in a linear trap quadrupole (LTQ) using default resonant excitation conditions (NCE=0% and Act=10 ms). Under these conditions, prompt bHDX occurs and accounted for ~20% of the original parent ion cloud (i.e., TIC). Observed species at m/z 197 (16%) and m/z 196 (3%) resulted from one and two bHDX, respectively. At 3000 ms, only 0.3% of TtEG<sub>d2</sub>D<sup>+</sup> survived, while the unlabeled TtEG<sub>d0</sub>H<sup>+</sup> ion reached more than 80% of the overall signal. The natural logarithm (Ln) evolution of the relative abundance of [TtEG<sub>d2</sub>+D]<sup>+</sup> ions displays a linear dependence with a negative slope in the ActT range from 10 ms to 800 ms confirming a pseudo-first order reaction. The slope decreased inversely to collision energy (up to NCE=10%).

The larger [PtEG<sub>d2</sub>+D]<sup>+</sup> ion exhibited higher bHDX, and its evolution Ln versus resonant excitation shows a higher bHDX rate constant than that observed for [TtEG<sub>d2</sub>+D]<sup>+</sup>.

These negative dependences on the excitation energy (ion kinetic increase) are expected for ion/molecule reactions. To calculate the water capture rate constant, residual gas phase water density (in molecule/ cm<sup>3</sup>) will be estimated using Gly-Gly<sub>d4</sub>D<sup>+</sup> as a standard whose HDX rate constant is well established. Finally, a possible relay mechanism can explain these bHDX. DFT calculations will be performed to confirm this model.

### Please explain why your abstract is innovative for mass spectrometry?

Rate constant of back HDX in ion trap combined to DFT calculations to evidence the exchange mechanism

### Co-authors:

Sandra De Sousa, Département de Recherche en sciences analytiques, L'ORÉAL Research & Innovation  
Ekaterina Darii, Génomique Métabolique, Génoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université, Paris-Saclay  
Annelaure Damont, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS)

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

*Denis Lesage, Sorbonne Université, Faculté des sciences et de l'ingénierie, Institut Parisien de Chimie Moléculaire (IPCM)*

*Yves Gimbert, Sorbonne Université, Faculté des sciences et de l'ingénierie, Institut Parisien de Chimie Moléculaire (IPCM), Université Grenoble Alpes*

*Nassera Lakhel, Département de Recherche en sciences analytiques, L'ORÉAL Research & Innovation*

*François Fenaille, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS)*

*Céline Ducruix, Département de Recherche en sciences analytiques, L'ORÉAL Research & Innovation*

*Jean-Claude Tabet, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), Sorbonne Université, Faculté des sciences et de l'ingénierie, Institut Parisien de Chimie Moléculaire (IPCM)*

Poster number: **IM-PB-043**

## STEREOSPECIFIC RECOGNITION OF AMINO ACIDS IN QUATERNARY COPPERED COMPLEXES VIA ENANTIOSELECTIVE REDUCTION

Abstract ID: **515****Presenting author: Clément Chalet, Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay**

### Introduction

Chirality poses a unique challenge in life sciences since individual enantiomers may exhibit different biological activity and toxicity, making it necessary to explore efficient chiral analytical methods. Enantiomeric amino acid (<sup>D</sup>AA and <sup>L</sup>AA) distinction based on the relative rate constants of competitive fragmentations of their respective quaternary coppered complexes (QCC) is known to be an efficient method for chiral differentiation. More recently, the use of non-natural amino acid phenylglycine (PhG) and proline (Pro) as chiral selectors improved the enantioselectivity of the dissociation of such QCC. We used phenylalanine (Phe) as a model compound to further investigate the dissociation based on the enantioselective reduction of homo- or heterochiral [Cu,<sup>D/L</sup>Phe,<sup>D</sup>PhG,<sup>D</sup>Pro-H]<sup>+</sup> complexes in gas phase.

### Methods

Mixtures of <sup>D</sup>AA or <sup>L</sup>AA and Cu<sup>II</sup> salt were prepared in H<sub>2</sub>O/methanol (1:1) and were directly infused in an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific) operating in positive ESI mode. The complexes were activated using a resonant mode (collision-induced dissociation, CID) while varying collision energy (CE) and activation time (ActT) to yield energy-resolved mass spectrometry (ERMS) as activation time dependent data. Complementary ion mobility data was collected with a TIMS-ToF mass spectrometer (Bruker). CID experiments were simulated, within the frame of RRKM theory, using MassKinetics Scientific Demo software.

### Preliminary data (results)

The main cleavage of the quaternary [Cu<sup>II</sup>,(Phe,PhG,Pro-H)]<sup>+</sup> complex (m/z 493) consists in the formal losses of PhG and (PhG-H)<sup>+</sup>, resulting in the expected ion [Cu<sup>II</sup>,(Phe,Pro-H)]<sup>+</sup> (m/z 342) and the reduced ion [Cu<sup>I</sup>,(Phe,Pro)]<sup>+</sup> (m/z 343), respectively. Enantioselective reduction is mostly occurring in homochiral complexes (i.e. <sup>D</sup>Phe,<sup>D</sup>PhG,<sup>D</sup>Pro or <sup>L</sup>Phe,<sup>L</sup>PhG,<sup>L</sup>Pro). The extent of the reduction was also found to be enhanced by increasing the activation time. Interestingly, the abundance of the reduced product ion m/z 343 was found to decay when increasing the CE. This behavior suggests that competition favors direct dissociations rather than reduction processes occurring through rearrangements. This phenomenon was also evidenced by simulation of the ERMS curves using MassKinetics software.

In addition, the abundance of the surviving QCC precursor ion in ERMS data was found to plateau before its complete disappearance. This behavior indicates that at least two forms of QCC co-exist, the most abundant of which leads to the chiral effects during dissociations described above. The minor surviving form was studied using different source conditions and sequential MS<sup>2</sup> and MS<sup>3</sup> experiments. The product ion spectra showed distinct fragmentation patterns from the most abundant form of QCC, hinting at different structures. Unfortunately, these two forms could not be distinguished by ion mobility.

### Please explain why your abstract is innovative for mass spectrometry?

Insights in the enantioselective reduction and gas-phase behavior of quaternary coppered complexes of amino acids

### Co-authors:

*Ekaterina Darii, Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay*

*Denis Lesage, Sorbonne Université, Faculté des Sciences et de l'Ingénierie, Institut Parisien de Chimie Moléculaire (IPCM)*

*Estelle Rathahao-Paris, Sorbonne Université, Faculté des Sciences et de l'Ingénierie, Institut Parisien de Chimie Moléculaire (IPCM)*

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



*Annelaure Damont, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS)*

*Alain Perret, Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris-Saclay*

*Fenaille François, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS)*

*Christophe Junot, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS)*

*Jean-Claude Tabet, Sorbonne Université, Faculté des Sciences et de l'Ingénierie, Institut Parisien de Chimie Moléculaire (IPCM)*

*Sandra Alves, Sorbonne Université, Faculté des Sciences et de l'Ingénierie, Institut Parisien de Chimie Moléculaire (IPCM)*

## Session: Separation & hyphenation; chromatography, electrophoresis

Poster number: **IM-PB-044**

### **MOLECULAR MARKERS PROFILES IN BLACK SEA MARINE SEDIMENTS OBTAINED BY GC/MS SYSTEM**

Abstract ID: 41

**Presenting author: Zaharie Moldovan, National Institute of Research and Development for Isotopic and Molecular Technology**

#### **Introduction**

The organic matter from marine sediment results from a complex combination of both natural and anthropogenic sources. In main these compounds when found provide a means of identification of sources of input and pathways of movement of chemicals through the ecosystem.

The concentrations of organic compounds in an environmental sample reflect both the original source of organic matter, as well as the alteration processes, which have occurred in the environment [1,2]. Several of occurring compounds may be used as tracers to study natural processes affecting the fate of original compounds in aquatic sediments.

[1] P. N. Polymenakoua, et al., Continental Shelf Research 25 (2005) 2196

[2] M. S. Zyla and L. Lubecki, Marine Chemistry 239 (2022) 104069

#### **Methods**

The procedure consists of ultrasonic solvent extraction followed by a clean-up operation and then analysis by a GC-MS method. The molecular markers are visualized by ion profile chromatogram. The compounds are identified based on mass spectra obtained by Electron Impact Ionization (EI) in full scan mode.

#### **Preliminary data (results)**

In order to differentiate the various inputs of organic matter, molecular marker approaches have been developed on the potential of molecules to be source specific. The purpose of the present paper is the characterization of organic molecular markers detected in Black Sea sediment by GC/MS analysis to evaluate original compound sources. The study presents data for several families of organic compounds used as molecular markers in sediment organic matter investigation: n-alkanes, acyclic isoprenoid, pentacyclic triterpanes (Hopanes), and steranes. This study focuses on the provenance of sedimentary organic matter deposited in the Black Sea basin based on the analysis of multiple specific markers in sediments from more than 1000 m level of deep and sediment depths below sea floor in the range 80–400 cm, sampled at few sites around of Danube estuary.

In Figure 1 is shown the GC/MS chromatogram at m/z 85 for visualization of n-alkanes, in the sample S1283m30 (1283 m deep, 30 cm under sea floor). The GC/MS chromatogram at m/z 213, 215, 231 and 174 for few markers from steranes family in the sample S1286m300 (1286 m deep, 300 cm under sea floor) is shown in Figure 2.

#### **Please explain why your abstract is innovative for mass spectrometry?**

The study focuses on organic matter origin deposited in the Black Sea basin by analysis of specific markers from high deepand sediment depths in the range 80–400 cm.

#### **Co-authors:**

Florina D Covaciu, National Institute of Research and Development for Isotopic and Molecular Technology  
 Gabriel Ion, Department of Seismo-Acoustic, Digital Cartography, GIS, Data Base, National Institute for Research & Development of Marine Geology and Geoecology

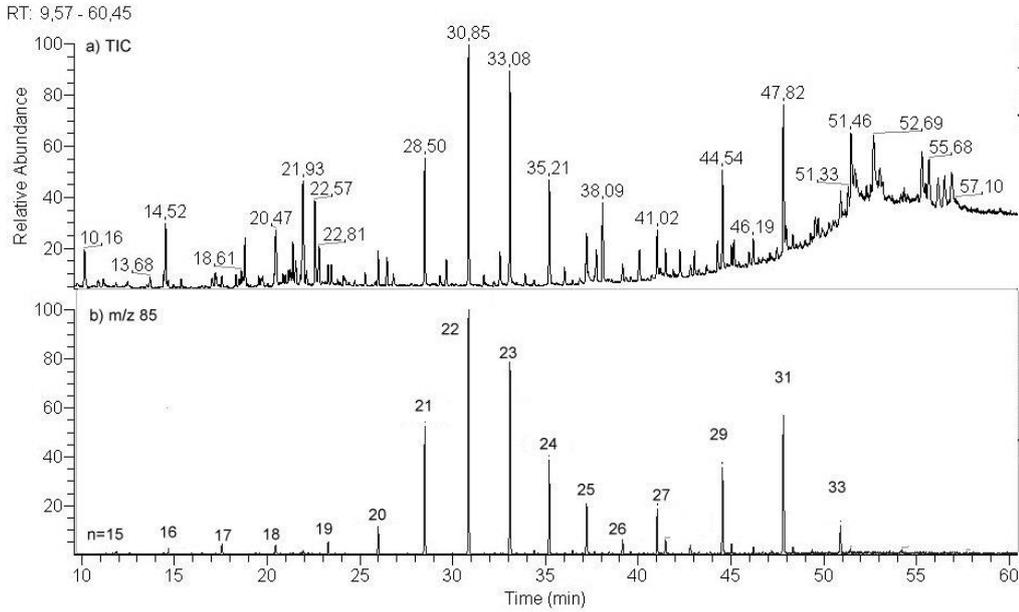


Figure 1. GC/MS Chromatogram at m/z 85 for n-alkanes on sample S1283m30

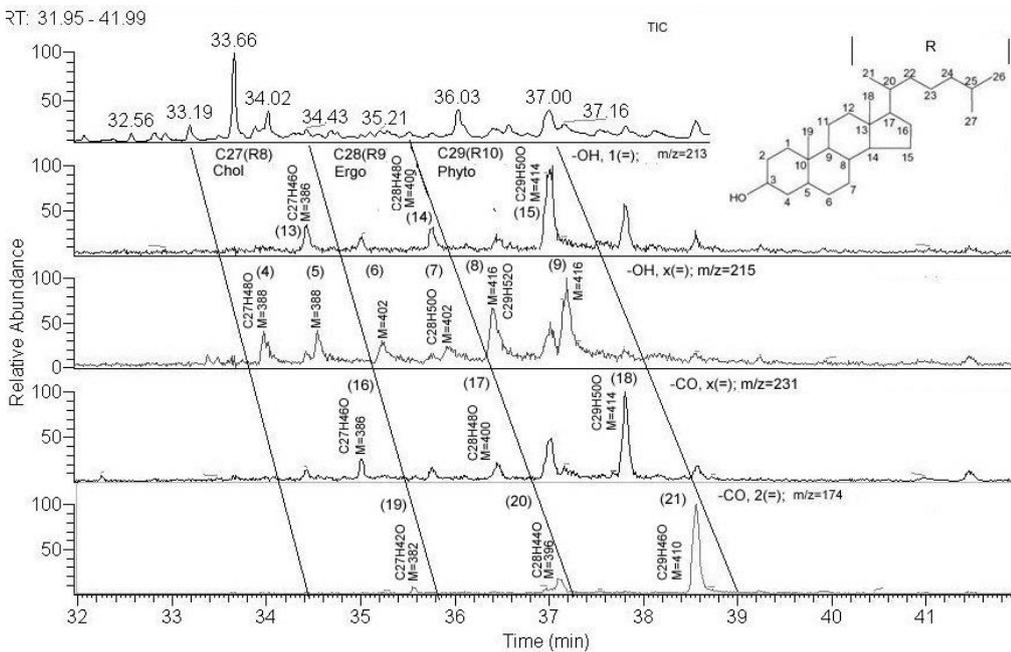


Figure 2. GC/MS Chromatogram at m/z 213, 215, 231 and 174 for sterane markers on sample S1286m300

Poster number: **IM-PB-045**

## LC-MS PROFILING OF STRONGLY COELUTING COMPLEX PHLOROTANNIN PATTERNS IN BROWN ALGAE

Abstract ID: 161

Presenting author: **Claudia Birkemeyer, University of Leipzig, Institute of Analytical Chemistry**

### Introduction

Accumulation of biologically active metabolites is a specific feature of plant biochemistry, directing the use of plants in numerous applications in the pharmaceutical and food industries with the plethora of phenolic compounds attracting particular interest. Phlorotannins are specific brown algal phenolic metabolites, the polymers of phloroglucinol. With different degrees of polymerization (DP) the molecular masses range from 126 g/mol of the monomer up to polymers of 650 kg/mol (DP>5000). Depending on the basic structure of the molecules, six major classes of phlorotannins have been identified so far. Consequently, the profiles of these secondary metabolites in algae can grow rather complex, and powerful methods with a high resolution are required to characterize such profiles.

### Methods

Brown algae samples of the family Fucaceae were collected in the Keret Archipelago (Kandalaksha Bay, White Sea, Russia). Plant material was incubated with aqueous acetone to extract intracellular phenolic compounds. Extracts were defatted partitioning three times against dichloromethane, and phlorotannins were extracted by five successive portions of ethyl acetate, subsequently dried in a speedvac and resuspended in water. For analysis of phenolic components, algal extracts were separated with reversed-phase HPLC coupled to a Bruker Esquire 3000 Plus ESI ion trap or, alternatively, to a Bruker Impact II QToF (Bruker Daltonics, Bremen, Germany) each time in negative ion mode.

### Preliminary data (results)

Each group of phlorotannins with a similar basic structure but different DP was observed as a specific m/z series with increments corresponding to multiples of the phloroglucinol moiety (124 u). As phlorotannins with DP>10 were not chromatographically separated, data analysis needed to be developed based on the observed MS data. We describe the great challenge in evaluating such complex extracts and present a strategy to systematically assess the semi-quantitative composition of samples beyond peak extraction when single components cannot be separated by chromatography anymore due to the relative similarity of species with increasing DP. An additional challenge is the occurrence of morefold charge states with increasing DP as frequently observed with electrospray ionization.

Using a six-step procedure omitting peak picking, we finally compiled and quantified 31 different phlorotannin series and used accurate mass spectrometry to assign tentative structures to the observed ions based on the confirmed sum formulas. This way, MS-analysis revealed the presence of five different classes of phlorotannins with three different types of modification and DP 3-50. In addition, complementary information from two different MS instruments can be used to add information on the pattern of the investigated phenolic polymers.

Research was supported by the Russian Foundation for Basic Research (project 20-04-00944) and the MS-UL core facility of the University of Leipzig.

### Please explain why your abstract is innovative for mass spectrometry?

Generally applicable evaluation strategy for LC-ESI-MS quantification of copolymers with a polydispersity >1.2 and mass differences of ~2% where incomplete peak separation is only used to improve ionization efficiency

### Co-authors:

*Valeriia Lemesheva, St. Petersburg State University, Faculty of Biology*  
*Susan Billig, University of Leipzig, Institute of Analytical Chemistry*  
*Elena Tarakhovskaya, St. Petersburg State University, Faculty of Biology*

Poster number: **IM-PB-046**

## HIGHER ORDER STRUCTURE OF HUMAN TELOMERIC G-QUADRUPLEXES AND PHOSPHOROTHIOATED ANALOGUES INVESTIGATED BY CAPILLARY ELECTROPHORESIS, ION MOBILITY MASS SPECTROMETRY, BREAKDOWN CURVE EXPERIMENTS AND COLLISION INDUCED UNFOLDING

Abstract ID: **165**Presenting author: **Johann Far, Mass Spectrometry Laboratory-ULiège**

### Introduction

Nucleic acids constitute an important family of biomolecules that handles genetic information through transfer, expression, and regulation. Nucleic acids can adopt various conformations such as triplexes, hairpins, and G-quadruplexes in addition to double helix conformation, G-quadruplexes have increasingly attracted interest due to the growing evidence supporting their implication in key biological processes such as mutagenesis or genome damage repair. Various biophysical methods were successfully introduced to characterize G-quadruplex and understand the physicochemical processes behind their respective functions. Despite their current implementation for G-quadruplex characterization, these methods come with their limitations.

### Methods

In this work, we introduce the use of Capillary Electrophoresis (CE) coupled to Ion Mobility Mass Spectrometry (CE-IM-MS) and Non-Equilibrium Capillary Electrophoresis of Equilibrium Mixture (NECEEM) for the investigation of G-quadruplexes in both solution (CE) and gas phase (IM-MS). To this end, the human telomeric sequence TAGGGTTAGGGT was used and compared to its phosphorothioated analog. Thermochemistry properties such as binding constant with the coordinating cations and kinetic constants could be determined.

### Preliminary data (results)

Collision Induced Dissociation (CID), Collision Induced Unfolding (CIU), with traveling wave Ion Mobility Mass Spectrometry (T-Wave IM-MS) was introduced to assess the stability and the resilience towards collisional activation. Energy resolved CID-MS (breakdown curve experiments) and Collision Induced Unfolding characterize the fragmentation channels and the respective dissociative pathway of the G-quadruplexes after respectively harsh or soft collisions with a neutral gas. The data obtained from CE-IM-MS and Non-Equilibrium Capillary Electrophoresis of Equilibrium Mixtures (NECEEM), breaking curve experiments and collision induced unfolding were processed with in-house GUI python scripts to easily extract several thermochemistry values including binding constant and koff (or k-1), the half dissociation (voltage at 50% survival yield) energy (V50) or equivalent features depending on the context of the experiments.

We are currently investigating the potential of the developed approaches to push as far as possible the characterization of physicochemical information that can be extracted from these in-solution and in-vacuo analytical techniques. Here we propose to present the data of these different works-in-progress.

### Please explain why your abstract is innovative for mass spectrometry?

First report of CE-IMS and NECEEM-IMS of G-quadruplex associated with V50 and CIU data of oligonucleotide and phosphorothioated analogues.

### Co-authors:

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Poster number: **IM-PB-047**

## A LOW-CODE PYTHON WEB APPLICATION FOR RETENTION TIME ALIGNMENT ACROSS LC SYSTEMS

Abstract ID: **181**

**Presenting author: Ian Reah, Waters Corporation**

### Introduction

Retention time shifts in liquid chromatography (LC) are unavoidable. Absolute retention times are highly dependent on column and analysis conditions which can become an obstacle to comparing chromatographic data across LC systems.

Here we demonstrate the ability to align multiple complex chromatograms from different LC systems in an interactive web application using relatively few lines of code and utilizing the Empower™ Chromatography Data Software (CDS) Software Development Toolkit (SDK).

The Empower CDS SDK can be used to monitor one or more Empower databases and extract data into a cloud environment where data from multiple projects, systems &/or Empower instances can be queried and accessed efficiently from a web application using cloud tools like Elasticsearch and Amazon S3.

### Methods

Chromatographic data was extracted from the Empower CDS system using the Empower SDK and converted to a JSON format. Metadata was sent to an Elasticsearch engine where it is indexed for flexible and efficient text-based querying via a web API. Raw chromatographic data was stored in Amazon S3 buckets.

The web application was developed in Python using Streamlit to build the UI.

Streamlit is a low-code web development framework for data science applications. It is used to build interactive web applications around data and machine learning models in Python using few lines of code without any knowledge of web development.

### Preliminary data (results)

Peptide mapping data sets using two different LC systems were processed. The conventional approach of using relative retention time (RRT) to account for retention time shifts (whereby retention times are expressed relative to the measured retention time of a single standard peak) doesn't always produce good peak alignment across the entire time range in these complex data sets.

The application allows for multiple common "anchor" peaks to be used as RRT standard peaks instead, providing much improved peak alignment between data from the different LC systems.

A small complication with this approach is the requirement to determine RRT values for the standard peaks relative to each other. The results from these data sets show that it is feasible to automatically calculate appropriate values for these based on analysis of the individual anchor peak absolute retention times.

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**Please explain why your abstract is innovative for mass spectrometry?**

Alignment of complex chromatograms across different LC systems in a highly interactive web application using relatively few lines of code

**Co-authors:**

*Pete Reay, Waters Corporation*

*Todor Petrov, Waters Corporation*

*Ian Morns, Waters Corporation*

*Jack Smith, Waters Corporation*

*Richard Chapman, Waters Corporation*

Poster number: **IM-PB-048**

## **OPTIMIZING POSTTRANSLATIONAL MODIFICATIONS CHARACTERIZATION BY LC-TIMS-MS**

Abstract ID: **255**

**Presenting author: Jean-Francois Greisch, Bruker Daltonics**

### **Introduction**

Liquid chromatography coupled to trapped ion mobility and mass spectrometry (LC-TIMS-MS) offers unique opportunities for the identification, characterization, and quantification of proteoforms – the different proteins resulting from genetic variations, alternatively spliced RNA transcripts, or post-translational modifications (PTMs). The ability to separate compounds based on their PTMs particularly benefits diagnostics, manufacturing, and forensics: PTM dysregulation can be associated with a given pathology or disease stage, PTMs affect therapeutic activity, and PTMs can also complement genetic information.

Overall, post-translational modifications impact the physico-chemical properties of proteins and peptides and, thereby, molecular and material interactions, chemical reactions, and mass spectrometric behavior. Here, we address how materials, TIMS-MS parameters, and processing affect the identification, characterization, and quantification of PTMs, with a special focus on glycosylation.

### **Methods**

The capabilities of a nanoElute – timsTOF Pro 2 have been leveraged to analyze PTMs. Samples ranging from glycosylated to phosphorylated peptides have been separated using a nanoElute equipped with different loops and columns coupled to a timsTOF Pro 2 equipped with a Captive Spray ion source. All these samples were subjected to trapped ion mobility separation prior to fragmentation. For protein digests, parallel accumulation-serial fragmentation (PASEF) was combined with both data dependent (DDA) and data independent (DIA) acquisition. ddaPASEF and diaPASEF modes are compared and dedicated PTM analysis is introduced.

### **Preliminary data (results)**

We describe the optimization of a nanoElute HPLC – timsTOF Pro 2 combination for the analysis of post-translationally modified samples. We address biocompatibility issues, such as sample loop material, reactivity issues, such as solvent composition, pH, and additives use, as well as method optimization for ion-mobility separation and fragmentation prior to mass analysis using dedicated software. Trends are reported that might contribute to improve sequence and PTM information for complex samples.

We conclude by highlighting the benefits of using LC-TIMS-MS for the analysis of complex protein digests incorporating PTMs.

### **Please explain why your abstract is innovative for mass spectrometry?**

We demonstrate the possibility to optimize a LC-IM-MS system to analyze post-translationally modified proteins thereby facilitating the analysis of complex protein samples.

### **Co-authors:**

*Florian Busch, Bruker Daltonics*  
*Bernhard Nemeč, Bruker Daltonics*

Poster number: **IM-PB-049**

## LOW-FLOW SIZE-EXCLUSION CHROMATOGRAPHY FOR ENHANCED NATIVE MS OF PROTEINS AND PROTEIN COMPLEXES

Abstract ID: **288****Presenting author: A Gargano, van't Hoff Institute for Molecular Sciences, Analytical Chemistry , Centre for Analytical Sciences Amsterdam**

### Introduction

We demonstrate the advantages of low-flow size-exclusion chromatography (SEC) coupled online to native mass spectrometry for the characterization of proteins and protein complexes. Lowering the elution flowrate to the micro/nano-electrospray ionization (ESI) regime provides a significant increase of the MS sensitivity allowing detection of higher-order protein species up to 230 kDa.

Under low-flow conditions, high ionic-strength mobile phases employing volatile salts (e.g. 400 mM) could be used, ensuring almost interaction-free SEC analysis. This is particularly useful for biotherapeutics (e.g. monoclonal antibodies) where non-specific interactions can induce peak tailing and/or adsorption. Additionally, efficient solvent evaporation can be achieved, allowing for softer ion desolvation conditions (lower activation energy) that prevent structural alterations or denaturation of proteins and their higher-order structures.

### Methods

We compared the performances of analytical-flow (4.6 mm ID; 0.2 mL/min), micro-flow (1 mm ID; 15  $\mu$ L/min) and nano-flow (0.2 mm ID; 0.5  $\mu$ L/min) SEC at pH 6.7 using ammonium acetate (50-400 mM). The separations were coupled to an Orbitrap QExactive+ Biopharma operated under High-Mass-Range mode. Electrospray gas temperatures and in-source CID were varied to allow coupling of the different flow rates.

To reduce injection band-broadening in low-flow SEC we used a trap&elute injection with self-packed capillaries (0.75 mm ID) ion-exchange traps. The analysis of reference proteins such as trastuzumab and pyruvate kinase are reported.

### Preliminary data (results)

We investigated the possible advantages of low-flow SEC separations coupled online to native MS for characterization of proteins and labile protein complexes. Lowering the flowrate provided a significant increase in the MS sensitivity and signal-to noise ratio allowing detection of low abundant impurities and higher-order species over 200 kDa (in the pmol range). Using micro-flow SEC (1 mm ID column and 15  $\mu$ L/min flow rate), the salt tolerance of the MS instrument was improved. High ionic-strength volatile salts (up to 400 mM) could be used to ensure almost interaction-free SEC analysis of proteins. Additionally, more efficient solvent evaporation was achieved using softer MS conditions (lower activation energy) preventing structural alterations or denaturation of the proteins and their higher-order structures. This was demonstrated by the analysis of Pyruvate Kinase (Figure 1; ~230 kDa) that was kept as intact tetramer using the low-flow setup.

Because of the reduced column dimensions, band broadening effects resulting from the injection volume became more critical. At higher injection volumes (exceeding 1% of the column pore volume) of more dilute samples, the peak shape and width was affected. Therefore, a new set-up was developed to pre-concentrate the injected proteins on an ion-exchange based trap prior to SEC-nMS analysis. This "trap&elute" set-up was able to eliminate adverse injection-volume effects in SEC while improving MS detection limits.

Results on the performance of the micro and preliminary results of nanoflow SEC-MS system will be discussed.

### Please explain why your abstract is innovative for mass spectrometry?

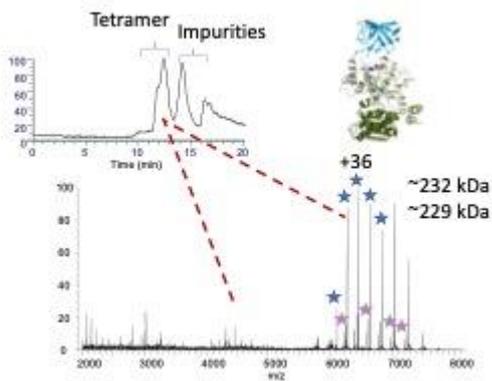
Microflow SEC allows coupling separations with high concentrations of volatile salts to MS and requires lower activation energy during ESI, allowing to maintain protein complexes in the gas phase

**Co-authors:**

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*G.W. Somsen, Division of BioAnalytical Chemistry, Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam*  
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TIC and MS of Pyruvate-Kinase by native micro-flow SEC-MS

Poster number: **IM-PB-050**

## A ROBUST HIGH-THROUGHPUT GC/TRIPLE QUADRUPOLE MS ANALYSIS OF 238 PESTICIDES IN UNDER 10 MINUTES

Abstract ID: **302****Presenting author: Remko Van Loon, Agilent Technologies**

### Introduction

Concern about trace-level food and environmental pollutants is driving the demand for more rapid and robust methods for the identification and quantitation of chemical residues. This work focuses on achieving fast GC/Triple Quadrupole MS (GC/TQ) analysis, while maintaining robust system performance in complex food matrices. Two system configurations described in this presentation provide analysis times of 10 minutes while maintaining sufficient chromatographic resolution for the analysis of 238 compounds. Method robustness is achieved using a mid-column backflush configuration. This presentation will discuss two configurations for achieving a 10-min analysis: A conventional 15 m x 15 m (0.25 mm x 0.25  $\mu$ m) and a narrow bore 10 m x 10 m (0.18 mm x 0.18  $\mu$ m) column configurations.

### Methods

The conventional 20-min retention time-locked method for 238 pesticides was created with an MRM database and used as a benchmark for the optimized fast analyses. To achieve faster analysis, two approaches were taken. First, the same 15 m x 15 m (0.25 mm x 0.25  $\mu$ m) conventional mid-column backflush column configuration was used with a faster oven ramp, yielding the analysis time of 10 min. Second, a narrow bore column 10 m x 10 m (0.18 mm x 0.18  $\mu$ m) mid-column backflush configuration was used enabling 10- and 8-min analysis time. The latter methods were precisely scaled using method translation.

### Preliminary data (results)

The use of the conventional 15 m x 15 m configuration allows choosing between a 20-min method for higher chromatographic resolution and analyzing up to 400 compounds at once, and a 10-min method that allows for maintaining similar chromatographic resolution while analyzing up to 250 compounds. Figure 1a shows the benchmark 20-min analysis of 238 pesticides commonly regulated in food. Figure 1b demonstrates the chromatogram acquired with the same configuration using a faster oven ramp. Retention index calibration was used to predict the new retention times as the relative elution order for some compounds changed.

Figure 1c and 1d show the chromatograms acquired with a 10 m x 10 m narrow bore configuration. The method translation technique allowed for preserving the relative elution order of the compounds, thereby accurately predicting retention times for both 10- and 8-min methods. The use of the narrow bore columns allowed for maintaining excellent chromatographic resolution (Fig. 2). Among the advantages provided by chromatographic resolution were reduced matrix interference and minimized interference between co-eluting analytes.

Mid-column backflushing used with both column configurations enabled method robustness by decreasing the need for column head trimming and the EI source cleaning. Also, when used with a temperature-programmable inlet, the liner can be changed much more rapidly compared to a conventional configuration with a column connecting the inlet directly to the mass spectrometer.

### Please explain why your abstract is innovative for mass spectrometry?

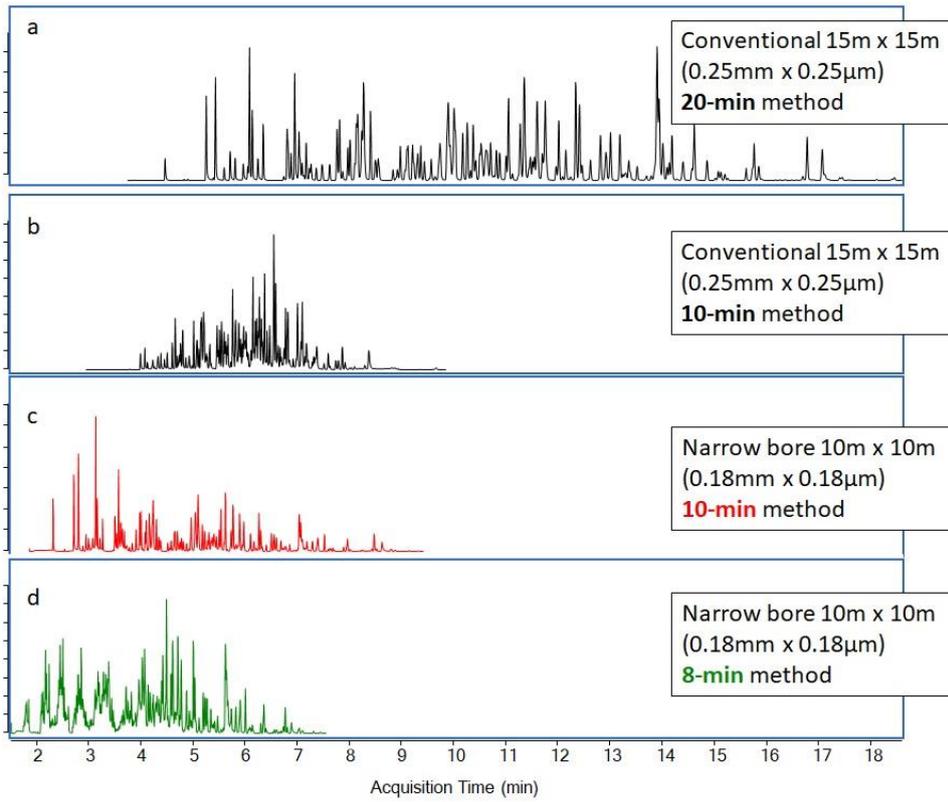
The two column configurations enable fast and robust analysis of 238 pesticides in under 10 minutes while maintaining chromatographic resolution similar to that achieved with the conventional 20-min analysis.

### Co-authors:

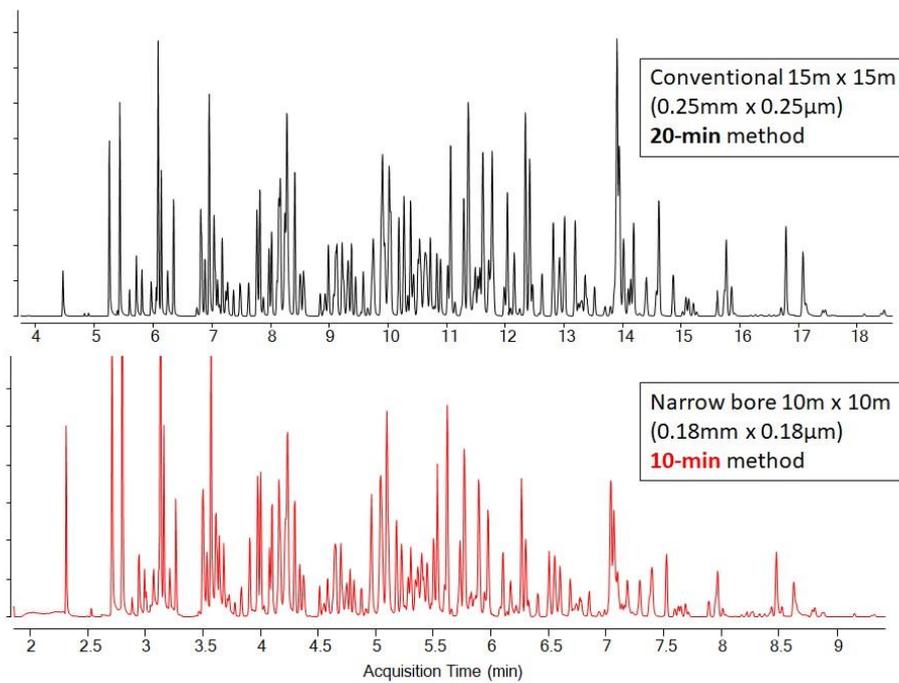
*Anastasia Andrianova, Agilent Technologies*  
*Bruce Quimby, Agilent Technologies*

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GC/TQ MRM total ion chromatograms (TICs) of 238 pesticides



Comparison of 20- and 10-minute methods for 238 pesticides

Poster number: **IM-PB-051**

## **NOVEL STRATEGY TO SCREEN MULTI-CLASSES OF HALOGENATED POLLUTANTS BY GAS CHROMATOGRAPHY-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION-TRAPPED ION MOBILITY-MASS SPECTROMETRY (GC-APCI-TIMS-TOFMS)**

Abstract ID: **348****Presenting author: Gauthier Eppe, Mass Spectrometry Laboratory-ULiège**

### **Introduction**

Over the past two decades, it has become increasingly evident that the presence of organohalogen contaminants in the environment, called persistent organic pollutants (POPs), is a growing concern. Competent authorities are implementing monitoring plans that require the screening of as many compounds as possible that are known and established to be toxic or potentially toxic. In addition, there is a need for policies that focus on compounds that are little or poorly studied at all, in order to address the hidden side of the problem. Thus, novel analytical methods and strategies that allow rapid screening and quantitation of multiple POPs simultaneously, while providing improved separation and identification capabilities, are in high demand.

### **Methods**

Gas chromatography (Bruker 456-GC; Rxi-5SilMS column, 30mx0.25mmx0.25m, Restek) has been hyphenated to atmospheric pressure chemical ionization (APCI, GC-APCI II, Bruker) and trapped ion mobility spectrometry (TIMS)-ToFMS (GC-APCI-TIMS-TOFpro, Bruker). A mix solution comprising 152 compounds was prepared in n-Nonane (99%, Alfa Aesar) and run by GC-APCI-TIMS-MS. This mixture contained 7 PCDDs and 10 PCDFs (NK-ST-B4 mix, Wellington), 82 PCBs (PAR-H mix, Wellington), 4 PXDDs and 2 PXDFs (where X are mixed Cl, Br substitution; individual standards, Wellington), 23 PAHs (EPA & EU-PAH-STK, Wellington), 20 BDEs (BDE-MXC, Wellington), 2 PBBs (individual standards, AccuStandard), BTBPE and DBDPE (Wellington).

### **Preliminary data (results)**

We propose to focus efforts mainly on ion mobility rather than on the ion mass. The research developed here aims at combining a method integrating the use of trapped ion mobility (TIMS) with a GC-APCI-TOFMS instrumentation. In TIMS mode, Collision Cross Sections (CCS) versus m/z trendlines were examined in details. The power fit functions (of the type  $y=ax^b$ ) highlighted that each class of contaminant was characterized by its own trendline. It was found that for given mass, the experimental CCS in nitrogen buffer gas of the chlorinated compounds were higher than that of the brominated compounds but lower than that of planar PAHs. In addition, the primary factor contributing the difference in CCS among a family of halogenated compounds was discernibly identified as the halogenation degree, while the fine ion mobility separation of isomeric congeners could also be related to key structural parameters such as the number of chlorine/bromine atoms in ortho position for PCBs or next to the oxygen atom for PCDFs. In addition, the ion mobility resolution provided by TIMS proved valuable in deconvoluting the signal from isobars compounds that were not separated by either the GC conditions used here or the high resolution ToF MS. Further exploration showed that correlation exist between ion mobility and GC retention time. We introduced a novel concept of mobility windows, which shows great potential to further enhance the ion mobility resolution and performance of the TIMS for the analysis of persistent organic pollutants and related emerging classes of compounds.

### **Please explain why your abstract is innovative for mass spectrometry?**

By coupling GC with APCI-TIMS-MS, a novel approach based on mobility windows has been developed to monitor non-targeted mixtures of halogenated POPs in complex samples.

### **Co-authors:**

*Hugo Muller, Mass Spectrometry Laboratory-ULiège*  
*Georges Scholl, Mass Spectrometry Laboratory-ULiège*  
*Alexandre Collgros, Bruker France*  
*Johann Far, Mass Spectrometry Laboratory-ULiège*  
*Edwin De Pauw, Mass Spectrometry Laboratory-ULiège*

Poster number: **IM-PB-052**

## DERIVATIZATION-TARGETED ANALYSIS OF AMINO COMPOUNDS WITH DIETHYL ETHOXYMETHYLENEMALONATE DERIVATIZATION AND NEUTRAL LOSS SCAN MODE BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

Abstract ID: **349****Presenting author: Larissa Silva Maciel, University of Tartu**

### Introduction

Derivatization consists of a reaction where a derivatization reagent reacts with a specific functional group present in the analyte, improving its chromatographic and detection behavior when compared to the underivatized analyte. Additionally, this approach can be used to detect the compounds that react with the derivatization reagent, if the derivatives present the same fragmentation pattern in LC-MS/MS.

In this work, diethyl ethoxymethylenemalonate (DEEMM) is the derivatization reagent of choice, targeting primary and secondary amino compounds. Its derivatives lose a neutral fragment (46, ethanol molecule) from the parent ion upon fragmentation, enabling the use of neutral loss scan mode. Such method was applied to hemp (*Cannabis sativa* L.) seed hull and sea buckthorn (*Hippophae rhamnoides*) pomace samples for the study of its amino compound content.

### Methods

Extraction of amino compounds was carried out with 2 mL of 30% methanol in 0.1 M HCl (hemp seed hull) and 50% acetonitrile (sea buckthorn pomace) for 15 minutes in an ultrasonic bath at room temperature. The extracts were derivatized with DEEMM and the amino compounds were detected with LC-ESI-MS in neutral loss scan mode (loss of 46) in the range  $m/z$  50-600.

### Preliminary data (results)

A combined amount of 37 amino compounds were detected in both samples by comparing the retention time and MS spectra of the peaks with injected reference standard substances. For quantification purposes, the influence of extraction parameters time (minutes), volume of solvent (mL) and temperature (°C) were studied by means of design of experiments (full factorial design). As a result, only the mass was a statistically significant parameter. Overall, higher amounts of glutamic acid, glycine and GABA were quantified in hemp seed hull and asparagine in sea buckthorn pomace.

### Please explain why your abstract is innovative for mass spectrometry?

Primary and secondary amino compounds can be detected in a sample after DEEMM derivatization and neutral loss scan mode in LC-MS/MS.

### Co-authors:

*Dunja Malenica, Estonian University of Life Sciences*  
*Marko Kass, Estonian University of Life Sciences*  
*Rajeev Bhat, Estonian University of Life Sciences*  
*Koiti Herodes, University of Tartu*

Poster number: **IM-PB-053**

## **DEVELOPMENT OF A METHOD FOR DETERMINATION OF EGG AND MILK ALLERGENS IN BAKERY PRODUCTS BY LC-MS/MS**

Abstract ID: **383**

**Presenting author: Ignazio Garaguso, PerkinElmer LAS**

### **Introduction**

Allergens pose a real health risk to consumers. Food products can be contaminated due to unintentional cross-contact with allergen sources during the production process. Therefore, food manufacturers and control laboratories require reliable methods for sensitive determination of allergens in food matrices. Indeed, methods based on LC-MS/MS offer a multitude of advantages: i) unambiguous identification of the allergen through the detection of signature peptides ii) multi-target analysis: detection of multiple compounds in one run iii) allergen quantification through the quantification of the produced signature peptides.

In this work we present the performance of a targeted LC-MS/MS method developed using a simple sample preparation and QSight triple quadrupole LC-MS/MS system for the quantification of milk and egg allergens in thermally processed bakery products.

### **Methods**

Samples were prepared according to a four steps method: (1) proteins extraction, (2) proteins purification by size exclusion chromatography, (3) tryptic digestion, (4) peptide purification and pre-concentration by solid-phase extraction. The instrumental parameters for chromatographic separation, MS/MS fragmentation and MRM acquisition were optimized with a mixture of synthetic peptides. Eight markers tracing for both egg and milk contamination were selected according to the review of current literature and monitored simultaneously within a 13 min elution gradient.

### **Preliminary data (results)**

With this method optimized on the QSight LC-MS platform, we achieved very challenging sensitivity with LODs as low as 0.1 µg/g for milk and 0.3 µg/g for egg, respectively. The method demonstrates a CV for intraday repeatability lower than 10% and inter-day repeatability lower than 9 %. The method recovery calculated with the aid of the reference material provided by MoniQA Association resulted to be around 57 % as determined for milk contamination.

### **Please explain why your abstract is innovative for mass spectrometry?**

High detection sensitivities for allergens in complex food matrices. Stay-clean source and HSID (Hot Surface Induced Desolvation) require less frequent cleaning permitting longer up-time of the mass spectrometer.

### **Co-authors:**

*Elisabetta De Angelisa, National Research Council of Italy, Institute of Sciences of Food Production, CNR-ISPA*  
*Rocco Guagnano, National Research Council of Italy, Institute of Sciences of Food Production, CNR-ISPA*  
*Linda Monaci, National Research Council of Italy, Institute of Sciences of Food Production, CNR-ISPA*

Poster number: **IM-PB-054**

## MULTICONDITIONRT (MCRT): PREDICTING LIQUID CHROMATOGRAPHY RETENTION TIME FOR EMERGING CONTAMINANTS FOR A WIDE RANGE OF ELUENT COMPOSITIONS AND STATIONARY PHASES

Abstract ID: 392

**Presenting author: Amina Souihi, Department of Environmental and Materials Chemistry, Stockholm University**

### Introduction

Liquid chromatography (LC) coupled to high resolution mass spectrometry (HRMS) is widely used in non-targeted screening. To identify the detected compounds at higher confidence levels, retention time provides an important orthogonal confirmation point to the HRMS spectra. Though many models have been developed to predict retention times from molecular descriptors and eluent compositions these are applicable to only one type of chromatographic columns and narrow range of eluent compositions.

### Methods

78 compounds were selected from NORMAN compounds in MassBank list (S1)<sup>1</sup> and they were analysed using four columns (C18 reversed phase, mixed mode, HILIC, and biphenyl columns), different pH-s (from 2.1 to 10), two organic modifiers (acetonitrile and methanol) and different additives.

(1) Schymanski, E.; Schulze, T.; Alygizakis, N.; Meier, R. S1 | MASSBANK | NORMAN Compounds in MassBank, 2019. <https://doi.org/10.5281/ZENODO.2621390>.

### Preliminary data (results)

Differences in retention time were observed for some compounds in mixed mode, C18 reversed phase and HILIC columns from one eluent composition to another (with a maximum difference of 10 min). Therefore, it was very important to include a wide variety of eluent compositions and columns to the retention time prediction models. Linear regression with backward selection, independent component regression, partial least squares regression, multivariate adaptive regression splines, *k*-Nearest neighbors and random forest models were trained on 61 compounds and compared on an independent test set (containing 14 compounds) to predict the retention time from the eluent compositions and molecular descriptors. The best performing random forest regression model yielded a root mean square error (RMSE) of 1.55 min for reversed phase, 1.79 min for mixed-mode, 1.93 min for HILIC, and 1.56 min for biphenyl column. MCRT can predict retention times in different gradient profiles using a general additive model-based calibration approach. The MCRT approach was validated internally using 84 compounds and externally using 324, yielding an RMSE of 2.68 and 2.32 min.

The results show that MCRT has slightly higher relative errors (from 14.4% to 26.9%) compared to the current models<sup>2</sup> but it can predict retention times for twenty different eluent compositions and four columns.

(2) Domingo-Almenara, X.; Guijas, C.; Billings, E.; Montenegro-Burke, J. R.; Uritboonthai, W.; Aisporna, A. E.; Chen, E.; Benton, H. P.; Siuzdak, G. The METLIN Small Molecule Dataset for Machine Learning-Based Retention Time Prediction. *Nat Commun* **2019**, *10* (1), 5811. <https://doi.org/10.1038/s41467-019-13680-7>.

### Please explain why your abstract is innovative for mass spectrometry?

MCRT could be used together with other tools such as SIRIUS to reduce the candidates list in non-targeted screening and identify compounds at higher confidence levels.

Poster number: **IM-PB-055**

## DEVELOPMENT OF UHPLC AND UHPSFC-MS ASSAYS TO DETERMINE THE CONCENTRATION OF BITREX™ AND SODIUM SACCHARIN IN HOMEMADE FACEMASK FIT TESTING SOLUTIONS

Abstract ID: 411

Presenting author: **Julie M Herniman, University of Southampton**

### Introduction

Fast and easily transferable chromatography-mass spectrometry QC assays were developed to detect and quantify denatonium benzoate known by the brand name Bitrex™ and sodium saccharin in homemade facemask fit testing solutions [1].

At the outset of the Covid-19 pandemic in 2020, demand for commercial spray solutions in the UK significantly increased in-line with increased usage of masks and respirators. As supplies to frontline healthcare workers became scarce, a team of chemists at the University of Southampton quickly produced multiple batches of the two test solutions following a method adapted from Fakherpour *et al.* [2] and made to the British Standard BS ISO 16975 3:2017 [3].

### Methods

Each batch solution was prepared at two concentrations, one to test the response of an individual, and a concentrated solution to test the facemask once fitted. Bitrex™ solutions were analysed using reversed phase ultrahigh-performance liquid chromatography mass spectrometry (UHPLC-MS). Separation was achieved using a 1.5-minute mobile phase gradient and selected ion monitoring (SIM) was used to detect positive ions for Bitrex™ and an internal standard. Sodium saccharin solutions were analysed using ultrahigh-performance supercritical fluid chromatography-MS (UHPSFC-MS). Separation was achieved using isocratic elution and SIM was used to detect negative ions for sodium saccharin and an internal standard.

### Preliminary data (results)

For each assay, calibration curves were created for the active ingredient together with a suitable internal standard over the linear response range of each instrument to give R<sup>2</sup> values > 0.99. Following preparation of the bulk solutions, 1 mL of each solution was taken for analysis. These pre-autoclave solutions were tested in triplicate and the concentrations calculated by plotting SIM peak area values for the active ingredient against the calibration curves. If the concentration of each solution was within ~10% of the required concentration, then the batch solution proceeded to a bottling and autoclave sterilisation stage. Further QC analyses were undertaken from a minimum of 10% of randomly selected sample bottles after autoclave sterilisation. Sample solutions that successfully gave repeatable measurements within 10% of the expected concentration were released for distribution to local hospitals and healthcare providers.

[1] Herniman J.M., Langley G.J. Development of ultrahigh-performance liquid chromatography/mass spectrometry and ultrahigh-performance supercritical fluid chromatography/mass spectrometry assays to determine the concentration of Bitrex and sodium saccharin in homemade facemask testing solutions. *Rapid Commun. Mass Spectrom.* 2020;34:e8848 doi:10.1002/rcm8848

[2] Fakherpour A, Jahangiri M, Yousefinejad S, Seif M. Feasibility of replacing homemade solutions by commercial products for qualitative fit testing of particulate respirators: a mixed effect logistic regression study. *MethodsX.* 2019; 6: 1313-1322. doi: 10.1016/mex.2019.05.034

[3] BSI Standards Publication BS ISO 16975-3: 2017 Respiratory protection devices. Selection, use and fit-testing procedures. 2017.

### Please explain why your abstract is innovative for mass spectrometry?

Use of novel chromatography and mass spectrometry assays to rapidly identify and quantify the active ingredients in facemask testing solutions.

### Co-authors:

G. John Langley, University of Southampton

Poster number: **IM-PB-056**

## UNRAVELING AND CONTROLLING AGGREGATE FORMATION IN CAENORHABDITIS ELEGANS PARKINSON'S DISEASE MODELS USING LOW FLOW SIZE EXCLUSION CHROMATOGRAPHY COMBINED WITH ION MOBILITY MASS SPECTROMETRY

Abstract ID: **438**

**Presenting author: Raya Sadighi, Vrije Universiteit Amsterdam, Division of BioAnalytical Chemistry, AIMMS Amsterdam Institute of Molecular and Life sciences**

### Introduction

Parkinson's Disease (PD) is the second most common and fastest-growing neurodegenerative disease at this moment, making it one of the largest health challenges we face in the 21st Century. This neurodegenerative disorder is characterized by protein aggregations comprised of  $\alpha$ -synuclein, where soluble proteins transform into insoluble fibrils. However, our understanding of this molecular process is lacking which significantly hampers the development of (new) treatments for Parkinson's Disease. We aim to develop a multidisciplinary MS-based approach that allows us to understand the mechanism of amyloid formation of  $\alpha$ -synuclein at the molecular level both from humans expressed in *Escherichia coli* and from *Caenorhabditis elegans* (*C. elegans*). Subsequently, we will use this knowledge and technological developments to search for small molecule therapeutic agents that inhibit the amyloid assembly.

### Methods

The first step is to develop our analytical workflow to separate and characterize  $\alpha$ -synuclein aggregation. Therefore, we combine native low-flow size exclusion chromatography (SEC) with trapped ion mobility mass spectrometry (TIMS-MS). Native SEC can separate the aggregates by size while keeping their structure intact. The  $\alpha$ -synuclein aggregates are subsequently analyzed by their mass-to-charge distribution and shape (3D structure) using TIMS-MS. To probe aggregation *in vivo*, nematode PD models that over-express human  $\alpha$ -synuclein are used. The worms are harvested at different stages during their life span. Different extraction protocols are accessed to purify the alpha-synuclein prior to native SEC-TIMS analysis.

### Preliminary data (results)

$\alpha$ -synuclein expressed in *E. coli* was used to develop the low-flow SEC-TIMS-MS method. In comparison to traditional SEC, low-flow SEC has a higher sensitivity and allows for higher concentrations to be analyzed. This step is used to separate different oligomer sizes, and functions as a sample clean-up stage prior to MS. The TIMS-MS method was optimized to prevent unfolding and ion heating, allowing the study of aggregates in a native state. Two distinct distributions are observed for  $\alpha$ -synuclein, i.e. a folded structure with a main charged state of +6 and an unfolded charge state of +14. Collision cross-sections of the 2 distributions show that the higher charge states correspond to different conformations.

Our recent work has shown that nematode PD models overexpressing  $\alpha$ -synuclein indeed show amyloid plaque formation in the head region. Here, we focus on the molecular effect in these PD models by extracting the aggregated  $\alpha$ -synuclein from worms at different life stages. Currently, different extraction mechanisms are explored, which aim to preserve the protein in its native form, while retaining a high recovery efficiency. Subsequently, we will use our developed low-flow SEC-TIMS-MS platform to study these aggregates.

In phase two, we aim to probe the effect of select inhibitors at the molecular level. *C. elegans* has been proven to be an ideal screening platform for small molecule inhibitors. By extracting the treated aggregates, and subsequently detecting and structurally characterizing the resulting disaggregated  $\alpha$ -synuclein monomers or oligomers, the effect of the small molecule drugs can be unraveled.

### Please explain why your abstract is innovative for mass spectrometry?

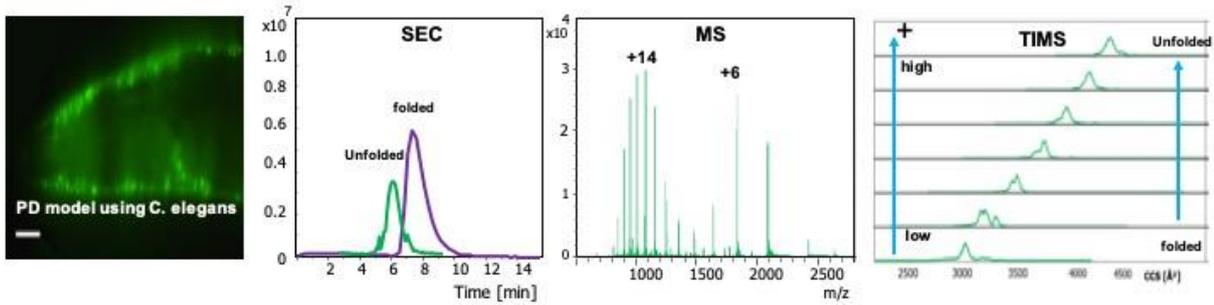
Development of a novel, hyphenated low-flow SEC-TIMS-MS approach to characterize different stages of aggregation of alpha-synuclein *in vivo* in Parkinson's Disease.

**Co-authors:**

*Christine Mathiesen, Vrije Universiteit Amsterdam, Division of BioAnalytical Chemistry*

*Samantha S Hughes, Vrije Universiteit Amsterdam, Division of environment and health, AIMMS Amsterdam Institute of Molecular and Life sciences*

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workflow of analyzing oligomers of  $\alpha$ -synuclein via low flow SEC-TIMS-MS

Poster number: **IM-PB-057**

## DIFFERENTIAL MOBILITY SPECTROMETRY COUPLED TO MASS SPECTROMETRY (DMS-MS) FOR THE CLASSIFICATION OF SPANISH PDO PAPRIKA

Abstract ID: 455

**Presenting author: Sonia Sentellas, Department of Chemical Engineering and Analytical Chemistry, University of Barcelona, Martí i Franquès 1-11, E08028 Barcelona, Spain, Research Institute in Food Nutrition and Food Safety, University of Barcelona, Av. Prat de la Riba 171, Edifici Recerca (Gaudí), E08921 Santa Coloma de Gramenet, Spain., Serra Hünter Lecturer, Generalitat de Catalunya, E08007 Barcelona, Spain**

### Introduction

Ion mobility spectrometry (IMS) has proved its potential in many research areas, especially when hyphenated with chromatographic techniques or mass spectrometry (MS). However, focusing on food analysis, very few applications have been reported following a fingerprinting approach. In this regard, the use of this technique for food classification and authentication purposes opens great opportunities. The present work aims at evaluating the applicability of direct infusion differential mobility spectrometry coupled to mass spectrometry (DMS-MS) for such a purpose, using Spanish paprika, produced under the protected designation of origin (PDO) label as a proof of concept.

### Methods

In this study, a total of 15 *La Vera* PDO, 10 *Murcia* PDO, and 10 *Mallorca* PDO samples were subjected to solid-liquid extraction (SLE) using water:acetonitrile (20:80, v/v) as the extracting solvent. The obtained extracts were directly introduced into the DMS-MS instrument. Separation voltage was fixed at 2500 V and the compensation voltage (CV) was scanned from -10 to 7 V in 0.1 V increments. Ions reaching the mass spectrometer were analyzed by full scan in negative mode from  $m/z$  100 to 650. Fingerprints obtained were subjected to principal component analysis and partial least squares regression-discriminant analysis.

### Preliminary data (results)

At first glance, the visual inspection of the obtained DMS-MS fingerprints allowed the detection of slight qualitative variations according to the sample geographical origin. Hence, the obtained PCA score plots showed a slight sample distribution depending on the origin. In this line, PLS-DA improved sample classification and allowed an apparent distinction between the three Spanish regions under study. External validation was carried out to evaluate the ability of the PLS-DA model established to classify paprika samples from the three Spanish regions distinguished with the PDO label. Thus, 60% of the samples were used as the calibration set and the remaining 40% as the external validation set. As a result, a classification accuracy of 100% was reached. Moreover, when building a PLS-DA model for a specific region, classification according to sample type (hot, sweet, and bittersweet types) was also achieved.

### Please explain why your abstract is innovative for mass spectrometry?

In this study DMS-MS was satisfactorily applied for the first time for food classification and authentication, demonstrating to be a reliable alternative to other currently applied techniques.

### Co-authors:

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Poster number: **IM-PB-058**

## THE ANALYSIS OF PESTICIDE PRODUCTS USING ULTRA-HIGH-PERFORMANCE SUPERCRITICAL FLUID CHROMATOGRAPHY-MASS SPECTROMETRY

Abstract ID: **530**Presenting author: **Rebecca Baker, University of Southampton**

### Introduction

New methods have been developed and optimised using Ultra-High-Performance Supercritical Fluid Chromatography (UHPSFC-MS) for the analysis of pesticides. These were achieved by using a small library of compounds, each selected to reflect the range of physicochemical properties of pesticide active ingredients. Pesticide products consist of number of components, including the active ingredient, formulants and impurities and the complexity often requires a highly efficient separation technique and a sensitive detection method that can quantify low level impurities. The high throughput and wide range of column chemistries available using UHPSFC has been utilised to separate a mix of pesticide active ingredients. Atmospheric Pressure Ionisation-Mass Spectrometry (API-MS) has been used to characterise the components of the mix and optimisation of MS parameters was performed to improve sensitivity.

### Methods

A mix of pesticide active ingredients was separated by UHPSFC-MS using a Waters Acquity UPC<sup>2</sup> TQD. The sensitivities of a number of API techniques were assessed for each component including electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI) and dopant-assisted atmospheric pressure photoionisation (APPI) using toluene as the dopant. Individual ion sources parameters were optimised to achieve maximal sensitivity. Data were recorded between  $m/z$  120 – 1000 and processed using MassLynx v4.1.

### Preliminary data (results)

UHPSFC conditions were developed to afford optimal separation of the components within the mixture. The pesticide active ingredients studied have a range of different functionalities within their structures, resulting in only a selection of them ionising by each technique and not one single ionisation technique was able to detect all compounds. The performance of the different ionisation techniques and the link to structure will be shown. Sensitivity varied significantly for the three ionisation techniques, and one technique was deemed to be unsuitable due to the low signal-to-noise ratio observed. Individual ion source parameters such as cone voltage, probe temperature and repeller voltage were optimised to maximise sensitivity.

### Please explain why your abstract is innovative for mass spectrometry?

UHPSFC-MS has been shown to separate and characterise a small library of pesticide active ingredients. A combination of ionisation techniques is required for the detection of all compounds by MS.

### Co-authors:

*John Langley, University of Southampton*  
*Julie Herniman, University of Southampton*  
*Chris Howson, Syngenta*  
*Lorraine Ezra, Syngenta*

Poster number: **IM-PB-059**

## **APPLICATION OF CHROMATOGRAPHY AND MASS SPECTROMETRY TO UNRAVEL THE COMPLEXITY OF DIESEL FUEL**

Abstract ID: **531**

**Presenting author: Molly Wilson, University of Southampton**

### **Introduction**

Changes have been made to fuel specifications and engine design to meet new legislative mandates; these changes have coincided with an increase in the number of reports detailing fuel delivery system failures due to blockages. New chromatography and mass spectrometry methods have been developed to detect and identify the materials responsible for these blockages. The new methods were also used to analyse the original fuel to either identify these materials or possible precursor molecules in the fuel. This knowledge can subsequently be used to develop mitigation strategies that will prevent blockages.

### **Methods**

New GC-MS, GCxGC-MS and SFC-MS methods were developed to detect, identify and, in some cases, quantify problematic materials within fuel or blocked samples – whether solid or liquid. Throughout method development, the different chemistries of suspect contaminants were considered as well as the specificity of chromatography and MS ionisation techniques.

### **Preliminary data (results)**

The analysis methods proved successful in accessing new sample specific information, leading to a deeper understanding of blockages in the fuel delivery system. In one case the new analytical methodology revealed a contaminant, this prompted a modified sample preparation that allows this material to be identified by GC-MS and SFC-MS using standard liquid injection. Analysis of the putative compound with these methodologies further improved confidence in assignment.

### **Please explain why your abstract is innovative for mass spectrometry?**

New chromatography and mass spectrometry methods to identify analytes within plugged fuel filters and associated fuels that may contribute fuel delivery issues within the diesel common rail fuel system.

### **Co-authors:**

*Jim Barker, Innospec*

*Jacqueline Reid, Innospec*

*Edward Wilmot, Innospec*

*Julie Herniman, University of Southampton*

*G. John Langley, University of Southampton*

Poster number: **IM-PB-060**

## NUMERICAL AND EXPERIMENTAL INVESTIGATIONS OF PROTON-BOUND ACETONE/WATER CLUSTERS IN DIFFERENTIAL ION MOBILITY SPECTROMETRY

Abstract ID: 611

**Presenting author: Walter Wißdorf, University of Wuppertal, Department of Physical and Theoretical Chemistry**

### Introduction

In differential ion mobility spectrometry (DMS), ions are separated by their differential ion mobilities with an asymmetric electric field. High electric fields lead to elevated effective ion temperatures, impacting the equilibrium state of chemically active systems, such as the proton-bound acetone/water cluster system. The compensation voltage (CV), which is used to compensate the net ion drift caused by the asymmetric field, is therefore influenced by the frequent cluster formation and dissociation as well as by the background gas temperature and acetone mixing ratio. The acetone/water cluster system is investigated numerically and experimentally. Results at different background gas temperatures and acetone mixing ratios are presented. Furthermore, the numerical results are compared with experimental data.

### Methods

DMS experiments were performed using a Sciex Triple Quad 6500 with SelexION® (Sciex, Ontario, Canada) and Turbo V™ ion source (APCI mode). The analyte (1:1 mixture of HPLC grade acetone and Millipore water) was added by a syringe pump into the DMS at a flow rate of 7  $\mu\text{L}/\text{min}$ . Numerical simulations were performed using the particle based simulation framework IDSimF. The chemical kinetics are modelled based on a Monte Carlo method, whereas the CV was calculated by an optimization process, which minimizes the net ion drift towards the electrodes, in combination with a simplified transport model.

### Preliminary data (results)

The effective ion temperature is modulated by the oscillating electric field caused by the separation voltage. Therefore, the dynamics of cluster formation and dissociation is governed by the oscillating ion temperature. Numerical simulations of the proton-bound acetone/water cluster system show that the concentration-time profiles of the individual cluster species are also oscillating. At low acetone mixing ratios, a strong cluster effect in the dispersion plot is observed experimentally. The ion mobility at the high field phase is higher due to cluster dissociation than in the low field phase, where cluster formation occurs. This is also observed in the numerical simulations, which are in a good qualitative agreement with experimental results. However, at higher acetone mixing ratios, the cluster effect is experimentally observed only at separation voltages higher than 2000 V. At lower SV, the CV vanishes. This is caused by the formation of the proton-bound acetone dimer at higher acetone mixing ratios, which does not form any ion-molecule clusters with water and therefore showing hard sphere behavior with increasing SV. The acetone dimer dissociates between the DMS cell and the mass spectrometer, leading to the observed dispersion plot. At high separation voltages, either dimer formation is inhibited, or the dimer dissociates until it reaches the DMS cell. Essentially, only the monomer is present at high SV, which can cluster with water and yields to negative CVs. It is planned to include the dimer formation in the numerical model and to compare the results with experimental data.

### Please explain why your abstract is innovative for mass spectrometry?

Experimental and numerical investigations of the chemical dynamics of the proton-bound acetone/water cluster system in a DMS cell at different background gas temperatures and solvent mixing ratios.

### Co-authors:

*Duygu Erdogan, University of Wuppertal, Department of Physical and Theoretical Chemistry*  
*Hendrik Kersten, University of Wuppertal, Department of Physical and Theoretical Chemistry*  
*Thorsten Benter, University of Wuppertal, Department of Physical and Theoretical Chemistry*

Poster number: **IM-PB-061**

## ISOMERIC O-GLYCOPEPTIDES CHARACTERIZATION USING $\mu$ LC, DIFFERENTIAL MOBILITY SPECTROMETRY, COLLISION INDUCED AND ELECTRON CAPTURE DISSOCIATION

Abstract ID: **614**Presenting author: **Charlotte Jacquet, University of Geneva**

### Introduction

Protein and peptides glycosylation cannot be accurately predicted making the determination of glycan position and composition challenging. O-glycan refers to a glycan bounded mainly on any serine (S) or threonine (T), thus, isomeric positional O-glycopeptide are highly probable. Collision induced dissociation (CID) and electron transfer dissociation (ETD)/electron capture dissociation (ECD) enable to characterize glycopeptide in term of glycan composition and position. However, detection of possible positional isomers can be challenging. Through this work different  $\mu$ LC-MS workflows are investigated to detect and characterize a set of three positional isomeric O-glycopeptide and the corresponding non glycopeptide using Differential Mobility Spectrometry (DMS), CID and ECD.

### Methods

Four standard peptides with the sequence SYISSQTNDTHK, non-glycosylated (0), glycosylated by an alphaDGalNAc on first serine (S1), fourth serine (S4) or seventh threonine (T7) were analysed using a QqTOF (TTOF 6600+, Sciex) equipped with a prototype chimeric cell and a differential mobility cell (Selexlon, Sciex). For infusion, C18 ZipTip was done prior analysis of the mix of four peptides. For  $\mu$ LC, column-switching was performed and separation was achieved in gradient mode (20  $\mu$ L/min, 15 min from 0 to 7% B) with a C18 column. Spectra analysis was performed with Byos software (Protein Metrics)

### Preliminary data (results)

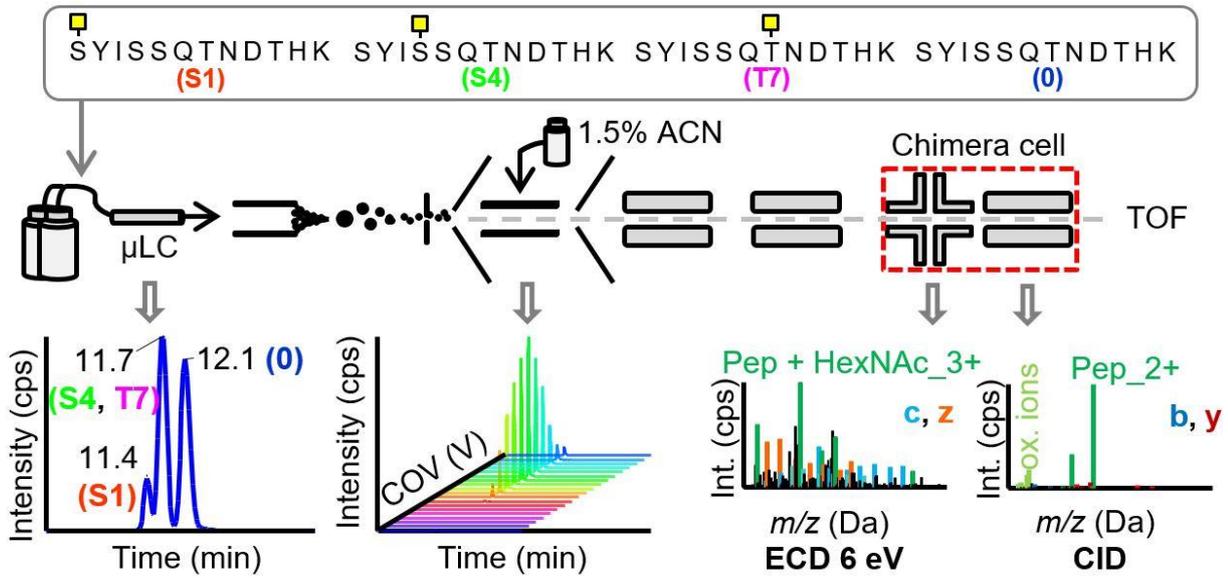
The four peptides were analysed by direct infusion (ESI, positive mode) and DMS-TOF using N<sub>2</sub>, 1.5% IPA, 1.5% ACN and 1.5% EtOH as modifiers. With N<sub>2</sub> and 1.5% ACN doubly and triply charged peptides (Pep\_3+, Pep+HexNac\_3+) were detected while with alcoholic modifiers only doubly charged peptides were identified. Baseline separation was not achieved but peak shapes showed a start of separation. Then, an LC-MS-CID-ECD workflow was developed including, TOF-MS, CID 20V Pep\_3+, ECD 6eV Pep\_3+, CID 20V Pep+HexNac\_3+, ECD 6eV Pep+HexNac\_3+ experiments. For mix analysis, T7 and S4 were coeluting allowing only identification of S1, S4 and 0. Focus was further done on glycopeptide due to clear LC separation of 0. A multidimensional LCxDMS separation was investigated. First, peptides were injected separately. After LC separation, COV was ramp and TOF-MS recorded to determine their COV optima: -5V, -2.5V and -7V for T7, S1 and S4 respectively. Then, similarly to LC-CID-ECD workflow, an LC-DMS-CID-ECD workflow was used with a fix value of COV to use DMS as filter and let only one of the glycopeptide go through the DMS cell. Three different injections of peptide mix were done. At -5V, -2.5V and at -7V where only T7, only S1 and only S4 were identified respectively. The combination of LC and DMS provide enough separation to detect and characterised three isomeric O-glycopeptides. ECD data were used to spot position of sugar and CID data to get information about sugar composition.

### Please explain why your abstract is innovative for mass spectrometry?

Improved separation and characterisation of isomeric O-glycopeptides using DMS with modifiers coupled to  $\mu$ LC, CID and ECD fragmentation.

### Co-authors:

*Gérard Hopfgartner, University of Geneva*



LC-DMS-ECD-CID workflow for identification and characterization of a peptide set

Poster number: **IM-PB-062**

## **GAS-PHASE ELECTROPHORESIS AND MASS SPECTROMETRY OF LIPOSOMES - TWO TECHNIQUES THAT PERFECTLY MATCH FOR VESICLE CHARACTERIZATION**

Abstract ID: 639

**Presenting author: Victor U. Weiss, Institute of Chemical Technologies and Analytics, TU Wien (Vienna University of Technology), Vienna, Austria**

### **Introduction**

Liposomes, vesicles consisting of a lipid shell and an aqueous core, are applied in e.g. the pharmaceutical industry for cargo transport. Particle surface modifications enable targeted delivery or increased lifetime of vesicles in biological systems, decreasing the overall drug burden to an organism as sustained release of active ingredients at their immediate site of action occurs.

However, for corresponding applications the thorough characterization of liposomes in terms of e.g. vesicle size, particle number-concentration, purity, lipid composition and particle heterogeneity is a necessary prerequisite. These questions we are targeting via gas-phase electrophoresis of a nano Electrospray Gas-phase Electrophoretic Mobility Molecular Analyzer (nES GEMMA) aka nES Differential Mobility Analyzer (nES DMA). Following separation, vesicles can be size-collected to allow for their subsequent characterization via MALDI MS.

### **Methods**

Liposomes were prepared from HSPC, DSPE and cholesterol via the thin lipid film hydration method (4:3:3 molar ratio). Ammonium acetate (40 mM, pH 8.4) was applied as electrolyte. Vesicles were extruded via 21 passes through a 100 nm pore-sized membrane. Subsequently, vesicles were characterized via nES GEMMA (TSI Inc, Shoreview, MN, USA) measurements. MALDI MS was on an Axima TOF<sup>2</sup> (Shimadzu Biotech, Manchester, UK) in reflector positive mode and employing THAP (2,4,6-Trihydroxyacetophenon) as matrix. Vesicles were collected at 1 mM total lipid concentration for 150 min at 85 nm surface-dry particle diameter on gold-coated silicon wafers.

### **Preliminary data (results)**

Liposomes were prepared via the thin lipid film hydration method. Subsequently, vesicles were characterized via gas-phase electrophoresis yielding surface dry particle diameters of nanoparticle material. Analysis of samples at different dilutions excluded detection of unspecific aggregates in nES GEMMA spectra. Hence, gas-phase electrophoresis yielded information on surface-dry vesicle size, particle number-concentration, repeatability of batch preparations and vesicle heterogeneity. It is of note that in contrast to other characterization methods like e.g. nanoparticle tracking analysis, gas-phase electrophoresis enabled us to also target smaller sized sample components, which we demonstrated to significantly impact cell viability.

Focusing on the MS based characterization of liposomes we were able to detect lipid species applied for liposome preparation in dispersion after vesicle formation. Moreover, gas-phase electrophoresis enabled us to size-collect surface-dry liposomes on gold-coated silicon wafers for subsequent characterization of monodisperse (as confirmed by atomic force microscopy) vesicle fractions via MALDI MS.

This approach was even possible for liposome samples doped with very low density lipoprotein (VLDL) particles. Only after nES GEMMA based size-selection the lipid composition of liposome species alone was accessible via MALDI MS, whereas MS of mixed liposome/VLDL samples naturally yielded mixed mass spectra. Therefore, we conclude that the offline hyphenation of gas-phase electrophoresis on a nES GEMMA instrumentation with subsequent MS based lipid identification offers unique vesicle characterization possibilities especially for mixed samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

Offline hyphenation of gas-phase electrophoresis on a nES GEMMA instrumentation with subsequent MALDI MS based lipid identification offers unique liposome characterization possibilities especially for mixed samples.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

**Co-authors:**

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*Peter Sandbichler, Institute of Chemical Technologies and Analytics, TU Wien (Vienna University of Technology), Vienna, Austria*

*Martina Marchetti-Deschmann, Institute of Chemical Technologies and Analytics, TU Wien (Vienna University of Technology), Vienna, Austria*

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Poster number: **IM-PB-063**

## COMBINING ULTRAHIGH RESOLUTION MASS SPECTROMETRY AND ELECTRONIC STRUCTURE CALCULATIONS WITH CHROMATOGRAPHY FOR STUDYING STRUCTURAL MOTIFS IN HIGHLY COMPLEX SAMPLES

Abstract ID: **653**

Presenting author: **Wolfgang Schrader, Max-Planck-Institut für Kohlenforschung**

### Introduction

Analytical methods have made enormous improvements in qualitative performance during the last few decades. They often are better in resolution, speed and overall output of information of the analyzed sample. This is also true for the information that can be gained about one of the most complex and difficult to cover samples: the heavy fraction of crude oil, so-called asphaltenes. Structural elucidation of compounds in crude oil mixtures is difficult to achieve due to the high complexity. The method of choice here is *collision induced dissociation* (CID) MS. Unfortunately, heavy fractions show a large number of signals per nominal mass, which is making elucidation of structural functionalities or motifs even more challenging. Different types of separations need to be utilized to simplify the sample.

### Methods

Different types of chromatography (ligand exchange chromatography and size exclusion chromatography) are being used to separate crude oil asphaltenes in combination with ultrahigh resolution mass spectrometry (Thermo Scientific Orbitrap Elite, Bremen, Germany). Single point electronic energy calculations were carried out at the DLPNO-CCSD(T)/def2-TZVPP level. Thermal corrections calculated at the DFT level were added to the DLPNO-CCSD(T) electronic energies. All calculations were made using the ORCA 4.0 program package.

### Preliminary data (results)

Complex samples are very difficult to analyze and even more difficult to really understand. For one, due to suppression and discrimination effects, they are causing problems during the analysis. Often only parts of the sample are really shown during one analysis and multiple methods need to be used to get a comprehensive look into the real sample. This means, that the representation of one sample is often biased by the analytical method. This is even more so if we want to gain structural information.

Here, the combination of ultrahigh resolution mass spectrometry with theoretical calculations allows a better understanding of the behavior of different compounds. We use the heaviest fraction of a complex crude oil mixture, so-called asphaltenes, to gain structural information. The difference can be made between aromatic cores and aliphatic side chains attached to the cores. The results indicate that the fragmentation of aliphatic side chains follows a simple mechanism: each side chain forms one single fragment, where the side chain is cleaved at the alpha carbon, forming an intermediate tropylium ion, which is a very stable fragment. The number of side chains and their length can be determined by doing MS<sup>n</sup>-studies.

Then the aromatic cores remains. Here theoretical calculations allow a better understanding of which structure is most stable and probable. Combining all these methods really can reveal a much better understanding of a complex mixture such as a crude oil. We show some very unique structural motifs that were discovered in these studies.

### Please explain why your abstract is innovative for mass spectrometry?

Revealing new structural motifs in a very complex mixture by HRMS and high end computational studies

### Co-authors:

Zahra Farmani, Max-Planck-Institut für Kohlenforschung  
Alessandro Vetere, Max-Planck-Institut für Kohlenforschung  
Correntin Poidevin, Max-Planck-Institut für Kohlenforschung  
Alexander A. Auer, Max-Planck-Institut für Kohlenforschung

Poster number: **IM-PB-064**

## ONE-POT DOUBLE DERIVATISATION TO IMPROVE THE LC-MS/MS SEPARATION AND SENSITIVITY OF VITAMIN D METABOLITES

Abstract ID: **654****Presenting author: Pascal Schorr, Department of Chemistry, Bioanalytical Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany**

### Introduction

The characterisation of vitamin D status is important since it plays a crucial role in many physiological and pathophysiological processes. Beyond vitamin D<sub>3</sub>, metabolites with high clinical importance are the epimers of 25-hydroxyvitamin D<sub>3</sub> (3 $\alpha$ -25(OH)D<sub>3</sub> and 3 $\beta$ -25(OH)D<sub>3</sub>), 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) and 24,25-dihydroxyvitamin D<sub>3</sub> (24,25(OH)<sub>2</sub>D<sub>3</sub>). LC-MS/MS is referred to as the "gold standard" method and derivatisation is often performed prior to analysis to increase the sensitivity of the method. The most commonly used derivatisation reagent is 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD), a Cookson-type reagent which attacks the diene moiety of vitamin D, due to its low price and easily performed reaction. Alternatively, derivatisation can be performed on the hydroxyl groups.

### Methods

A double derivatisation using both a Cookson-type reagent and a derivatisation for hydroxyl groups was used. The analytes initially reacted with PTAD (0.5 mg/mL) followed by acetylation using acetic anhydride, catalysed by 4-dimethylaminopyridine (DMAP) at room temperature. Acetylation is crucial since vitamin D<sub>3</sub> metabolites vary in the amount of hydroxyl groups. DMAP concentration determines the reactivity and was optimized in the range of 1–20 mg/mL. LC-MS/MS experiments were performed on an Agilent 1290 Infinity II UHPLC coupled to a Sciex QTRAP 6500+, using a Thermo Hypersil Gold C18 column with methanol/water gradient elution.

### Preliminary data (results)

In reversed-phase LC, C-18 column is the most commonly used. The separation of the epimers after PTAD derivatisation cannot be performed on a C-18 column. Acetylation of the C3 hydroxyl group showed a solution to this problem, as chromatographic selectivity increase. Until now, the double derivatisation for 3 $\alpha$ -25(OH)D<sub>3</sub> and 3 $\beta$ -25(OH)D<sub>3</sub> is not new to LC-MS measurements of 25(OH)D<sub>3</sub>. However, until now it was only performed for the epimers of 25(OH)D<sub>3</sub>, where 3 $\alpha$ -25(OH)D<sub>3</sub> was not detectable, due to low concentrations. In our study, we propose an optimisation of the derivatisation reaction which becomes easier, faster, does not require high temperature and increases the sensitivity compared to other protocols. Furthermore, we expand the application beyond 25(OH)D<sub>3</sub> to the other highly interesting metabolites as 1,25(OH)<sub>2</sub>D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub> and vitamin D<sub>3</sub> and were able to detect all five metabolites in clinical human samples. The second derivatisation step can take place at room temperature as the first one; and without stopping PTAD reaction followed by evaporation of the solvent which is usually a time-consuming step. Our protocol is feasible to be applied in large batches of samples because it minimises the sample preparation process. Also, the two steps derivatisation is ideal when the separation of the above-mentioned vitamin D<sub>3</sub> metabolites is required, apart from that the differentiation of the isobaric metabolites 1,25(OH)<sub>2</sub>D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> is also possible by MS/MS.

### Please explain why your abstract is innovative for mass spectrometry?

This one-pot double derivatisation is suitable for LC separation of isobaric vitamin D<sub>3</sub> and furthermore for MS/MS distinction of the isobaric metabolites 1,25(OH)<sub>2</sub>D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub>.

### Co-authors:

*Anastasia Alexandridou, Department of Chemistry, Bioanalytical Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany*

*Dietrich A. Volmer, Department of Chemistry, Bioanalytical Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany*

Poster number: **IM-PB-065**

## **ANALYSIS OF DIASTEREOMERIC PYRROLIZIDINE ALKALOIDS IN TEA SAMPLES BY LIQUID CHROMATOGRAPHY AND SUPERCRITICAL FLUID CHROMATOGRAPHY VACUUM DIFFERENTIAL MOBILITY SPECTROMETRY - MASS SPECTROMETRY**

Abstract ID: **667****Presenting author: Maria Fernanda Cifuentes Girard, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva**

### **Introduction**

Recently, the monitoring of pyrrolizidine alkaloids (PAs), classified as phytotoxic compounds, in food matrices has gained interest. PAs are a complex mixture of diastereomers and more than 600 PAs and their N-oxides forms were identified in over 6000 plants. Many LC-MS and GC-MS assays have been developed but require extensive sample preparation and long analyses times, and methods with higher throughput are needed. In the present work, the potential of vacuum DMS (vDMS) for reducing LC analysis time while maintaining good selectivity is investigated for the analysis of diastereomeric PAs in tea samples. In addition, the performance of supercritical fluid chromatography-mass spectrometry (SFC-MS) is an attractive alternative to LC for reducing analysis time and is capable of resolving the diastereomeric PAs.

### **Methods**

Diastereomeric PAs were analysed in tea samples. Epi-jacobine, atropine were used as internal standards. Samples were extracted using H<sub>2</sub>SO<sub>4</sub>, centrifuged and filtered. Extracts were analysed on: 1) short LC-vDMS-MS, 50 x 1.0 mm C<sub>18</sub> column (5min) in trap/eluted setup using MeOH/H<sub>2</sub>O (15/85; v/v), 0.1%FA 2) LC-MS/MS method using C<sub>18</sub> column, 150 x 2.1 mm (12min). 3) SFC-MS/MS method (7min) using CHIRALPAK® IG-3, 3.0 x 100 mm, 3 µm SFC column. Analyte detection was performed either on LCMS-8050 QqQ or on 8060 QqQ mass spectrometer (Shimadzu) and prototype vDMS cell (33mbar pressure).

### **Preliminary data (results)**

Four sets of PA diastereomers were investigated. Intermedine, echinatine, lycopsamine, indicine (*m/z* 300) and their N-oxide forms; intermedine-N-oxide, echinatine-N-oxide, indicine-N-oxide, lycopsamine-N-oxide (*m/z* 316) have no selective fragments under CID and coeluted in LC. Senecivernine and senecionine (*m/z* 336) cannot be distinguished by MS/MS and they partially coeluted in LC. Jacobine is the only diastereomer (*m/z* 352) that has a selective fragment (*m/z* 352>155) and can be distinguished from retrorsine, while senecivernine-N-oxide and senecionine-N-oxide (*m/z* 352); have different retention times.

For SFC-MRM/MS all diastereomers are resolved using the CHIRALPAK® IG-3 SFC column under ternary gradient. vDMS has shown to be a powerful approach for the separation of isomeric analytes and to improve detection selectivity. Solvent, correction, and dispersion voltage, temperature, and pressure were optimized in vDMS to achieve separation of diastereomers. The combination of short LC column with trap/elute setup and vDMS and detection in SIM mode enabled simplified sample preparation, reducing the analysis time and improved selectivity compared to classical LC-MS method. In addition, LC-vDMS-MS enables accurate quantitation of 10/14 diastereomeric PAs with higher throughput (<1h), compared to reported LC-MRM/MS method in which 9/14 diastereomers could be separated and SFC-MRM/MS in which all diastereomers are fully resolved but requires time-consuming sample preparation (>3h). Finally, PA diastereomers were successfully analysed in 20 tea samples using the short LC-vDMS-MS method and compared to LC-MRM/MS and SFC-MRM/MS methods.

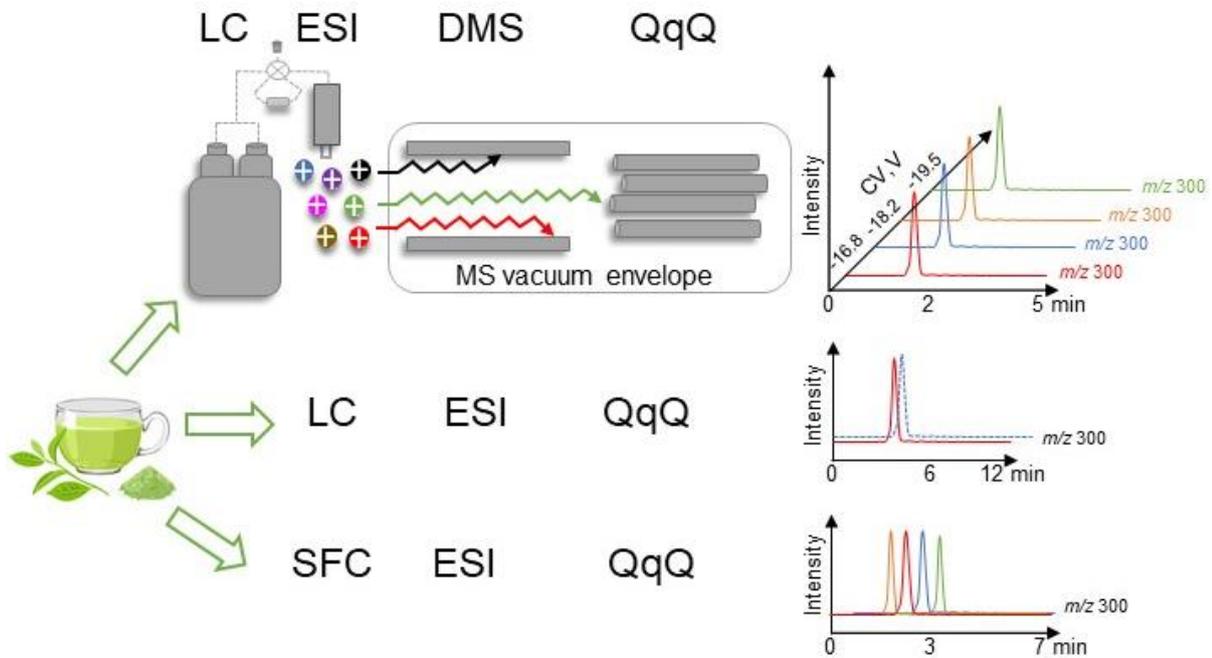
**Please explain why your abstract is innovative for mass spectrometry?**

**Quantitative analysis of diastereomeric pyrrolizidine alkaloids using vacuum Differential Ion Mobility with short LC in trap/elute setup for higher throughput.**

**Co-authors:**

Patrick Knight, Shimadzu Research Laboratory  
 Roger Giles, Shimadzu Research Laboratory

G rard Hopfgartner, Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva



Analysis workflow of pyrrolizidine alkaloids in tea samples.

Poster number: **IM-PB-066**

## EXPLORATORY INORGANIC SPECIATION OF PINE WOOD FAST PYROLYSIS BIO-OIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY HYPHENATED TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (HPLC - ICPMS)

Abstract ID: 710

**Presenting author: Wladimir Ruiz, Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, UMR 5254, 2 Av. Pr. Angot, Pau, France, TOTAL Refining & Chemicals, Total Research & Technology Gonfreville, BP 27, F-76700 Harfleur, France, International Joint Laboratory iC2MC: Complex Matrices Molecular Characterization, Total Research & Technology, Gonfreville, BP 27, 76700 Harfleur, France**

### Introduction

Bio-oils (BO) are among the most important alternative energy sources to cope with climate change, they are the product of pyrolysis or hydrothermal liquefaction of biomass. BO from lignocellulosic materials such as pinewood presents a complex matrix of oxygenated compounds that have been studied by liquid chromatography with UV, ELS, and MS detectors. BO also contains traces of inorganic species with elements like Na, Mg, P, Fe, etc., which can cause problems in catalytic upgrading process, and storage stability. The approach in inorganic analysis of BO involves the total quantification of metals/metalloids by ICP-AES/MS. Nonetheless, the way how inorganic species are distributed in BO has not been widely reported. Consequently, this work aims at the inorganic speciation of pinewood pyrolysis BO by HPLC-ICPMS.

### Methods

In this regard, a Dionex HPLC system with a Viridis HSS C18 SB Column (100Å, 1.8 µm, 3 mm x 100 mm), has been hyphenated to an ICP MS (7700 series, Agilent, Santa Clara, CA). The mobile phase consisted of a gradient of a mixture of polar solvents HPLC-grade with MiliQ water. Oxygen gas was mixed with the argon carrier gas, to prevent carbon formation in the platinum cone and skimmer of the ICP-MS. The samples analyzed were pinewood pyrolysis BO from RTI (research triangle institute) dissolved in HPLC-grade methanol. The isotopes of interest were <sup>11</sup>B, <sup>23</sup>Na, <sup>24</sup>Mg, <sup>31</sup>P, <sup>56</sup>Fe.

### Preliminary data (results)

The HPLC chromatograms of the species tracked by the isotopes <sup>11</sup>B, <sup>23</sup>Na, <sup>24</sup>Mg, <sup>31</sup>P, <sup>56</sup>Fe with the ICP-MS showed interesting results. The main observation was that the fingerprint of the inorganic species was different for the different isotopes. This diversity in the way how the metals and metalloids are distributed in the BO might be the key in the optimization of hydrodemetalization processes. The number of peaks was also different considering the different isotopes of interest, which might be linked to the fact that some heteroatoms might not be inside the structure of some species, instead they might be as contra ions of ionic organic species. However, further analysis must be carried out to better understand these observations.

### Please explain why your abstract is innovative for mass spectrometry?

In contrast to total analysis quantification, the inorganic speciation of BO through hyphenated techniques as HPLC-ICPMS allows a more complete overview of the way these problematic trace compounds are distributed.

### Co-authors:

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Poster number: **IM-PB-067**

## **DEVELOPMENT OF A ROBUST UPLC-MS/MS METHOD FOR THE ANALYSIS OF POLYAMINES IN BIOFLUIDS FROM NEURODEGENERATIVE DISEASES**

Abstract ID: 751

**Presenting author: Michele Iannone, Neuroscience Department, Janssen Pharmaceutical Companies of Johnson & Johnson, Turnhoutseweg 30, Beerse, Belgium., Discovery and Exploratory BA Laboratory, DMPK Department, Janssen Pharmaceutical Companies of Johnson & Johnson, Turnhoutseweg 30, Beerse, Belgium.**

### **Introduction**

Parkinson disease's (PD) is a common neurodegenerative disorder. Loss of dopaminergic neurons together with accumulation of  $\alpha$ -synuclein aggregates are its major histopathological signatures. While several molecular mechanisms are implicated in PD, accumulating evidence puts lysosomal dysfunction at the center stage of PD pathogenesis.

It was demonstrated that loss of activity of the lysosomal polyamine transporter ATP13A2 leads to accumulation of polyamines leading to lysosomal and mitochondrial dysfunction [1, 2]. Based on the above, the aim is to understand the role of polyamines in PD. Hence, we describe the development, validation and application of a liquid chromatography – tandem mass spectrometry (LC-MS/MS) method for the quantification of endogenous polyamines in biofluids and various tissue types and cell lysates to correlate their levels with the ATP13A2 activity.

### **Methods**

Sample pre-treatment is articulated in protein precipitation with methanol, performed only in the case of plasma and cerebrospinal fluid analysis, derivatization with benzoyl chloride and liquid-liquid extraction with ethylacetate. The chromatographic separation is achieved on a Acquity BEH C18 column using an acetonitrile – water (with 0.1 % of formic acid) gradient while the mass spectrometric analysis is performed using a triple quadrupole as mass analyzer operating in selected reaction monitoring mode. The method was validated in terms of selectivity, linearity, limit of quantification (LOQ), recovery, matrix effect and carry over, using both surrogate and biological matrices.

### **Preliminary data (results)**

Ten target compounds (cadaverine, ornithine, putrescine, spermidine, spermine, N1 acetylcadaverine, N1 acetylputrescine, N1 acetylspermidine, N1 acetylspermine and N1N8 diacetylspermidine) and six labelled internal standards (L-ornithine- $^{13}\text{C}_5$ , putrescine-d8, spermidine-d8, spermine-d8, N8 acetylspermidine-d3 and N1 acetylspermine-d3) are included in the method.

During the method development process, different protein precipitation reagents (methanol, ethanol, acetonitrile, a methanol/acetonitrile 50:50 v/v mixture, sulfosalicylic acid and trichloroacetic acid), derivatization reagents (benzoyl chloride, isobutylchloroformate) and chromatographic columns (Acquity BEH C18 2.1 x 100 mm; 1.7  $\mu\text{m}$ , Acquity HSS T3 C18 1 x 100 mm; 1.8  $\mu\text{m}$ , Kinetex C18 2.1 x 100 mm; 2.6  $\mu\text{m}$ ) were tested. The introduction of a liquid/liquid extraction step after the derivatization reaction for sample clean-up was also evaluated.

Our results indicated that, the ideal conditions for the identification and quantification of the target compounds are obtained after the use of methanol as protein precipitation reagent and of a derivatization reaction performed with benzoyl chloride followed by a liquid-liquid extraction with ethylacetate. The benzoyl derivatization reaction improves chromatographic resolution and at the same time enhances the MS/MS fragmentation yields.

The LLOQ values are between 0.005 and 0.01 ng/mL. The method is linear in the range 0.005/0.01 ng/mL - 1000 ng/mL. For certain compounds, linear dynamic range extension was achieved using isotopologue transitions. No carry over and only negligible matrix effect were observed.

The analysis of plasma, cerebrospinal fluid and cell samples confirmed the applicability of the developed method.

**Please explain why your abstract is innovative for mass spectrometry?**

## POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

Due to its sensitivity (LLOQs and extended linear dynamic range) this approach could be easily adapted for the analysis of samples collected in the framework of other diseases (e.g., cancer).

### **Co-authors:**

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Poster number: **IM-PB-068**

## CHEMICAL SPECIATION OF CHROMIUM IN WATER SAMPLES: METHOD DEVELOPMENT AND VALIDATION

Abstract ID: 766

**Presenting author: Jelle Verdonck, Environment and Health, Department of Public Health and Primary Care, KU Leuven, ON5 Herestraat 49 - box 952, Belgium**

### Introduction

Chromium (Cr) is a transition element that exists in oxidation states ranging from - 2 to +6. The common stable ones in the environment are trivalent Cr(III) and hexavalent Cr(VI) chromium. Cr(III) is an important micronutrient for the human body, while Cr(VI) is highly toxic and carcinogenic. The environmental concentrations of both oxidation states are low. Due to the differences in toxicity between Cr(VI) and Cr(III) compounds, speciation of Cr is very important.

### Methods

Therefore, an improved sensitive and robust method for the simultaneous determination of Cr(III) and Cr(VI) in water samples has been developed. The method uses a hyphenated micro liquid chromatography ( $\mu$ LC) system coupled to inductively coupled plasma mass spectrometry (ICP-MS).

### Preliminary data (results)

The optimised method incorporates a pH adjusted EDTA complexation step to stabilise Cr(VI) and Cr(III). The  $\mu$ LC system uses an anion exchange micro-sized column to separate the Cr species. Cr(III) and Cr(VI) were separated with different retention times at 170 and 230 sec, respectively. The method was optimized and validated by spiking Cr(III) and Cr(VI) in various water samples. Furthermore, the method was validated using a drinking water proficiency testing material sample.

### Please explain why your abstract is innovative for mass spectrometry?

The developed method can be used for rapid routine determination of chromium species with high precision and reliability.

### Co-authors:

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*Lode Godderis, IDEWE, External Service for Prevention and Protection at Work, Interleuvenlaan 58, 3001, Heverlee, Belgium, Environment and Health, Department of Public Health and Primary Care, KU Leuven, ON5 Herestraat 49 - box 952, Belgium*

Poster number: **IM-PB-069**

## LOWERING THE DETECTION LIMITS BY IMPLEMENTING HADAMARD TRANSFORM MULTIPLEXING WITH HIGH KINETIC ENERGY ION MOBILITY SPECTROMETRY

Abstract ID: **822****Presenting author: Cameron N. Naylor, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology**

### Introduction

Because of the operating principles of the ion mobility experiment, traditional signal averaged drift tube IMS experiments operate with low duty cycles (< 1%). Therefore, there is a clear advantage to increasing the ion throughput by increasing the duty cycle in the ion mobility experiment in cases where ionization efficiency is low, the amount of analyte sample is limited or when ions are transferred to a mass spectrometer after mobility separation. Multiplexed ion gating schemes (e.g. Fourier and Hadamard techniques) represent one series of approaches to theoretically increase the duty cycle up to 50%. The High Kinetic Energy IMS (HiKE-IMS) already is capable of high signal-to-noise ratio and high resolving power, however, increasing these metrics further is possible through multiplexing.

### Methods

Hadamard transform (HT) multiplexing techniques were chosen because virtually no instrument modifications are required. Terpenes (i.e. limonene, pinene, myrcene, camphor, humulene all from Sigma-Aldrich) and non-alcoholic beers were measured and ionized primarily via the  $O_2^+$  reactant ions in air as the drift gas. A commercially available, low-cost microcontroller (Teensy 4.1) with custom programming was used to generate and select pseudo-random gate-pulsing sequences ranging from 5-bit to 10-bit or an equivalent length signal averaging experiment. Each HT experiment was performed twice to allow for blank subtraction to eliminate any gate-pulsing interference with the ion signal.

### Preliminary data (results)

After circular deconvolution, the absolute signal of the terpenes was increased by over a factor of two when compared to the signal averaged spectra. These signal gains approach the theoretical maximum SNR gain after accounting for decrease in the duty cycle from overstretching the sequence to fit within the time scale of the IMS experiment. Another interesting aspect of implementing HT multiplexing on HiKE-IMS is the selection of sequences because of the short gate pulse widths. Standard operating conditions in the HiKE-IMS use a 1  $\mu$ s gate pulse with a tristate pulsing technique. These short gate pulse widths allow for the implementation of larger sequences and thus a higher duty cycle and theoretical signal-to-noise-ratio gain than on any other IMS platform. However, due to the short drift times of the ions, lower bit (<5-bit) overstretched sequences offer no multiplexing advantages because the ion mobilities are fully resolved. Additionally, smaller fragments generated at higher E/N in the reaction region and ion-neutral clusters at lower reduced electric field strengths are more easily observed with these gains in signal. These smaller peaks can be confirmed by eliminating artifacts using masked-multiplexing deconvolution techniques. By adjusting the pressure within the HiKE-IMS, the ion signal can be further increased. Through this first effort, the groundwork is established for applying multiplexing techniques to HiKE-IMS which can allow for future efforts of analyzing more complex sample matrixes and coupling to mass spectrometers.

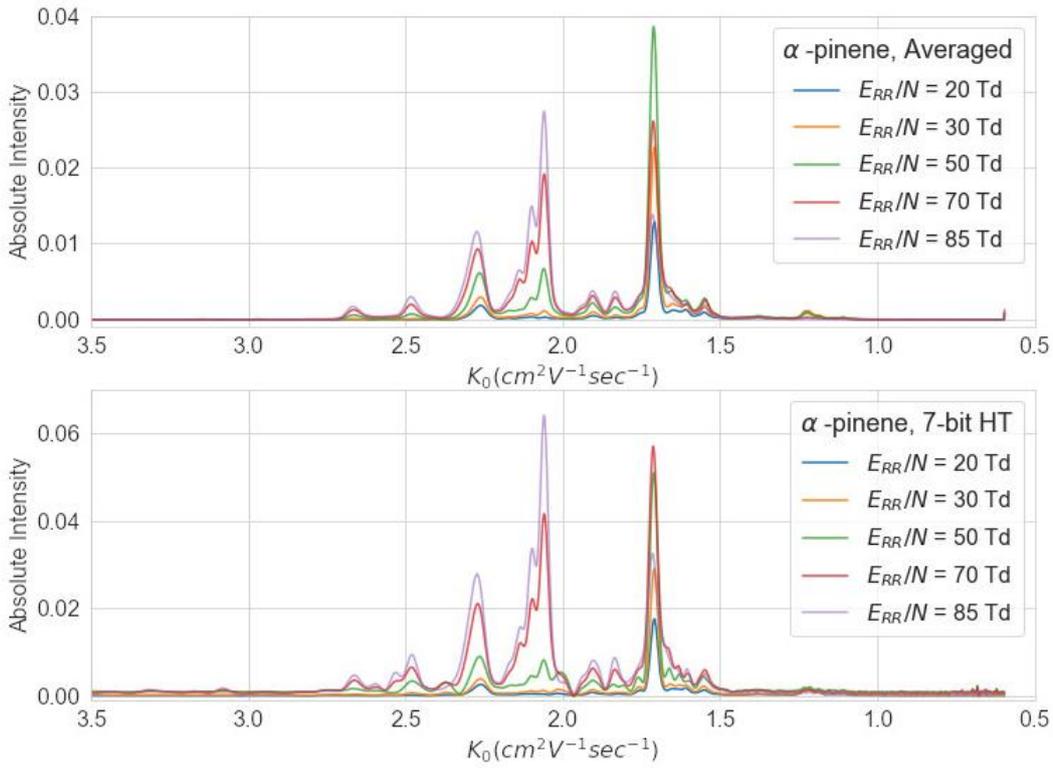
Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 318063177 and 458829155.

### Please explain why your abstract is innovative for mass spectrometry?

Implementing HT multiplexing allows for higher ion throughput through the HiKE-IMS and requires higher bit sequences than have been implemented on IMS before allowing for lower limits of detection.

### Co-authors:

*Brian H. Clowers, Washington State University, Department of Chemistry*  
*Florian Schlottmann, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology*  
*Stefan Zimmermann, Leibniz University Hannover, Institute of Electrical Engineering and Measurement Technology*



Example signal-averaged spectra is compared to 7-bit Hadamard sequence

Poster number: **IM-PB-070**

## **COUMARIN-BASED DERIVATIZATION REAGENT FOR THE LC-MS ANALYSIS OF AMINO ACIDS**

Abstract ID: **843**

**Presenting author: Ngan Nguyen Kim Bui, University of Tartu, Institute of Chemistry, Ravila 14a, Tartu 50411, Estonia**

### **Introduction**

This poster reports a fluorogenic derivatization reagent named Coumarin151-N-succinimidyl Carbamate (Cou151DSC) for primary and secondary amino acids – using high-performance liquid chromatography (HPLC) compatible with 2 detectors: fluorescence (FLD) or ultraviolet (UV) and electrospray ionization - tandem mass spectrometry (ESI-MS/MS).

We optimized the derivatization condition and validated an analytical method to determine 24 amino acids in traditional Estonian soft drinks - Kali. The derivatized amino acids' UV - fluorescent characteristics and MS/MS fragmentation behavior were examined with 23 amines. The results were compared with derivatization using 6-Aminoquinoly-N-Hydroxysuccinimidyl Carbamate (6-AQC), commonly employed in different applications.

### **Methods**

Reagent Cou151 N-Succinimidyl Carbamate (Cou151DSC) was successfully synthesized. Its pH-dependent fluorescent properties were observed.

Analysis of derivatized standard or sample solutions was carried out using a UHPLC system coupled to an Agilent QqQ MS/MS or a fluorescent/ ultraviolet detector.

Two UPLC 5 cm columns, Biphenyl type (Kinetex) and C18 (Agilent Zorbax RRHD) were compared using different mobile phases. Fragmentation of derivatized amino acids and amino compounds was investigated using product ion scan mode.

The derivatization was optimized, including dilution solvent, buffer type, time, temperature, sonication effect, and reagent concentration. The method was validated using Kali drinks.

### **Preliminary data (results)**

Under the derivatized condition, amino acids have an excitation wavelength of 355 nm and an emission wavelength of 486 nm and different MS/MS fragmentation patterns. When changing solvent from moderate to higher polarities, the Cou151DSC offers a red-shifted high fluorescence intensity. A solution of 0.2% acetic acid in a mixture of acetonitrile: water (1:1) was chosen as a pH modifier to end the reaction.

Compared to 6-AQC, this study offers a faster and milder reaction condition, at 40°C for 5 minutes instead of 15 minutes at 55°C. The limit of quantitations (LOQ) using dynamic multiple reaction monitoring mode (dMRM) (in pmol on column) from this work is lower than 6-AQC one order of magnitude for all investigated amino acids.

By using certified reference material (CRM), the accuracy of the proposed method was within a range of 83 – 107% with good relative standard deviations (RSDs) of less than 6% in all measurements.

The proposed method gave excellent recoveries in 4 different spiked concentrations in 4 types of Kali, the total range was from 82 – 120%, and the repeatabilities were between 0 – 14%. The intra- and inter-day precision of less than 13% and 18%, respectively.

### **Please explain why your abstract is innovative for mass spectrometry?**

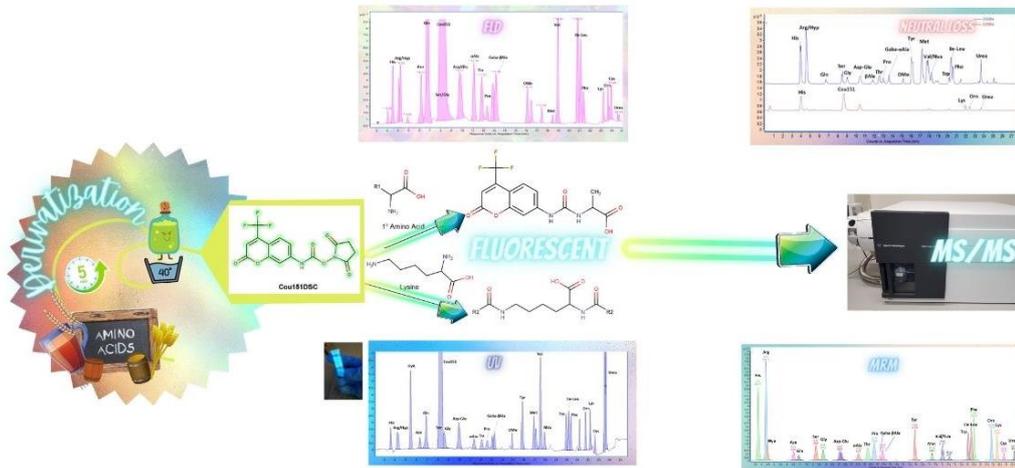
The MS/MS fragmentation of Cou151 derivatives (25 amino acids and 23 amines) followed characteristic patterns in contrast to 6-AQC (only at 171 m/z).

## POSTER SESSION B

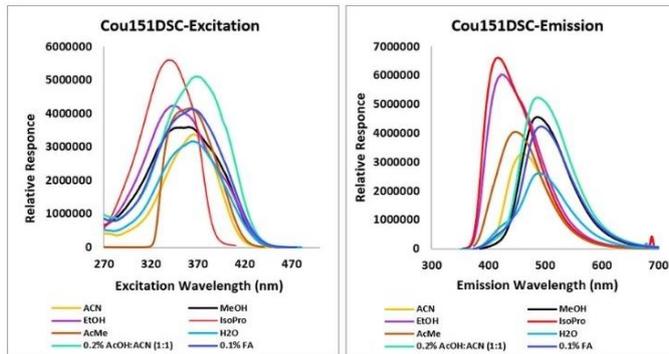
Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

### Co-authors:

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Koit Herodes, University of Tartu, Institute of Chemistry, Ravila 14a, Tartu 50411, Estonia  
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General derivatization reactions and chromatograms of Cou151 derivatives



Excitation and emission wavelengths of Cou151DSC in different solvents

Poster number: **IM-PB-071**

## **DETERMINATION OF SUSPECTED ALLERGENS IN BABY WET WIPES**

Abstract ID: **863****Presenting author: Adnan Al-Mussallam, Head of Department at Saudi Food and Drug Authority**

### **Introduction**

Generally, a fragrance substance can release and possess a pleasant and aromatic odour that is formed up of synthetic chemicals and essential oils. Particularly in baby skin, there are certain concerns about skin irritation and sanitation. Previously, wet wipes were considered to be generally safe for use on infant skin. However, recent studies have found that wet wipes might sensitize some infant, while some cases might lead to acute allergies. According to few studies, wet wipes are related to the infant allergy by 71. The aim of this study is to evaluate the prevalence of wet wipes as a source of contact allergy, and to study the most commonly associated allergens in wet wipes available.

### **Methods**

Basically, the most common ingredients including 26 allergens were analysed by using Agilent5975Series Gas Chromatography-Mass Spectrometry GCMS. Helium was employed as carrier gas at a constant column flow of 1.0 mL min<sup>-1</sup>. The temperatures of the transfer line and the ion source were set at 150 and 230 °C, respectively. The identification was carried out by scan monitoring from 35 to 500 amu. In addition. The wet wipes samples were prepared by collecting a liquid from 10-20 wipes into a clean glass, then samples were diluted 1:1 by ethanol along with addition to the internal standard.

### **Preliminary data (results)**

In this study, 47 samples of baby wet wipes were analyzed by using gas chromatography-mass spectrometry. The study demonstrated the importance of the quantitation of 26 allergens in wet wipes that intended to use for children. Importantly, during the data interpretation, we found that linalool, benzyl alcohol, and benzyl benzoate were the most common ingredients among all samples. Linalool was contributed in 40% of samples, while benzyl alcohol was found in 60% and finally, benzyl benzoate was found in 38%. Of note, linalool, benzyl alcohol, and benzyl benzoate collectively are linked to potential skin sensitization, especially when they exceed the limits of 10 µg/ml in leave-on products. Consequently, the potential of risk assessment of these ingredients on dermal should be addressed and calculated. We assessed the dermal exposure by calculating the Risk Characterization Ratio (RCR), margin of safety (MOE), and dermal exposure.

### **Please explain why your abstract is innovative for mass spectrometry?**

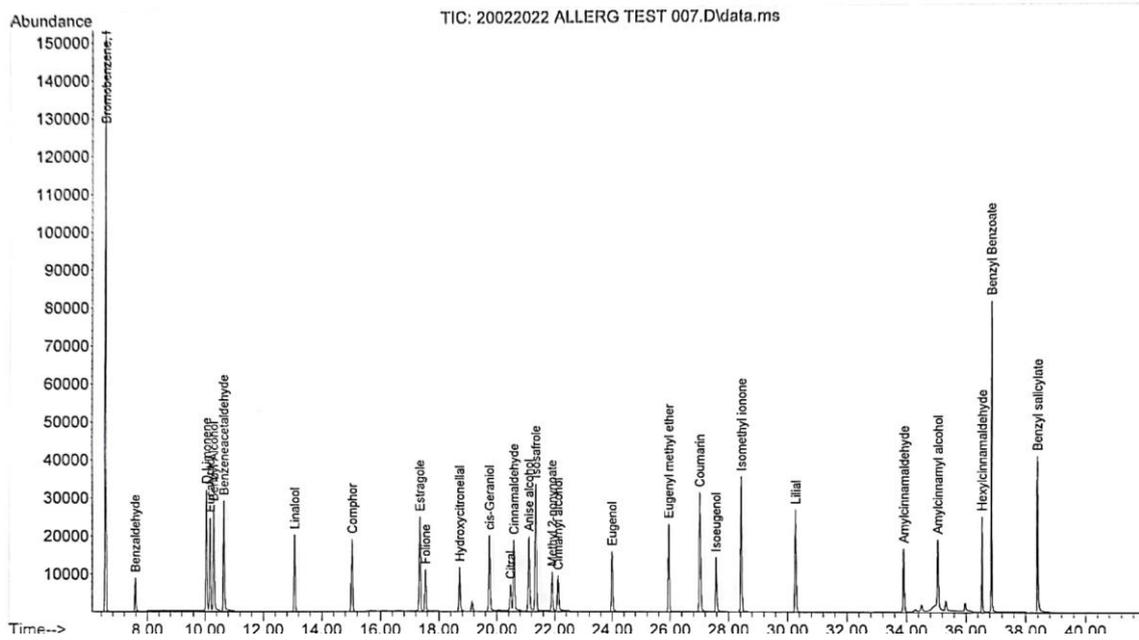
It is important that industry controls and regulations by competent authorities prevent the risk of allergic reactions in babies. It contains irritating allergens, such as linalool, and benzyl benzoate.

### **Co-authors:**

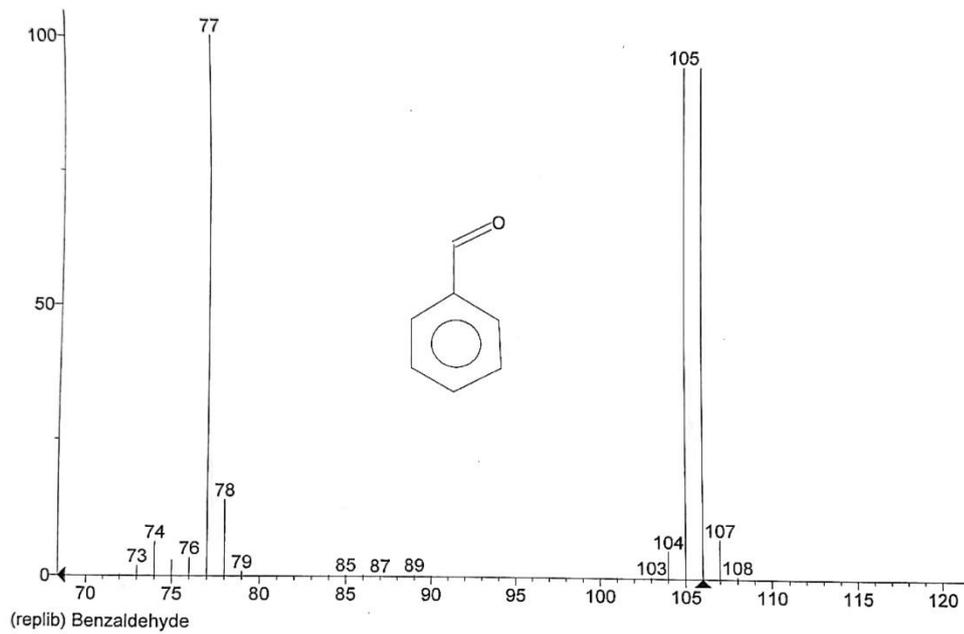
*Fahad Al-Dawsari, Director Manager at Saudi Food and Drug Authority*  
*Abdullah Bawazir, Lab Expert Researcher at Saudi Food and Drug Authority*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
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26 allergens peak High resolution Mass Spectrometry SIM Mode



Mass Spectrum Structure for Benzaldehyde.

Poster number: **IM-PB-072**

## A PROTOCOL TO MONITOR PERFORMANCE OF HIGH-FLOW LC-HRMS SYSTEMS

Abstract ID: **864****Presenting author: Nicole Zehethofer, Thermo Fisher Scientific (Bremen) GmbH**

### Introduction

Evaluating liquid chromatography-mass spectrometry (LC-MS) instrument status prior to the analysis of unknown samples is of critical importance to data quality. While there are many protocols to monitor the status of individual components of LC-MS systems, a combined protocol, which tests the entire LC-MS system, is highly desirable. The Small Molecule System Suitability (SMSS™) standard contains 9 different compounds, ranging from  $m/z$  75 to  $m/z$  1222, that can be analyzed in positive and negative ion mode. The aim of this initiative is to design methods and establish acceptance criteria to use the SMSS for routine performance monitoring, system evaluation and troubleshooting on the Thermo Scientific™ Orbitrap Exploris™ 120 MS and the Thermo Scientific™ Orbitrap Exploris™ 240 MS.

### Methods

The SMSS standard was diluted 10-fold in water with 0.1% formic acid. LC gradient was a 5.5-minute binary gradient, 5% water with 0.1% formic acid to 95% methanol at a flow rate of 500  $\mu$ L/min. Separation was on a 2.1 x 50 mm Thermo Scientific™ HypersilGOLD™ column. Three MS methods were developed specifically for the analysis of the standard.

- Detection limit determination (positive ion mode using Full Scan and targeted SIM scans)
- MS2 performance evaluation (negative ion mode using targeted MS2 scans)
- System evaluation/troubleshooting (positive and negative ion mode using data dependent MS2 scans)

### Preliminary data (results)

The methods, which were developed for detection limit determination and MS2 performance evaluation, were used to evaluate the performance of more than 40 Orbitrap Exploris 120 MS systems using two different LC systems. Acceptance criteria were determined and templates for automatic data analysis and report generation are available for Thermo Scientific™ Chromeleon™ software.

The system evaluation/troubleshooting method was run on more than 50 Orbitrap Exploris MS instruments. Inter-system and intra-system variability of peak areas, MS2 ions ratios, mass stability and retention times are reported.

### Please explain why your abstract is innovative for mass spectrometry?

Development of methods and acceptance criteria to use the new SMSS for fast system evaluation of LC-HRMS performance and perform whole system troubleshooting.

### Co-authors:

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*Bhavin Patel, Thermo Fisher Scientific*  
*Alan Atkins, Thermo Fisher Scientific*  
*Kay Opperman, Thermo Fisher Scientific*

Poster number: **IM-PB-073**

## MONITORING REACTION INTERMEDIATES TO PREDICT ENANTIOSELECTIVITY USING MASS-SPECTROMETRY

Abstract ID: **868****Presenting author: Roelant Hilgers, Wageningen University & Research**

### Introduction

Enantioselective reactions are at the core of chemical synthesis. Their development mostly relies on prior knowledge, laborious product analysis and post-rationalization by theoretical methods. We developed a simple and fast method to determine enantioselectivities based on ion mobility-mass spectrometry.

### Methods

The method is based on ion mobility separation of diastereomeric intermediates, formed from a chiral catalyst and prochiral reactants, and delayed reactant labeling experiments to link the mass spectra with the reaction kinetics in solution. The data provide rate constants along the reaction paths for the individual diastereomeric intermediates, revealing the origins of enantioselectivity. Using the derived kinetics, the enantioselectivity of the overall reaction can be predicted.

### Preliminary data (results)

We illustrate the method for the Michael addition of cyclopentadiene (CP) to an  $\alpha,\beta$ -unsaturated aldehyde catalyzed by a diarylprolinol silyl ether. In this reaction, two enantiomeric products are formed via competing isomeric reaction pathways. We successfully separated the diastereomeric intermediates, and monitored their intensities over time, using TIMS-TOF-MS. By using delayed reactant labeling, and subsequent fitting of the ion intensities of labeled vs. unlabeled reaction intermediates, rate constants were obtained for the individual isomeric intermediates of the reaction. Based on these rate constants, we predicted an enantiomer ratio of 86.6:13.4 (R:S) for the final product, which was in close agreement with the ratio of 88.5:11.5 (R:S) that we determined after large-scale synthesis using chiral HPLC. We believe that this method can become a useful tool to track the reaction pathways of asymmetric reactions, and can be used to rapidly screen for optimal reaction conditions without the need to perform multiple syntheses.

### Please explain why your abstract is innovative for mass spectrometry?

We used ion mobility-MS in a conceptually novel manner. For the first time, we show that ion mobility-MS can be used to determine rate constants of individual isomeric reaction intermediates.

Poster number: **IM-PB-074**

## **USE OF THE DESIRABILITY FUNCTION TO OPTIMIZE THE SEPARATION OF OLIGONUCLEOTIDE IMPURITY POSITIONAL ISOMERS BY ION PAIR-RP LCMS AND MS/MS**

Abstract ID: **872**Presenting author: **Stilianos Roussis, Ionis Pharmaceuticals**

### **Introduction**

Small alkyl amines as ion pair (IP) reagents have recently shown the capability for enhanced separation of oligonucleotide isomers, which can be highly beneficial in quality control applications, and the elucidation of the mechanisms of impurity formation. The addition of a small alkyl acid and a salt can further improve the separation capabilities of the method. However, extensive empirical method optimization is required, and each optimized method is generally limited to a single family of isobaric impurities. Ideally, a single method would be desirable for the separation of all isomers regardless of their chemical impurity family. In the present work, the concept of the desirability function is employed to determine the best combination of experimental factors for a single, optimized method.

### **Methods**

Experiments were conducted with an Agilent Technologies 1290 Infinity II series HPLC system interfaced to a 6495 triple quadrupole mass spectrometer. The collision energy was 20 eV. The instrument was operated in the full scan, SIM, and MS/MS modes. Also, an Agilent 1290 Infinity HPLC system coupled to a 6224 TOF mass spectrometer equipped with a dual nebulizer ESI source was used for the experiments. Both mass spectrometers were operated in the negative ionization mode and data were collected in the profile format. Desirability function analysis and optimization were done using JMP and Design-Expert software.

### **Preliminary data (results)**

Oligonucleotide isomeric impurities, such as the n-1 composite impurity family, consist of groups of positional isomers having the same nominal mass based on the loss of the same nucleotide from different positions of the sequence (e.g., groups of n-dMeC/T, n-dG, etc). Complete chromatographic separation of each impurity is generally impossible, and MS and MS/MS are often employed. Nevertheless, significant challenges may remain due to co-elution. For an improved understanding of the IP mechanism, and the role of each reagent on the separation of the members of each n-1 impurity group, many reagent combinations were examined to determine the boundary conditions, and their separation capabilities. We found widely different performance depending on the impurity group and the reagent combination used. A globally optimized method was obtained by using the concept of the desirability function. First, the best model was determined for each individual response (measured resolution between a given peak doublet) as a function of the factors (reagents). Then, the individual prediction formulas were simultaneously used to optimize the overall desirability objective function (maximize resolution), producing a single optimum solution (1 mM PA, 30 mM ABC, 1 mM C8A). The optimization methodology, conditions, results obtained, along with the chemical rationalization and the impact of the approach to future research and applications will be presented.

### **Please explain why your abstract is innovative for mass spectrometry?**

Development of an optimized method for the global separation of oligonucleotide isomers regardless of their chemical family.

Poster number: **IM-PB-075**

## **COMPATIBILIZING HIGH SALT CONCENTRATIONS FOR OPTIMAL CHROMATOGRAPHY WITH ELECTROSPRAY IONIZATION MASS SPECTROMETRY USING AN ION EXCHANGE MEMBRANE SUPPRESSOR**

Abstract ID: **915****Presenting author: Sam Wouters, Janssen R&D, Drug Metabolism and Pharmacokinetics, Vrije Universiteit Brussel, Dept. of Chemical Engineering**

### **Introduction**

This study reports on the potential of using ion-exchange suppressor technology in liquid chromatography - electrospray ionization mass spectrometry workflows. The aim was to overcome the incompatibility of using high salt concentrations to improve separation performance, with electrospray ionization, where this leads to significant ion-suppression. We apply the concept to the hydrophilic interaction liquid chromatography separation and detection of taurine and glycochenodeoxycholate sulfate, endogenous biomarkers allowing the assessment of organic anion transporter protein inhibition. Previous observation demonstrates that inhibition of two major renal organic anion polypeptide transporters (OAT) decreases the renal clearance of taurine and glycochenodeoxycholate sulfate (GCDCA-S) significantly, suggesting these compounds may serve as diagnostic biomarkers of OAT inhibition.

### **Methods**

The compounds of interest were separated using HILIC and detected via SRM on a API4000 QqQ, while applying 10 mM and 100 mM ammonium acetate to the mobile phase. The latter improved chromatography but resulted in significant ion suppression. This was mediated using a cation-exchange membrane suppressor post-column prior to the ESI interface, targeting the depletion of ammonium ions from the HILIC mobile phase. A dilute solution of a strong acid (sulfuric acid) was applied in the regenerant as a source of protons. To overcome sticking of zwitterionic compounds, a switching valve was implemented to selectively use the suppressor.

### **Preliminary data (results)**

To achieve the desired chromatographic selectivity for the separation of taurine, GCDCA-S and the isotopically labelled GCDCA-D4 structural analogue of GCDCA-S, the amount of ammonium acetate was increased from 10 mM to 100 mM. This however led to a sharp 6.4-fold reduction in MS sensitivity due to ion suppression for GCDCA-S. Taurine was less sensitive to changes in retention but also undergoes significant (3.2-fold) reduction in MS sensitivity.

When implementing the suppressor in the workflow, ions negatively affecting MS sensitivity were actively removed post-column from the solvent via a charged partially permeable membrane and replaced with protons, resulting in an up to 10-fold increase in detection sensitivity for GCDCA-S, despite an increase in peak width by a factor 1.4, which is to be expected with the added dead volume of the suppressor. However, the peak shape of taurine was significantly degraded, likely caused by the zwitterionic character of taurine and consequently, ionic interactions between the positively charged amine of taurine and the negatively-charged membrane. To overcome the chemical incompatibility of taurine with the ion-stripper, a flow diverter valve was integrated, allowing taurine to selectively bypass the ion-stripper.

We have provided a proof-of-concept showing the advantages one can expect when implementing suppressor technology for taurine and GCDCA-S as example biomarkers. We determined the robustness, linearity, and precision as well as the parallelism between the calibrators and the authentic analyte in the biological matrix.

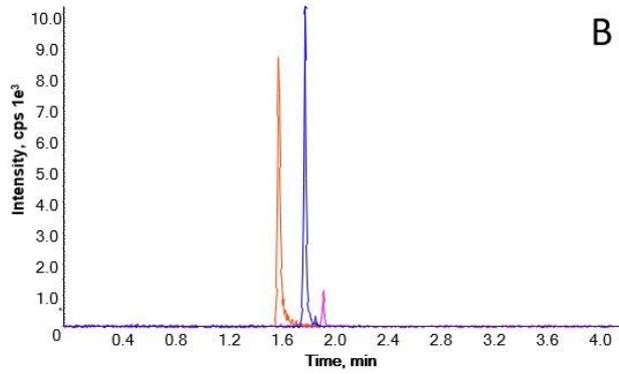
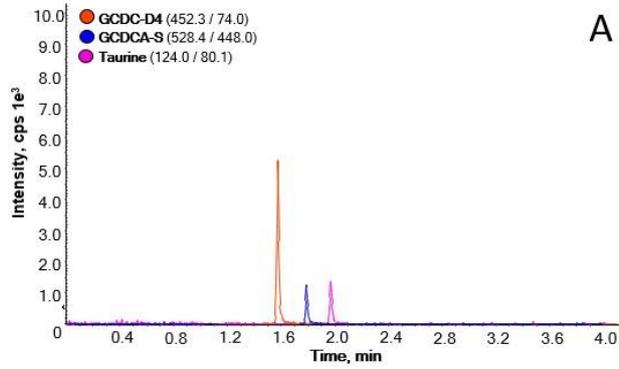
### **Please explain why your abstract is innovative for mass spectrometry?**

Membrane suppressors provide a facile methodology to implement less "MS-friendly" buffers allowing one to tune selectivity using high concentration ion-pairing agents, without compromising MS detection sensitivity.

### **Co-authors:**

*Ils Pijpers, Janssen R&D, Bioanalysis Discovery and Development Sciences*  
*Lieve Dillen, Janssen R&D, Bioanalysis Discovery and Development Sciences*

Filip Cuyckens, Janssen R&D, Drug Metabolism and Pharmacokinetics  
 Sebastiaan Eeltink, Vrije Universiteit Brussel, Dept. of Chemical Engineering



HILIC-MS separation without and with suppressor to enhance ESI-MS response.

Poster number: **IM-PB-076**

## **CYCLIC ION MOBILITY SPECTROMETRY FOR THE STRUCTURAL CHARACTERIZATION OF OLIGOSACCHARIDES**

Abstract ID: **934**

**Presenting author: Bram van de Put, Wageningen University and Research**

### **Introduction**

The structural characterization of complex oligosaccharide mixtures is an analytical challenge. Current LC-MS approaches provide some insights in the structure of these samples but fall short in resolving the complete structure including: sequence, linkage type, and internal anomeric configuration of individual oligosaccharides. Due to the high degree of isomerism in complex oligosaccharide mixtures, only tentative assignments of both precursor and fragment ions can be made based on relative fragment abundancies.

Ion mobility spectrometry (IMS) can alleviate this ambiguity in oligosaccharide assignments by adding an additional dimension of separation for isomeric precursor and fragment ions. We propose a number of tools and approaches to exploit these isomer selective separations and the unique IMS<sup>2</sup> capabilities of the cyclic ion mobility mass spectrometer for the characterization of oligosaccharides.

### **Methods**

Characterization of mono-, and disaccharide standards and mixtures was performed by direct infusion in the cyclic ion mobility mass spectrometer. Ionization was carried out in electrospray mode. The influence of different adducts ([M-H]<sup>-</sup>, [M+Na]<sup>+</sup>, [M+Li]<sup>+</sup>) on the ion mobility separation and oligosaccharide fragmentation behavior have been assessed. Different types of complementary data were acquired in various CID fragmentation modes including: pre IMS-CID, post IMS-CID and IMS<sup>2</sup>. The multi-pass feature of the cyclic IMS was used to increase the resolving power until sufficient separation for distinguishment was achieved.

### **Preliminary data (results)**

Determination of the monosaccharide composition of individual oligosaccharides was achieved by fragmentation into monosaccharide structures, followed by ion mobility separation. These fragments could then be compared to a select set of monosaccharide standards. Distinguishing partially separated isomers was achieved by fragmentation after the ion mobility cell followed by multivariate curve resolution (MCR). Using this approach, closely related isomers, such as  $\alpha$  and  $\beta$  anomers of disaccharides could be recognized, and their individual Collisional Cross Sections (CCSs) and fragmentation spectra were recorded. The structural characterization of mixtures of isomers was done by tandem ion mobility spectrometry. This unique feature of the cyclic IMS system allows isolating a fraction of an ion mobility spectrum, performing fragmentation on it, and recording the ion mobility spectrum of the formed fragments. Taking narrow fractions across the spectrum provided meaningful information about partially separated structures. This approach facilitates the sequencing of oligosaccharides, even if they fragment into very similar fragment ions.

The developed approaches will aid the structural characterization of hard to separate oligosaccharide isomers. This will help build our understanding of the structure-function relationships of these illustrious sugars for their use in health, nutrition, materials, and other fields.

### **Please explain why your abstract is innovative for mass spectrometry?**

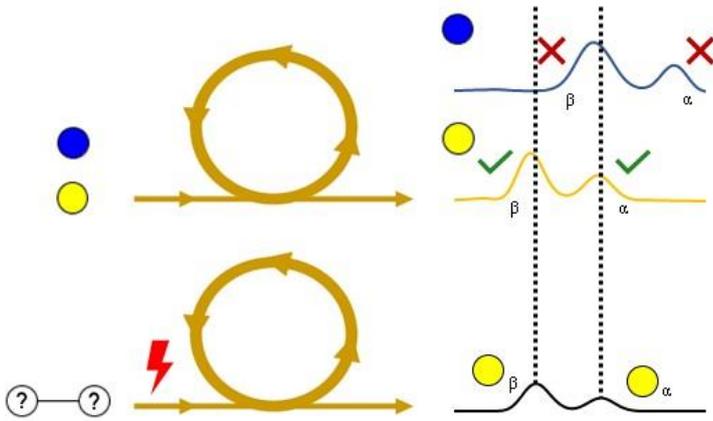
This work proposes several approaches using cyclic ion mobility spectrometry for the fast and complete structural characterization of complex oligosaccharide structures, including: monosaccharide sequence, linkage type, and anomeric configuration.

### **Co-authors:**

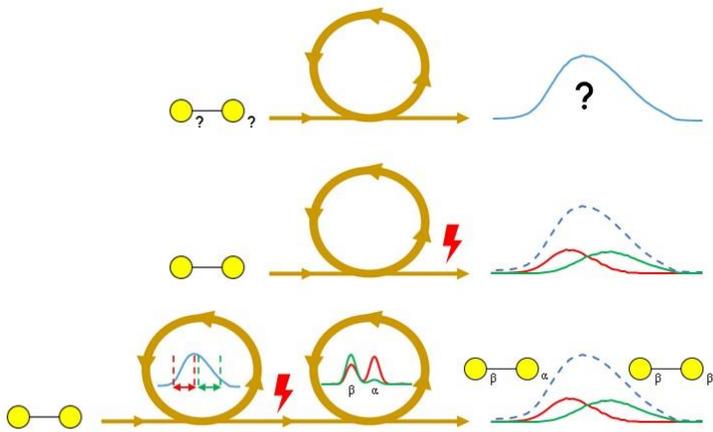
*Wouter de Bruijn, Wageningen University and Research*  
*Edwin Bakx, Wageningen University and Research*  
*Henk Schols, Wageningen University and Research*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Ion mobility reveals the monomer composition of a disaccharide.



Deconvolution of hard to separate isomers and anomeric form determination.

Poster number: **IM-PB-077**

## LC/MS STUDY OF DICLOFENAC DECARBOXYLATION AND ARTIFACTS FORMATION AT THE HOT GAS CHROMATOGRAPH INJECTION PORT

Abstract ID: **987**

Presenting author: **Elena Stashenko, Universidad Industrial de Santander**

### Introduction

Diclofenac is a widely used anti-inflammatory drug and it is synthesized from an indolinone derivative [1]. Due to the thermal instability of diclofenac, an intramolecular cyclization product (impurity A) [2] may be formed, or the oxidation of the decarboxylated diclofenac product to an alcohol (-CH<sub>2</sub>-OH, impurity C), an aldehyde (-CHO, impurity B), and other derivatives [3].

### Methods

GC injection port with temperature controller; carrier gas type (air or helium), temperature (100-300 °C), and different solvents (ethyl acetate, methanol and dichloromethane) were studied. The splitless injection mode was used, a flow rate was 1 mL min<sup>-1</sup> and an injection volume, 2 µL. A DB-5MS column (0.12 m x 0.25 mm x 0.25 µm) was connected to the injection port, and the column outlet was introduced into ethyl acetate (0.5 mL); the sampling time was 1 min. Each eluate was concentrated to dryness, reconstituted in water: methanol (1:1) and analyzed by UHPLC-ESI<sup>+</sup>-Orbitrap-HRMS.

### Preliminary data (results)

The best analytical response obtained by LC/MS for diclofenac and its impurities A, B, C was obtained using water and methanol as mobile phase, with 0.2% (v/v) formic acid, and the capillary voltage set at 3.5 kV. The variation coefficients were in the range of 0.04-0.2% and 0.6-4% for retention times and chromatographic areas, respectively. Detection and quantification limits for diclofenac and impurities were 0.2-0.9 µg L<sup>-1</sup> and 0.6-3 µg L<sup>-1</sup>, respectively. It was shown that helium gas favored the formation of impurity A (γ-lactam), while in the presence of air, impurities B and C predominated, through the formation of the possible intermediate 2,6-dichloro-*N*-(*o*-tolyl) aniline. This study helps to understand the diclofenac thermal decomposition and could contribute to the elucidation of different reactions, which labile compounds can eventually experiment in the hot GC injection port.

1. Roy, J.; Islam, M.; Khan, A.; Das, S.; Akhteruzzaman, M.; Deb, A.; Alam, A. Diclofenac sodium injection sterilized by autoclave and the occurrence of cyclic reaction producing a small amount of impurity. *Journal of Pharmaceutical Sciences*, **2000**, 90(5), 541-545.
2. Stashenko E.E.; González A.; Martínez J.; *et al.* Hallazgo de diclofenaco en un producto fitoterapéutico a base de caléndula comercializado en Colombia. *Salud UIS*, **2020**, 52(3), 261-284.
3. Giordano, F.; Rossi, A.; Pasquali, I.; Bettini, R.; Frigo, E.; Gazzaniga, A.; Sangalli, M.; Mileo, V.; Catinella, S. Thermal degradation and melting point determination of diclofenac. *Journal of Thermal Analysis and Calorimetry*, **2003**, 73(2), 509-518.

### Please explain why your abstract is innovative for mass spectrometry?

The explanation that low signal response in diclofenac GC/MS analysis is due to thermal decomposition.

### Co-authors:

Andrés González, Universidad Industrial de Santander  
Miguel Arias, Universidad Industrial de Santander  
Jairo Martínez, Universidad Industrial de Santander

Poster number: **IM-PB-078**

## **ADVANCING NANOLC-MS SENSITIVITY TOWARDS FOR SINGLE CELL PROTEOMICS USING SOLID SILICON MICRO-PILLAR ARRAY COLUMN TECHNOLOGY**

Abstract ID: **998****Presenting author: Jeff Op De Beeck, Thermo Fisher Scientific**

### **Introduction**

The practice of ultrasensitive MS-based proteomics has seen remarkable breakthroughs in the last few years. Improvements at different stages involved in the proteomics workflow have pushed sensitivity to a level which nowadays allows scientists to successfully identify and quantify more than 1000 proteins from a single mammalian cell. Optimized sample handling, maximum separation efficiency at low nanoLC flow rates and high resolution mass spectrometry are key technologies to achieve maximum sensitivity. In this contribution, we will describe a proof-of-concept study where LC separation conditions for a non-porous microfabricated pillar array column type were optimized to get maximum proteome coverage from sub nanogram protein digest samples.

### **Methods**

Human proteome samples were analyzed using an Orbitrap Exploris 240 mass spectrometer coupled to a Vanquish Neo instrument. RPnanoLC separation was performed on a 50 cm  $\mu$ PAC Neo Limited Sample column. A dilution series (0.5, 1, 2 ng) was separated using a range of flow rate ramping methods, starting at 750 nL/min and ramping down to respectively 250 and 125 nL/min at the point where peptides start eluting. LC-MS/MS data were searched using Thermo Scientific Proteome Discoverer. Spectra were searched against the UniProt human database, peptide spectral matches were filtered to a 1% false-discovery rate using Percolator.

### **Preliminary data (results)**

We report on the use of a novel type of micro pillar array nanoLC column, specifically designed for limited sample workflows. By combining separation channels that contain 2,5  $\mu$ m diameter high aspect ratio etched pillars, non-porous C18 functionalized surfaces and a separation length of 50 cm, a column with a volume of 1.5  $\mu$ L and flow rates between 100 and 750 nL/min was developed. The high flow rate flexibility offers the possibility to define and optimize LC gradient profiles on both flow rate and organic modifier percentage. By using higher flow rates in the beginning of the gradient, we can speed up analyses and bring peptide elution down to 2 min after sample injection. As soon as peptides start eluting, flow rates are reduced to respectively 250 and 125 nL/min to take advantage of increased ionization efficiency associated with low end nanoflow LC. For both flow rates, LC methods that can handle respectively 20 to 100 samples per day have been defined and LC performance and proteome coverage were compared. For low amount human proteome samples, a significant increase in ionization efficiency was observed when reducing eluting flow rate from 250 to 125 nL/min. The full potential of this approach was revealed when comparing 40 and 20 sample per day workflows, resulting in respectively 2600 and 2800 versus 2300 and 2500 protein group id's from as little as 1 ng human proteome digest sample.

### **Please explain why your abstract is innovative for mass spectrometry?**

Development of high throughput, low flow bottom-up proteomics methodology that allows increasing detection sensitivity and proteome coverage for low input samples.

### **Co-authors:**

*Natalie Van Landuyt, Thermo Fisher Scientific*  
*Tabiwang N Arrey, Thermo Fisher Scientific*  
*Robert Van Ling, Thermo Fisher Scientific*  
*Remco Swart, Thermo Fisher Scientific*  
*Paul Jacobs, Thermo Fisher Scientific*

Poster number: **IM-PB-079**

## HIGH ASPECT RATIO PILLAR ARRAY COLUMNS FOR DEEP PROTEOME PROFILING AT MODERATE LC PUMP PRESSURES

Abstract ID: **1016**Presenting author: **Robert Van Ling, Thermo Fisher Scientific**

### Introduction

The quality of LC-MS based proteomics research relies to a large extent on the resolving power, scanning speed and sensitivity that HRAM mass spectrometers can provide to identify and quantify proteins with high confidence. The impact of the resolving power achieved with LC separation of enzymatically digested proteins must however not be underestimated. In search of increased separation power, LC column technology has been continuously evolving towards using smaller packing materials to present a continuous feed of peptides to the mass spectrometer. In this contribution, we report the evaluation of a novel type of pillar array column where the combination of reduced inter pillar distance and increased etching aspect ratio result in improved separation performance at moderate operating pressures.

### Methods

Chromatographic performance metrics were determined for different pillar array formats with varying pillar sizes. Digested Cytochrome C was injected with a Thermo Scientific Ultimate 3000 nanoRSLC instrument and detected using a 3 nL UV cell. Human proteome samples were analyzed using an Orbitrap mass spectrometer equipped with a FAIMS Pro interface. A dilution series (200, 500, and 1000 ng) was separated (300 nL/min) using non-linear solvent gradients (60-180 min). The resulting LC-MS/MS data were searched using Thermo Scientific Proteome Discoverer. Spectra were searched against UniProt human database, peptide spectral matches filtered to a 1% false-discovery rate using Percolator.

### Preliminary data (results)

Previously, we have reported on a novel generation of pillar array columns where pillar dimensions had been scaled down by a factor of 2 to increase resolving power. Even though separation performance was improved by a factor of up to 1.75, column permeability was decreased by a factor of 12. This seriously limited the range of flow rates at which columns could be operated, but also the maximum length at which they could be designed. By modifying the aspect ratio (AR, pillar height/inter pillar distance) of the separation bed, permeability could again be increased by a factor of 4, opening up opportunities to design LC columns with increased separation length and wider LC flow rate acceptances. Using a 2<sup>nd</sup> generation pillar array column with a length of 110 cm peak capacities ( $n_c=(T_c/FWHM)+1$ ) up to 1600 could be obtained. In agreement with the UV based chromatographic performance evaluation, significantly more precursors could be characterized when performing single data-dependent LC-MS/MS analyses of a tryptic digest of a human cell line with FAIMS, namely near 20% more proteins groups and over 40% more unique peptides were identified. With LC-MS run times of 90, 120 and 150 min, we respectively identified 6521, 7165 and 7539 protein groups with 1000 ng of sample loaded on column. We will further evaluate the 2<sup>nd</sup> generation pillar array columns with optimized MS methods to maximize the proteome coverage within single LC-MS/MS analyses.

### Please explain why your abstract is innovative for mass spectrometry?

Increases in nanoLC separation performance to facilitate comprehensive proteome coverage

### Co-authors:

Jeff Op de Beeck, Thermo Fisher Scientific  
Natalie Van Landuyt, Thermo Fisher Scientific  
Joshua Silveira, Thermo Fisher Scientific  
David Bergen, Thermo Fisher Scientific  
Tabiwang N Arrey, Thermo Fisher Scientific  
Romain Huguet, Thermo Fisher Scientific  
Xuefei Sun, Thermo Fisher Scientific  
Brandon Robson, Thermo Fisher Scientific  
Paul Jacobs, Thermo Fisher Scientific

Poster number: **IM-PB-080**

## QUANTITATIVE DETERMINATION OF OCHRATOXIN A AND OCHRATOXIN ALPHA IN DRIED BLOOD SPOTS OF CHICKEN USING LC-MS/MS

Abstract ID: **1024**Presenting author: **Barbara Streit, BIOMIN Research Center**

### Introduction

Ochratoxin A (OTA) is a major mycotoxin and produced by some *Aspergillus* and *Penicillium* species. It is considered to be the most toxic member of the ochratoxin group with various effects on the health of human and animal. Furthermore, it is classified as a possible human carcinogen. Exposure routes of OTA include among others cereals and cereal based products. Natural detoxification of OTA in less-/non-toxic metabolites such as ochratoxin alpha (OT $\alpha$ ) and phenylalanine is possible by microbes and enzymes. We developed and validated a method for the quantification of OTA along with OT $\alpha$  in dried blood spots (DBS) from chicken using LC-MS/MS at biological relevant levels of OTA and OT $\alpha$ .

### Methods

Spiked DBS from chicken were prepared on protein saver cards. Extraction is based on ACN/water (70/30 v/v) on a rotary shaker followed by drying and reconstitution in ACN/water/formic acid (50/49/1 v/v/v). As internal standard <sup>13</sup>C-labelled analytes were added. For measurement an HPLC 1290 Infinity II from Agilent coupled to a Sciex QTRAP 6500+ mass spectrometer is used. Chromatographic separation was achieved within 3 min total run time. Validation parameters included accuracy and precision, linear quantification range, limit of detection and quantification as well as stability of samples ready for measurement.

### Preliminary data (results)

The aim of this work was to develop and validate a simple method for the extraction and quantification of OTA and OT $\alpha$  in DBS from chicken at biological relevant concentrations. For method development and validation blank whole blood from chicken was spiked at different concentrations, spotted on protein saver cards and extracted. The analyte concentration was determined by reversed phase LC-MS/MS using external calibration in neat solvent and <sup>13</sup>C-labelled internal standard correction. In DBS samples the limit of detection was 0.3 ng/mL and the limit of quantification was 1 ng/mL for OTA and OT $\alpha$ . The range of analysis for both analytes was from 1-500 ng/mL in the blood. Accuracy was evaluated at LOQ and the upper range of analysis and  $\pm 15$  % of the initially spiked concentration. The maximum relative standard deviation was < 10 % calculated either from a triplicate extracted on the same day or from extractions on different days. The stability of processed samples was evaluated at different temperatures and storage periods. After storage analyte recoveries between  $\pm 10$  % compared to the initial measured concentrations were obtained.

### Please explain why your abstract is innovative for mass spectrometry?

A very fast and easy to establish method for the quantitative determination of OTA and OT $\alpha$  in DBS of chicken using LC-MS/MS was developed.

### Co-authors:

*Tibor Czabany, BIOMIN Research Center*

*Georg Weingart, BIOMIN Research Center*

*Martina Marchetti-Deschmann, TU Wien*

*Shreenath Prasad, BIOMIN Research Center*

Poster number: **IM-PB-081**

## **ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION ON-LINE COUPLED TO NATIVE MASS SPECTROMETRY FOR DETAILED CHARACTERISATION OF BIOPHARMACEUTICALS**

Abstract ID: 1032

**Presenting author: Alina Astefanei, Van 't Hoff Institute for Molecular Sciences, Faculty of Science, University of Amsterdam, Science Park 904, 1098 XH, Centre of Analytical Sciences Amsterdam, Science Park, 904, 1098 XH Amsterdam**

### **Introduction**

To help understand protein aggregation, we report for the first time an online platform based on the simultaneous coupling of a conventional (analytical) asymmetrical flow field-flow fraction (AF4) channel to UV-absorbance, multi-angle-light-scattering and differential-refractive-index (UV-MALS-dRI) detectors and native mass spectrometry (nMS).

AF4 is considered a “soft” separation technique able to preserve the native structure of intact proteins and their higher-order structures (HOS). The platform allows to study proteins and large molecules in their native state by using eluents that mimic physiological conditions.

The platform is applied to obtain detailed information about critical quality attributes of anti-cancer drugs (i.e. monoclonal antibodies and asparaginase).

### **Methods**

A commercially available Postnova AF4-RI-UV-MALS system was online coupled to a Orbitrap QExactive plus HRMS (Thermo Scientific). A smart stream splitting (S3) was employed to reduce sample dilution. The effluent flow after the UV detector was divided 1:1 between MALS-dRI and electrospray-ionization (ESI) MS.

### **Preliminary data (results)**

For antibodies, to better understand the experimental parameters that influence the final result, a design-of-experiments (DoE) approach was chosen. The cross flow, protein load, and ionic strength were identified as crucial parameters to control. Under optimized conditions, robust and true size-based separations were obtained independently of the mAb. This allowed to quantify fragments and aggregates present. The DoE together with the AF4-MS platform enabled the characterization of glycosylation heterogeneity and is applicable to a large panel of mAbs.

For asparaginase, various stress conditions (pH, temperature, agitation, addition of ammonium bicarbonate) were studied to gain insight in the stability of the various oligomers and the denaturation pathway. Exposing asparaginase to high pH led to dissociation of the HOS. AF4 and MALS-dRI did not suffice to fully separate the various formed species and to accurately determine their respective molar masses. Involving on-line nMS, correlation between the results obtained in the liquid and the gas phase in a single run provided information across an extended mass range. Dissociation of the HOS showed to result in formation of monomeric ( $3.5 \times 10^4$  g/mol), tetrameric ( $1.4 \times 10^5$  g/mol) and pentameric species ( $1.7 \times 10^5$  g/mol).

### **Please explain why your abstract is innovative for mass spectrometry?**

MS-compatible AF4 of such protein assemblies has not been yet reported and has not been routinely performed. Moreover, it allowed us to identify small protein modifications (deamidation, glycosylation).

### **Co-authors:**

*Iro Ventouri, Van 't Hoff Institute for Molecular Sciences, Faculty of Science, University of Amsterdam, Science Park 904, 1098 XH*

*Maria Hayder, Van 't Hoff Institute for Molecular Sciences, Faculty of Science, University of Amsterdam, Science Park 904, 1098 XH*

*Bart de Spiegeleer, Drug Quality and Registration (DruQuaR) Group, Department of Pharmaceutical Analysis, Faculty of*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

*Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000*

*Florian Meier, Postnova Analytics GmbH, Max-Planck-Str. 14, 86899 Landsberg, Germany*

*Govert W Somsen, Vrije Universiteit Amsterdam, Amsterdam Institute of Molecular and Life Sciences, Division of BioAnalytical Chemistry, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

*Peter J Schoenmakers, University of Amsterdam, van 't Hoff Institute for Molecular Sciences, Analytical Chemistry Group, Science Park, 904, 1098 XH Amsterdam, The Netherlands*

*Rob Haselberg, Vrije Universiteit Amsterdam, Amsterdam Institute of Molecular and Life Sciences, Division of BioAnalytical Chemistry, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands*

## Theme: Life sciences and health

### Session: Cellular signaling processes and MS in Systems biology

Poster number: LS-PB-001

#### REVEALING A UNIQUE INHIBITION MECHANISM OF THE POTENTIAL DRUG TARGET CHOLINE ACETYLTRANSFERASE USING MASS SPECTROMETRY TOOLS

Abstract ID: 151

**Presenting author: Tomas Bergström, Swedish Defence Research Agency (FOI)**

##### Introduction

Choline acetyltransferase (ChAT) is a ubiquitous enzyme in the animal kingdom and a central actor in acetylcholine (ACh) metabolism within cholinergic neurons [1]. ChAT is also a potential target for pharmacotherapy for a range of medical conditions, including cholinergic overstimulation caused by organophosphorus nerve agents [2]. Arylvinyropyridiniums (AVPs), are the most widely studied class of ChAT inhibitors [3], but their mechanism of action has until recently been unknown [4]. Performed X-ray studies of ChAT with CoA and AVP present, indicated a hydrothiolation event within the active site tunnel as part of the inhibition mechanism.

In this study, mass spectrometry tools were used to investigate AVPs function as ChAT inhibitors, to confirm the suggested reaction with CoA, and to explore kinetic properties of this inhibitor assembly.

##### Methods

The reaction between ChAT, CoA and the AVP (figure 1) was monitored using ultra high performance liquid chromatography–high resolution mass spectrometry (UHPLC-HRMS). A representative subset of AVPs was selected, and the reaction with ChAT and CoA characterized. The pre-steady-state kinetics of the AVP-CoA assembly was explored by LC-MS after discontinuous sampling of reactions mixtures kept on ice.

##### Preliminary data (results)

Our LC-MS results confirmed that AVPs and CoA are substrates in a hydrothiolation reaction, and that the inhibition of ChAT attributed AVPs actually are due to an in situ synthesis of the adduct AVP-CoA. This mechanism was shown to be common for a representative subset of AVPs, and time-resolved analysis of the reaction revealed a pseudo-first order association kinetics model.

Conclusively, we could in this work show that AVPs, the most widely studied class of ChAT inhibitors, act as substrates in an unusual coenzyme A-dependent and ChAT catalysed hydrothiolation reaction.

The slow trafficking of the inhibitors within the ChAT tunnel may provide an opportunity to develop nonreactive, tunnel spanning inhibitors with prolonged target residence time.

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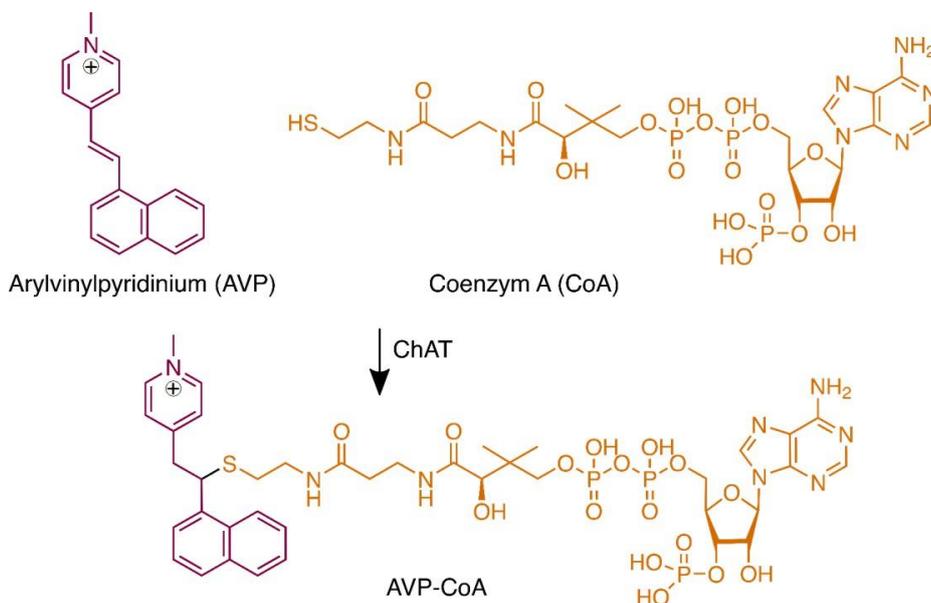
4. D. Wiktelius, A. Allgardsson, T. Bergström, N. Hoster, C. Akfur, N Forsgren, C. Lejon, M. Hedenström, A. Linusson, F. Ekström, *Angew. Chem. Int. Ed.* 2021, 60, 813–819

**Please explain why your abstract is innovative for mass spectrometry?**

Different LC-MS approaches were key contributors in the findings of a unique inhibition mechanism providing new directions for the development of improved ChAT inhibitors.

**Co-authors:**

*Daniel Wiktelius, Swedish Defence Research Agency (FOI)*  
*Fredrik Ekström, Swedish Defence Research Agency (FOI)*



**Figure 1)** ChAT-catalysed hydrothiolation reaction, producing the enzyme inhibitor AVP-CoA.

Poster number: **LS-PB-002**

## PHOSPHONATE HANDLES FACILITATE SITE-SPECIFIC ACTIVITY-BASED PROTEIN PROFILING

Abstract ID: **260****Presenting author: Wouter van Bergen, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University**

### Introduction

Mapping the target profile of small molecule drugs aids future drug development by improving our understanding of drug effectiveness and the identification of potential off-target proteins. Activity-based protein profiling (ABPP) coupled to mass spectrometry-based proteomics enables the identification of inhibitor targets. ABPP utilizes an activity-based probe (ABP) to interrogate the activity status and occupancy of active site pockets in proteins. A biotin-based enrichment approach is most used for the identification of ABP-bound proteins. However, the exact binding site of the ABP to the protein hereby remains often elusive. Identification of drug binding sites is key to understand and predict how drugs affect protein function. To address this challenge, we developed a strategy that uses IMAC-enrichable phosphonate tags for the enrichment of activity-based probe-bound peptides.

### Methods

Building on the phosphonate handle designed for specific tagging of proteins of interest by copper-catalyzed alkyne-azide cycloaddition (CuAAC), we now adapt this for ABPs (figure 1). Live cells and lysates were treated with an alkynylated derivative of the anti-cancer drug Afatinib. CuAAC allows introduction of the handle on probe-labeled residues in complex lysates. After trypsin digestion, the peptides were enzymatically dephosphorylated circumventing co-enrichment of endogenously phosphorylated peptides. Efficient enrichment of phosphonate-probe-labeled peptides was achieved adapting automated IMAC, commonly used for the enrichment of phosphopeptides. Enriched fractions were analyzed with LC-MS/MS and MaxQuant was used for probe binding site identification.

### Preliminary data (results)

Using the new approach, termed PhonIX (phosphonate-assisted inhibitor binding site exploration), over 500 unique binding sites of the alkynylated Afatinib were reproducibly identified and quantified. PhonIX allowed the investigation of differences in binding sites between lysate and live cell drug treatment revealing several different binding sites on the target proteins. Moreover, drug binding pockets were further explored using computational 3D protein modelling techniques, such as protein-ligand docking. Overall, we find that PhonIX provides a complementary method to biotin-based enrichment for the detection of inhibitor and ABP binding sites in ABPP studies, which should facilitate the further investigation into the exact binding mechanisms of drugs and expedite the identification of possible unwanted off-target proteins.

Reference:

Kleinpenning, F., Steigenberger, B., Wu, W. & Heck, A. J. R. Fishing for newly synthesized proteins with phosphonate-handles. *Nat. Commun.* **11**, 1–10 (2020).

### Please explain why your abstract is innovative for mass spectrometry?

Bio-orthogonal chemical approaches using phosphonate handles coupled with highly efficient automated IMAC enrichment enable the detection of binding sites of inhibitors combining mass spectrometry-based proteomics with activity-based protein profiling.

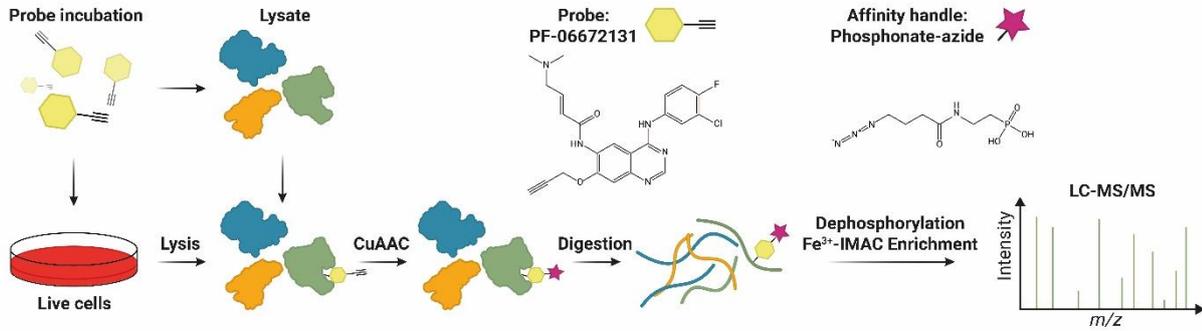
### Co-authors:

*Johannes Hevler, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University*  
*Wei Wu, Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

Marc Baggelaar, *Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University*  
Albert Heck, *Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University*



Identification of drug binding-sites by phosphonate-assisted Inhibitor binding-site exploration (PhonIX).

Poster number: **LS-PB-003**

## **MORE THAN MEETS THE EYE: EXTRACELLULAR VESICLE CHARACTERIZATION VIA NANO ES DIFFERENTIAL MOBILITY ANALYSIS, NANOPARTICLE TRACKING ANALYSIS AND MS/MS REVEALS CO-ISOLATED PROTEINS**

Abstract ID: **642**

**Presenting author: Stephanie Steinberger, Institute of Chemical Technologies and Analytics, TU Wien, Vienna, Austria**

### **Introduction**

Extracellular vesicles (EVs), released by cells and consisting mainly of a lipid bilayer and an aqueous lumen, are in demand due to the presence of biomarkers and as therapeutic platforms. They play a significant role in cellular processes from cargo transport to cell-to-cell communication. However, their heterogeneity makes their characterization highly challenging.

In this context, vesicles were analyzed via gas-phase electrophoresis on a nano Electrospray Differential Mobility Analyzer (nES-DMA) aka nES Gas-phase Electrophoretic Mobility Molecular Analyzer (nES-GEMMA), determining their surface-dry particle diameter with particle-number based detection. Compared to common techniques, gas-phase electrophoresis enables the detection of smaller analytes next to larger ones. This led to information on sample constitution and purity. Nanoparticle tracking analysis (NTA) and proteome analysis corroborated our results.

### **Methods**

EVs from human blood were isolated via ultracentrifugation (UC). The sample buffer (PBS) was exchanged to 40 mM ammonium acetate, pH 8.4, with centrifugal filters (10 kDa, MWCO, polyethersulfone membrane). Analyses were performed on NTA (Particle Metrix) and nES-GEMMA instrumentation (TSI Inc.) Vesicle loss during sample preparation was monitored via NTA.

Proteinaceous contaminants detected in nES-DMA were verified with SDS-PAGE and tryptic in-gel digest followed by MALDI TOF/RTOF. Contaminants were depleted via size exclusion chromatography (SEC) after ultracentrifugation and Annexin V-positive fractions were pooled (+SEC). Samples prior and after SEC were analyzed via timsTOF MS/MS (Bruker).

### **Preliminary data (results)**

We demonstrate that gas-phase electrophoresis on a nES-DMA / nES-GEMMA instrument is highly suitable for the quality assessment of EV containing samples in respect of vesicle integrity and data on co-purified material. Such information is important for quality control and assurance especially when considering future clinical applications.

Focusing on gas-phase electrophoresis of blood-derived EVs we exchanged the sample buffer (PBS) to volatile ammonium acetate, necessary for nES-based techniques. This solvent exchanged led to a particle loss of 60-90%, starting with  $10^{11}$ - $10^{12}$  vesicles/mL, as observed by NTA. Subsequent gas-phase electrophoresis revealed a broad nanoparticle size distribution from 20 nm to 90 nm electrophoretic mobility diameter (EMD, i.e. the surface-dry particle diameter). Concomitantly, mostly co-isolated proteins were detected in the lower analyte size range ( $\leq 20$  nm EMD). These co-isolated proteins were not measurable by NTA.

MALDI TOF/RTOF MS identified the main co-isolated proteins as hemoglobin,  $\alpha$ -2-macroglobulin, and  $\beta$ -actin-(like)-protein.

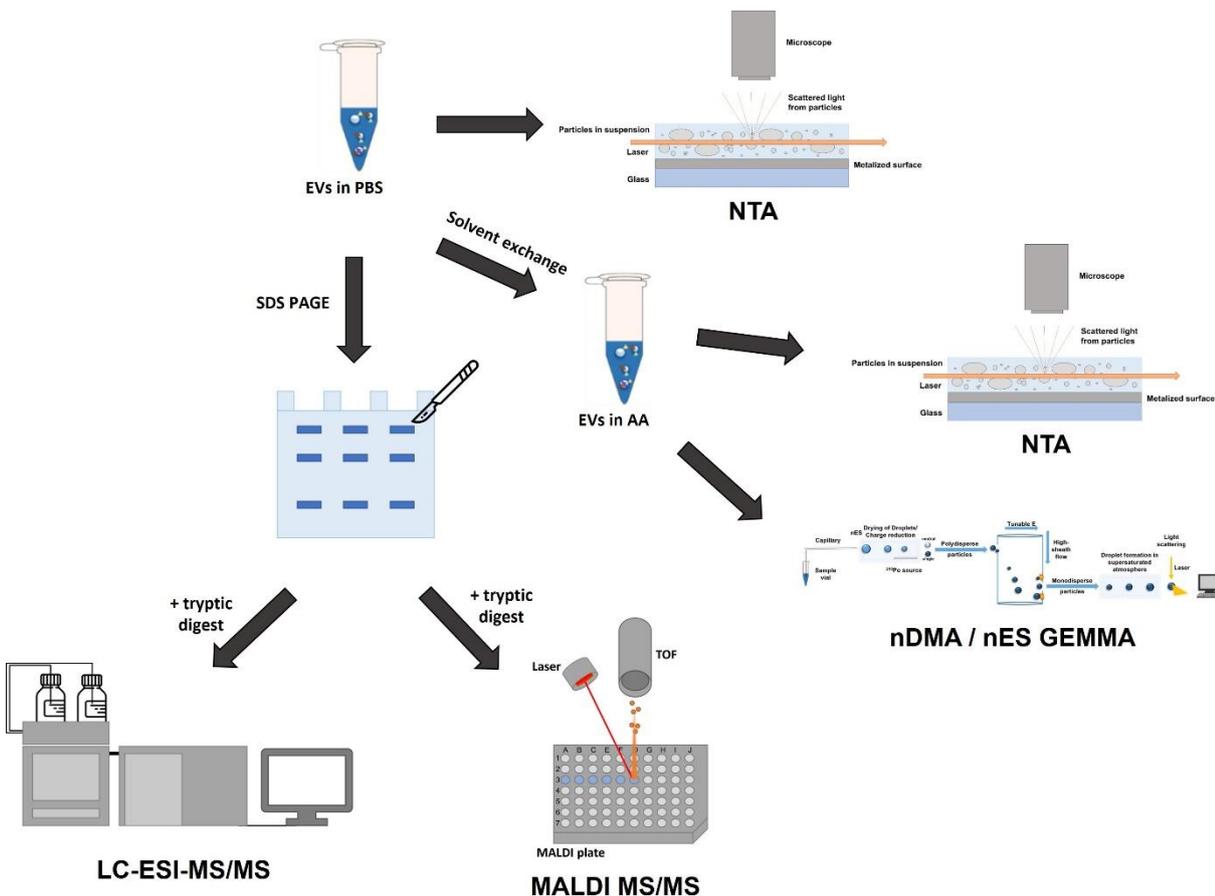
An additional SEC purification results in a significant depletion of co-isolated proteins, evident in a drop of particle numbers  $\leq 20$  nm EMD in nES-GEMMA spectra. At the same time, the overall EV hydrodynamic size distribution and the sample proteome was not affected. The latter was verified by an LC-ESI-MS/MS approach. However, SEC contributes to a further loss of vesicle numbers. Also, SEC samples showed an increased number of spikes due to lipid fragments upon nES-GEMMA measurements indicating rupture and damage of vesicles. Therefore, gas-phase electrophoresis enables the assessment of the condition and stability of EVs in solution.

**Please explain why your abstract is innovative for mass spectrometry?**

EV characterization via nES-GEMMA and MS to investigate sample quality, co-purified contaminants and the influence of sample preparation on the vesicle integrity.

**Co-authors:**

- Sobha Karuthedom George, Center for Biomedical Technology, Department of Biomedical Research, Danube University Krems, Krems, Austria*
- Lucia Lauková, Center for Biomedical Technology, Department of Biomedical Research, Danube University Krems, Krems, Austria*
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Workflow of EV containing sample analysis

Poster number: **LS-PB-004**

## **CONNECTING (PHOSPHO)PROTEOMICS AND DRUG PHENOTYPES UNCOVERS THE MODES OF ACTION OF KINASE INHIBITORS IN SARCOMA CELLS**

Abstract ID: **814****Presenting author: Chien-Yun Lee, Chair of Proteomics and Bioanalytics, Technical University of Munich**

### **Introduction**

Understanding the cellular mode of action (MoA) of drugs is essential for precision medicine. However, tumors are often molecularly heterogeneous and show varied drug responses. Combining omics technologies and drug response measurements has proven useful to understand drug MoA. Kinase inhibitors (KIs) are important cancer drugs but often display polypharmacology that is not molecularly understood. To approach this systematically, we combine full- and phospho-proteomic analysis and high-throughput phenotypic drug screening to study the interactions of 150 clinical kinase inhibitors and signaling pathways in sarcoma cells.

### **Methods**

The proteomes and phosphoproteomes from 17 sarcoma cell lines (12 entities) were characterized by micro- and nano-LC-MS/MS. High-throughput cell viability assays were performed on these cell lines in 384-well plates for 150 cancer drugs, 139 of which are kinase inhibitors. The activity landscape of 17 cell lines was calculated by integrating the kinase abundance, kinase phosphorylation, and substrate phosphorylation. The abundances of protein and phospho-sites were further integrated with the drug responses by simple correlation, sparse multiblock partial least square regression, and elastic net regression.

### **Preliminary data (results)**

We present deep (phospho)proteomic profiling consisting 9,200 to 10,500 proteins and 10,300 to 27,100 phospho-sites per cell line as well as 2,550 phenotypic drug profiles, covering broad signaling landscapes and distinct drug responses in sarcoma respectively. We showed that the kinase-based activity landscape often explains the drug sensitivity in our screening. The connectivity between (phospho)proteomics data and drug responses further reveals known and novel MoAs of many drugs. For data mining by the scientific community, we will provide an interactive web application. In conclusion, our study provides a rich resource for (1) understanding active signaling pathways in sarcoma cells, (2) identifying treatment response predictors, and (3) revealing novel MoA of clinical KIs and non-KIs. These findings serve as the first step to building a knowledge base for precision medicine.

### **Please explain why your abstract is innovative for mass spectrometry?**

The integration of drug responses and (phospho)proteomes identifies molecular markers that often explain the drug sensitivity or resistance.

### **Co-authors:**

*Matthew The, Chair of Proteomics and Bioanalytics, Technical University of Munich*

*Johanna Streubel, Chair of Proteomics and Bioanalytics, Technical University of Munich*

*Chen Meng, Chair of Proteomics and Bioanalytics, Technical University of Munich*

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*Julia Rechenberger, Chair of Proteomics and Bioanalytics, Technical University of Munich*

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Poster number: LS-PB-005

## IDENTIFYING NOVEL TRANSCRIPTIONAL REGULATORS USING AFFINITY PURIFICATIONS COUPLED TO QUANTITATIVE MASS SPECTROMETRY

Abstract ID: 881

**Presenting author: Cathrin Graewe, Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Oncode Institute, Radboud University Nijmegen, Nijmegen, The Netherlands**

### Introduction

Gene expression is driven by the binding of transcription factors to regulatory elements in the genome, such as enhancers and promoters. In recent years, DNA-protein interactions have been extensively studied and many essential transcription factors have been identified. Nevertheless, the mammalian genome contains numerous ‘orphan’ motifs for which the interacting transcription factors are still unknown. A powerful technique to identify transcription factors binding to orphan motifs is DNA affinity purification coupled to quantitative mass spectrometry. Classic affinity purifications provide information about binding specificity; however, binding of transcription factors also depends on binding affinity. To obtain this information, we recently developed a technique called PAQMAN that uses a series of DNA affinity purifications to determine apparent binding affinities proteome-wide.

### Methods

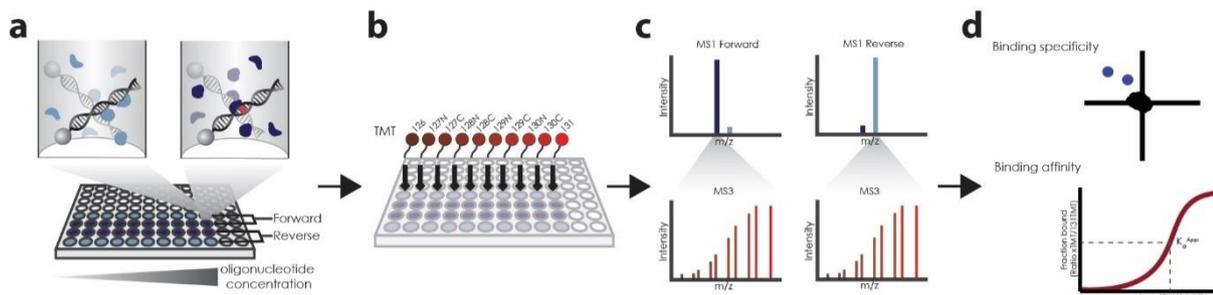
We recently expanded our PAQMAN workflow to obtain information about binding specificity and affinity in a single experiment. To this end, we combine quantitation at the MS<sup>1</sup> level with quantitation at the MS<sup>2</sup> level, a strategy that is known as higher order multiplexing (Fig. 1).

### Preliminary data (results)

We applied classic DNA affinity purifications and PAQMAN, as well as our new expanded PAQMAN, to the orphan CGCG motif. We identified BANP binding to the unmethylated CGCG motif with high affinity (Fig. 2) and showed that BANP is a strong transcriptional activator. This example illustrates that classic DNA affinity purifications in combination with PAQMAN are a powerful technique to investigate DNA-protein interactions in a proteome-wide, unbiased manner. With our expanded PAQMAN method, the same information can be obtained from just a single experiment.

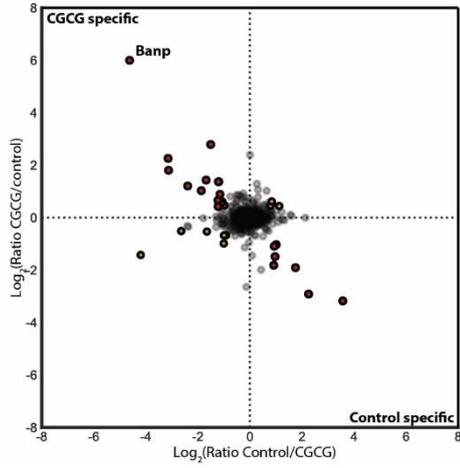
### Please explain why your abstract is innovative for mass spectrometry?

Here we show that higher order multiplexing can be applied to AP-MS experiments. In the future, we anticipate that our new workflow will be useful to investigate transcription factor biology.

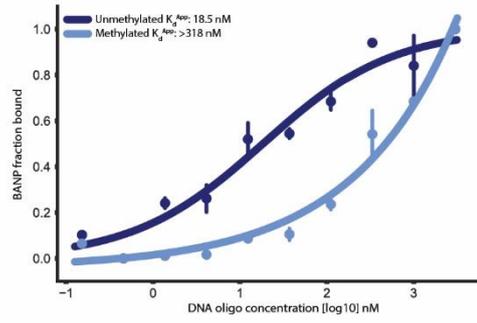


Obtaining binding specificity and affinity from a single experiment.

**a**



**b**



BANP binds to the unmethylated CGCG motif with high affinity.

Poster number: **LS-PB-006**

## **SIMULTANEOUS QUANTIFICATION OF THE NAD METABOLOME USING A TARGETED UHPLC-MS/MS METHOD**

Abstract ID: **986****Presenting author: Esther Kristianto, Victor Chang Cardiac Research Institute Innovation Centre, Sydney, New South Wales, Australia**

### **Introduction**

Nicotinamide adenine dinucleotide (NAD) is a key metabolic intermediate found in all cells, and perturbances in the NAD metabolome are linked to many disease conditions such as cancer, diabetes, and neurological and cardiovascular diseases. Furthermore, NAD deficiency during pregnancy is a cause of congenital malformations and recurrent miscarriage. Mouse models to explore the disease causation and potential prevention strategies have been established. To better understand how the NAD metabolome is perturbed and explore pathway-related biomarkers, a method to simultaneously quantify as many NAD-related metabolites as possible is required. Here, we developed a fit-for-purpose targeted method to quantify 26 NAD-related metabolites in mouse plasma, blood, and liver tissue. The method covers the 3 NAD synthesis pathways (Figure 1), dietary precursors, intermediates, enzymatic cofactors, and excretion products.

### **Methods**

The method chosen for this purpose is a targeted analysis using Ultra-High Performance Liquid Chromatography – Tandem Mass Spectrometry (UHPLC-MS/MS), which is more selective and sensitive compared to an open/untargeted methodology, since we aim to quantify a specified set of NAD metabolites. The total compounds captured simultaneously is 26 metabolites plus 21 isotope-labelled internal standards. Additionally, the data acquisition workflow is simpler and faster compared to similar previous methods. Finally, the sample extraction protocol was also kept simple and inexpensive for the different matrices analysed (blood, plasma, and liver tissue; Figure 2).

### **Preliminary data (results)**

We investigated several published UHPLC-MS/MS methods for NAD metabolome and optimised them into one single method that can capture as many compounds as possible in the pathways of interest. The chromatography showed a good separation of the 26 analytes (Figure 2). The precision, accuracy and specificity of the method were established by including technical and analytical replicates, as well as quality control samples with defined levels of spiking concentration and pooled samples interspersed periodically within a worklist. The relative standard deviation (RSD) was satisfactory for the consecutive injections (2%-20%) and the periodic injections (2%-25%). The percentage recovery in most analyte for the different matrices was also good, ranging from 60% - 120%, with some analytes exhibiting matrix suppression (<60%) and a few with matrix enhancement (>120%). A short-term stability test for 7- and 21-day storage period at autosampler temperature (4°C) showed that while the extracted metabolites are stable over the course of a long sequence of injections, storage of non-frozen samples for a week or longer is not advisable. Lastly, the limit of quantification (LOQ) and linear calibration range was determined for all 3 matrices (mouse plasma, whole blood, and liver tissue) and deemed fit-for-purpose. In summary, the method is found to be robust, selective, and sensitive to detect the metabolites of interest in the different matrices analysed. In future, the method can easily be adapted to quantify NAD-related metabolites in other mouse tissues as well as human plasma and whole blood samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

The method is unique in its simple sample processing protocol combined with the capability to simultaneously capture the largest number of metabolites directly linked to NAD metabolism.

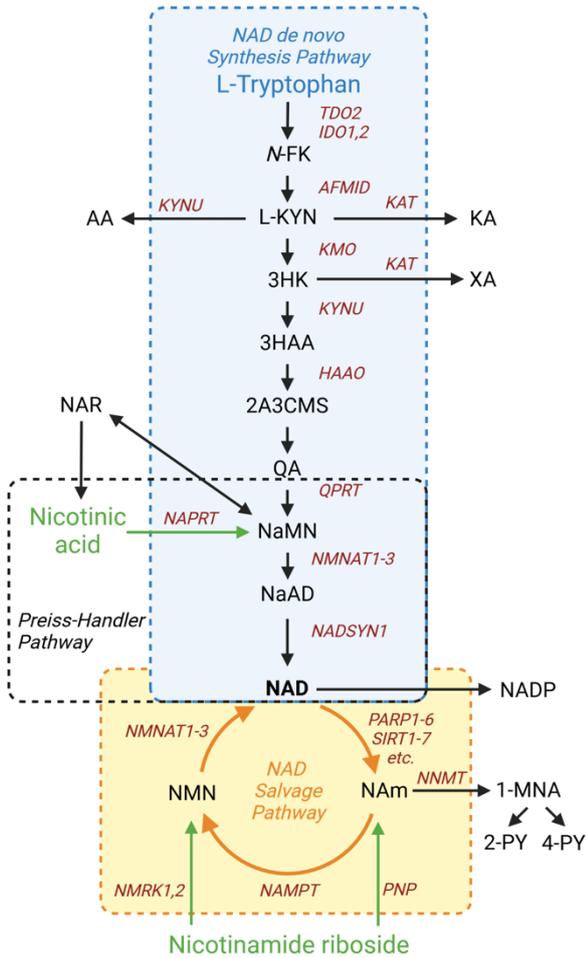
### **Co-authors:**

*Hartmut Cury, Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Sydney, New South Wales, Australia, Faculty of Medicine, University of New South Wales, Sydney, Australia*

*Mark Hodson, Victor Chang Cardiac Research Institute Innovation Centre, Sydney, New South Wales, Australia, School of Pharmacy, University of Queensland, Woolloongabba, Queensland, Australia*

*Sally Dunwoodie, Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Sydney, New*

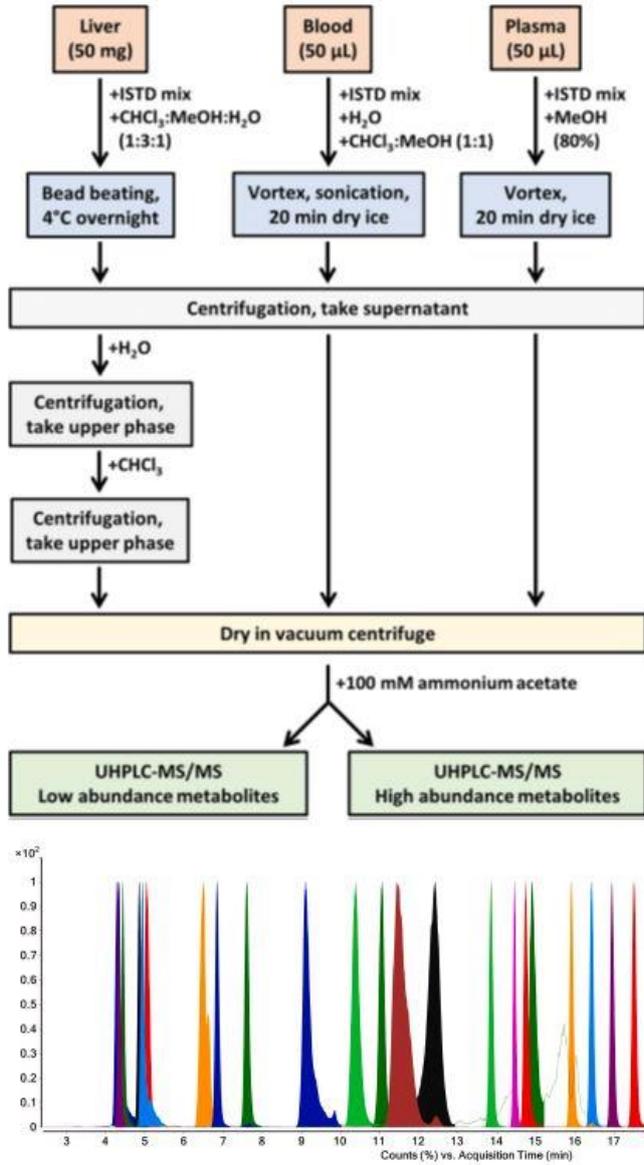
South Wales, Australia, Faculty of Medicine, University of New South Wales, Sydney, Australia, Faculty of Science, University of New South Wales, Sydney, Australia



The NAD biosynthesis pathways.

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Schematic of sample extraction protocols and chromatogram the metabolites' transitions.

## Session: Cross-omics, data integration and bioinformatics for MS

Poster number: LS-PB-007

### PROTEOMIC, METABOLOMIC, AND LIPIDOMIC ANALYSES OF LUNG TISSUE EXPOSED TO MUSTARD GAS

Abstract ID: 9

**Presenting author: Elizabeth S Dhummakupt, US Army DEVCOM Chemical Biological Center**

#### Introduction

Sulfur mustard, also known as mustard gas, is a chemical warfare agent that was introduced in World War I. This agent is called a vesicant or blistering agent and causes blistering of skin, eyes, and respiratory tract. Exposure to sulfur mustard is not typically lethal; however, there is no antidote or specific care for sulfur mustard exposure beyond supportive care. Despite most exposures not being fatal, there are long-term health consequences to exposure to sulfur mustard. This study aims to elucidate the proteomic, lipidomic and metabolomic changes that occur in the respiratory tract upon exposure to sulfur mustard in an effort to open avenues of treatment and long term care.

#### Methods

Lung tissue cultures were purchased and equilibrated for 24 hours before exposure to sulfur mustard at 0.1 mg/mL for three hours. The cultures were then rinsed and placed back in the incubator for a time course study. The tissue cultures were frozen in liquid nitrogen at 3 and 24 hours post exposure. The tissues were ground up and extracted for proteomic, lipidomic and metabolomic data analyses. Proteomics was performed using a Dionex Ultimate 3000 LC on a Thermo Orbitrap Eclipse Tribrid MS. Lipidomics and metabolomics were performed using a Thermo Vanquish Duo LC system on Thermo Orbitrap QExactive Plus.

#### Preliminary data (results)

Principal component analysis (PCA) was performed on control samples and HD-exposed samples at both 3- and 24-hours post-exposure. Figure 1 demonstrates separation between the 3-hour post-exposure control and exposed samples, which indicate molecular changes are occurring at an early time point. Figure 2 adds the 24-hour control and exposed samples, and the greatest separation is seen between the 24-hour exposed samples and all other samples. Using Gene Ontology (GO) enrichment on the proteomic data, multiple genes associated with DNA binding, RNA metabolic processes, and histone binding were shown to be significantly affected by mustard gas exposure. In addition to GO enrichment, there are several proteins that significantly down-regulated. For example, NF-kappa-B-activating protein, which is a transcriptional repressor, is heavily down-regulated in exposed samples at 24 hours, and proteins associated with RNA polymerase II mediation are down-regulated in exposed samples at both 3 and 24 hour time points. These results indicate processes involved in transcription, translation, and quality control of these pathways are heavily affected by mustard gas exposure. Initial metabolomics analysis indicates pathways associated with tRNA metabolic processes, nucleotide metabolic processes, and nucleotide biosynthetic processes are significantly dysregulated. Further analysis of the metabolites and lipids will be conducted and incorporated into the overall analysis.

#### Please explain why your abstract is innovative for mass spectrometry?

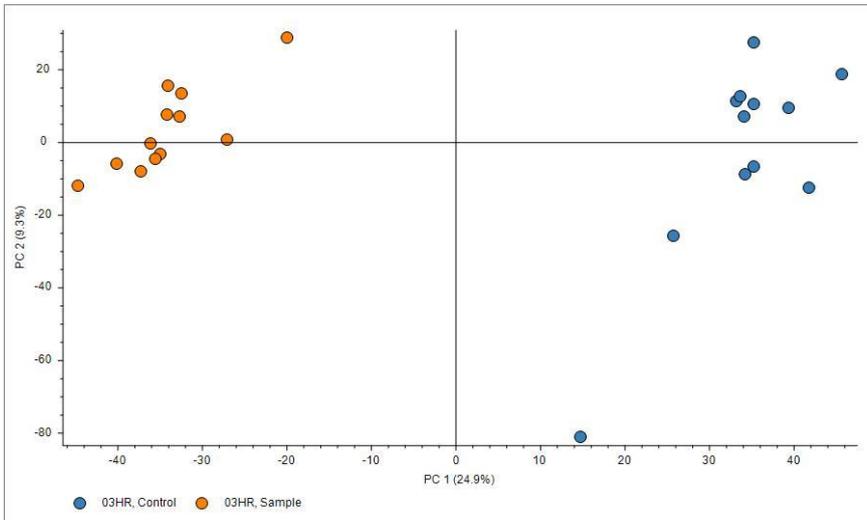
First use of multi-omic analysis of human tissues exposed to mustard gas with focus on low-level exposures.

#### Co-authors:

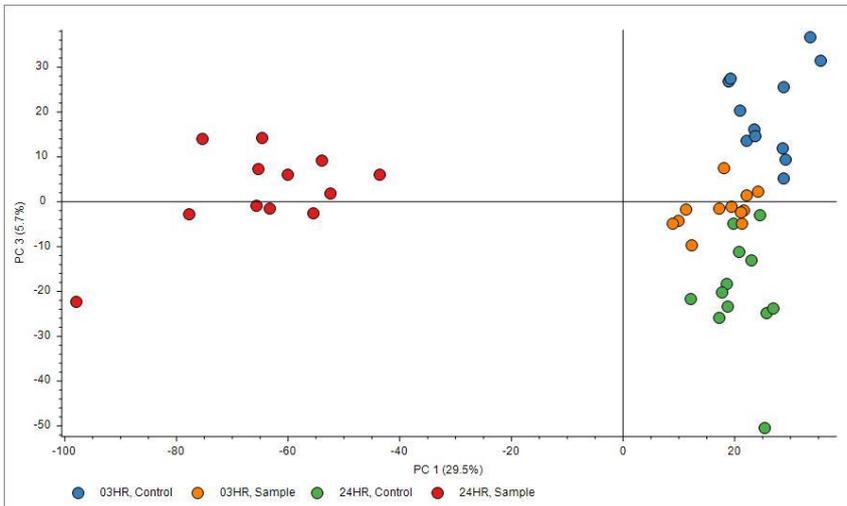
*Conor Jenkins, US Army DEVCOM Chemical Biological Center*  
*Gabrielle Rizzo, US Army DEVCOM Chemical Biological Center*  
*Daniel Carmany, US Army DEVCOM Chemical Biological Center*  
*Amber Prugh, US Army DEVCOM Chemical Biological Center*  
*Julie Renner, US Army DEVCOM Chemical Biological Center*  
*Daniel Angelini, US Army DEVCOM Chemical Biological Center*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



PCA 3-hour HD post-exposure (orange) and control (blue) samples.



PCA 3-hour post-exposure, control samples, 24 hour post-exposure, control.

Poster number: **LS-PB-008**

## MZMINE 3 - A TOOL FROM AND FOR THE MASS SPECTROMETRY COMMUNITY

Abstract ID: **235****Presenting author: Tomáš Pluskal, IOCB Prague**

### Introduction

We present MZmine 3, the third generation of the popular open-source, platform-independent software framework for MS data processing and visualization. Since the introduction of MZmine 2 a decade ago, the project has matured into a community-driven, collaborative platform, and its functions have been expanded with a variety of new modules for data preprocessing, feature detection, compound identification, spectral libraries, ion mobility separation, mass spectrometry imaging, ion identity molecular networking (Schmid et al., *Nat Commun*2021 doi:10.1038/s41467-021-23953-9), and others.

### Methods

MZmine 3 is implemented as a multi-platform Java application. It involves a completely new data model that provides the flexibility to process any type of mass spectrometry data ranging from chromatography (e.g., LC or GC)-MS to mass spectrometry imaging and both combined with ion mobility spectrometry (IMS). While offering more flexibility, work was also devoted to trace memory issues and bottlenecks for increased performance of rate limiting steps like sample alignment and gap-filling.

### Preliminary data (results)

MZmine enables research by spectral preprocessing, feature detection, and various options for compound identification, including chemical formula prediction, (epi)lipid identification, and spectral library querying and creation. Its modern graphical user interface and interactive charts facilitate data exploration and validation of results from every processing step. Since its first introduction in 2005, MZmine has received thousands of citations and tens of follow-up publications. An unbiased evaluation using 1100 standard compounds spiked into a biological matrix also confirmed that MZmine provides outstanding quantification accuracy (Li et al., *Anal. Chim. Acta* 2018 doi:10.1016/j.aca.2018.05.001).

### Please explain why your abstract is innovative for mass spectrometry?

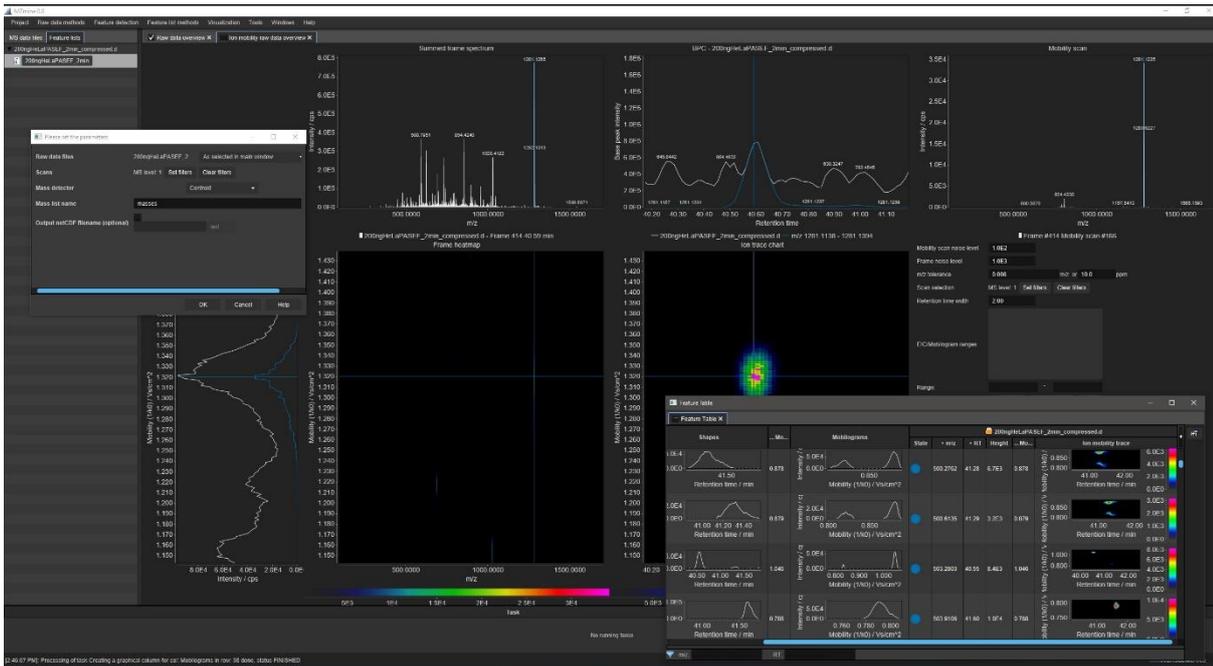
Besides support for ion mobility and MS imaging, MZmine 3 provides good integration with the GNPS platform for molecular networking and with the SIRIUS suite for molecular structure prediction.

### Co-authors:

*Robin Schmid, UC San Diego*  
*Steffen Heuckeroth, University of Münster*  
*Ansgar Korf, University of Münster*  
*Aleksandr Smirnov, UNC Charlotte*  
*Roman Bushuiev, IOCB Prague*  
*Xiuxia Du, UNC Charlotte*  
*Heiko Hayen, University of Münster*  
*Uwe Karst, University of Münster*  
*Pieter C. Dorrestein, UC San Diego*

## POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



The graphical user interface of MZmine 3.

Poster number: **LS-PB-009**

## **AN AI-DRIVEN LEAP FORWARD IN PEPTIDE IDENTIFICATION THROUGH DECONVOLUTION OF CHIMERIC SPECTRA**

Abstract ID: **242****Presenting author: Martin Frejno, MSAID GmbH**

### **Introduction**

Chimeric spectra are estimated to constitute >40% of DDA data, violating the assumption that one spectrum represents one peptide. Here, we describe a new intelligent search algorithm (CHIMERYYS) that rethinks the analysis of tandem mass spectra from the ground up. It routinely doubles the number of peptide identifications and reaches identification rates of >80%.

### **Methods**

Our new algorithm uses accurate predictions of peptide fragment ion intensities and retention times provided by a deep learning framework (INFERYYS). All candidates in the isolation window of a given tandem mass spectrum are considered simultaneously and compete for measured fragment ion intensity in one concerted step. The algorithm aims to explain as much measured intensity with as few candidate peptides as possible, resulting in the deconvolution of chimeric spectra. FDR-control is performed using Percolator. Searches can be triggered from laptops and conventional workstations (via a node in Proteome Discoverer 3.0 software) and are parallelized in the cloud.

### **Preliminary data (results)**

Analyzing a HeLa tryptic digest (1 hour gradient) with our new algorithm identified 114k PSMs, 61k unique peptides and 7,300 unique protein groups at 1% FDR. This is a 3.5-, 2- and 1.5-fold increase compared to SequestHT, respectively, resulting on average in 2.5-fold more identified peptides per protein (up to 30-fold in individual cases).

We successfully demonstrated the fidelity of our new algorithm in four experiments: I) entrapment searches focusing on FDR-estimation, II) dilution experiments focusing on expected ratio distributions, III) comparisons with multiple search engines focusing on the overlap of identifications, IV) simulation experiments focusing on the deconvolution of chimeric spectra.

Our new algorithm is compatible with older mass spectrometer generations, but profits disproportionately from the increased sensitivity of recent instruments and measurements using wider isolation windows. It substantially outperforms other search engines (e.g. SequestHT) on data of different complexity such as body fluids and organisms from all kingdoms of life.

### **Please explain why your abstract is innovative for mass spectrometry?**

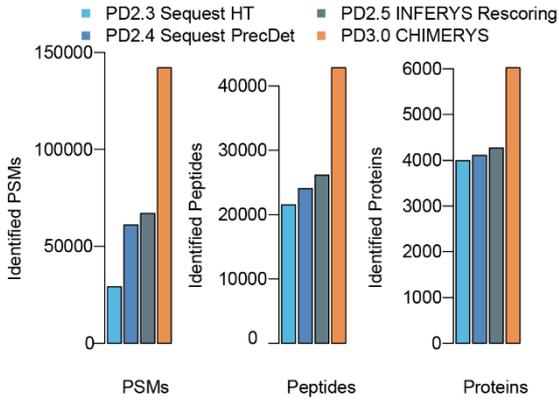
An AI based intelligent search algorithm that enables a leap forward in peptide identification.

### **Co-authors:**

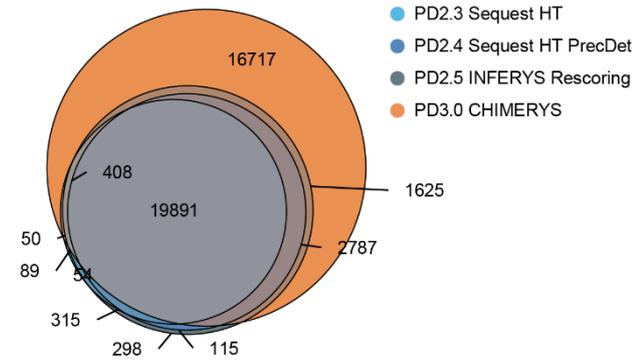
*Daniel P. Zolg, MSAID GmbH*  
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*Siegfried Gessulat, MSAID GmbH*  
*Michael Graber, MSAID GmbH*  
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 Bernhard Kuster, Technical University of Munich  
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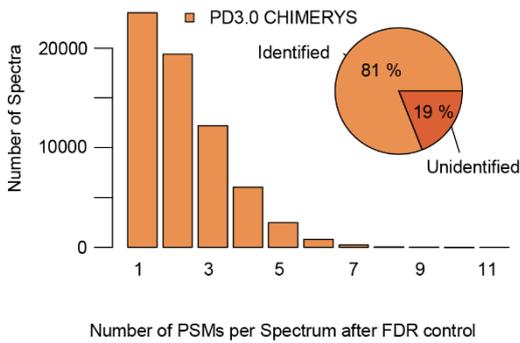
a)



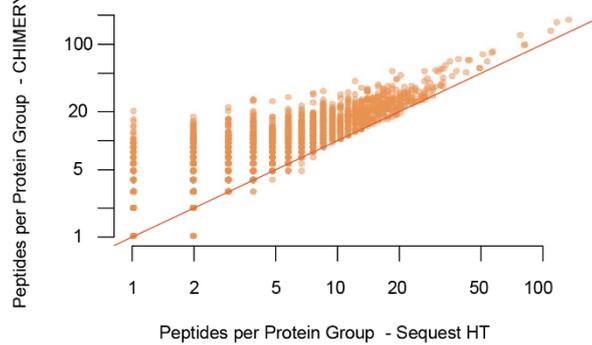
b)



c)



d)



Poster number: LS-PB-010

## BOOSTED CONVOLUTIONAL NEURAL NETWORKS FOR SUB-TISSUE CLASSIFICATION OF GLIOBLASTOMA TISSUES IN MASS SPECTROMETRY IMAGING

Abstract ID: 301

**Presenting author: Shad Mohammed, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, Institute for Medical Technology, Heidelberg University and Mannheim University of Applied Sciences**

### Introduction

Mass spectrometry imaging (MSI) enables simultaneous untargeted molecular characterization of biological tissues. It is used as one of the methods of choice to distinguish various tissue types and conditions and has been heavily researched as an orthogonal method supporting histopathological tissue investigations. Manual histopathological annotation of tissues can be both labor-intensive and time-consuming, as it is relying heavily on expert knowledge. To alleviate this problem, MSI has been applied before for sub-tissue classification in histopathological investigations, however, the high inter-measurement variability posed a true challenge for such applications. In this study, we propose boosted convolutional neural networks for inter-sample sub-tissue classification for histopathological annotation based on MSI.

### Methods

Glioblastoma human tissue samples were analyzed by MALDI-FTICR-MSI, and adjacent sections were expertly annotated according to their histopathological attributes. Annotations were registered and transformed into the corresponding MSI datasets. These were fed into a boosted convolutional neural network. The model learned representations about the training set and made decisions on the testing set. The model learned about the features in the 1D-Convolutional Neural Network layers, and overfitting was prevented due to the ensemble boosted layer. The model was tested for consistency over different Glioblastoma samples collected in different dates, as it was fed data incrementally.

### Preliminary data (results)

The results showed that multiple sub-tissue regions could be classified using our model and related features to each subclass were extracted. These results were then validated based on ground truths from histopathological annotations.

### Please explain why your abstract is innovative for mass spectrometry?

The model accounts for inter-sample variability and sub-tissue classification over a large cohort of glioblastoma tissue samples using MSI data as input.

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Poster number: LS-PB-011

## ION MOBILITY SPECTROMETRY IN MZMINE 3

Abstract ID: 347

**Presenting author: Steffen Heuckeroth, University of Münster**

### Introduction

Ion mobility spectrometry (IMS) separates ions based on their size-to-charge ratio within milliseconds, enabling hyphenation of liquid chromatography (LC)-IMS-mass spectrometry (MS) in a single setup. The resulting four-dimensional datasets (retention time, mobility,  $m/z$ , and intensity) easily reach millions of spectra per sample which presents new challenges to MS data processing tools. Despite the introduction of various IMS-MS instruments in recent years, only a few commercial or open-source software tools were adjusted for IMS compatibility.

With MZmine 3, IMS-MS support is introduced to one of the most-used and open-source software packages for MS data processing, offering stand-out capabilities to users of various IMS-MS platforms.

### Methods

Ion mobility data support was introduced to MZmine 3. With several new and multiple IMS-adapted algorithms, performant 4D feature detection was introduced and integrates seamlessly with the original platform and its existing capabilities. MZmine 3 supports IMS-MS data imported from the open-source mzML as well as native Bruker Daltonics tdf format. Overall, MZmine 3 received a major update, including a new user interface and various new processing modules.

### Preliminary data (results)

MZmine 3 offers four-dimensional feature detection workflows, aiding the discovery of isomers and separation of isobars, which typically remain hidden using conventional LC-MS analysis (see fig. 1) and would lead to chimeric features. The new IMS-MS 4D-feature detection integrates seamlessly with existing processing workflows, allowing every module to take advantage of the additional dimension provided by IMS. Furthermore, processing results can be filtered for specific  $m/z$ -to-mobility ratios, which, e.g., highly simplifies the annotation of anthropogenic substances due to heteroatom related shifts in their  $m/z$ -to-mobility ratio. For example, per- and polyfluorinated substance (PFAS) annotation can be streamlined due to compound class specific trajectories in the mobility- $m/z$  plot. Thereby, features can be extracted from these compound class-specific trajectories, which can be performed directly from the user interface. Additionally, CCS-aware spectral library matching provides increased confidence during compound annotation, on top of spectral similarity scores. All analysis results can be exported to other open-source software such as SIRIUS or GNPS for compound annotation or generation of molecular networks. Several new visualisation modules with interactive charts enable the investigation of IMS-MS data at every stage of processing from the raw data level up to individual features, allowing quick overviews as well as thorough investigations of the most complex samples.

### Please explain why your abstract is innovative for mass spectrometry?

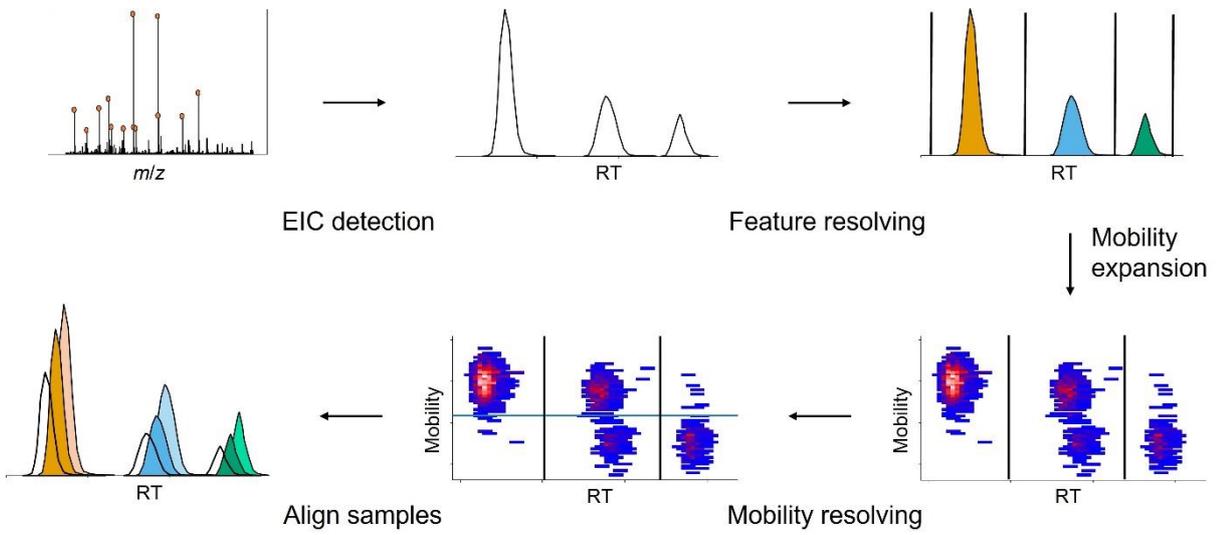
MZmine 3 is the next generation of the popular open-source MS data processing platform, providing performance enhancements, a new user interface and support for IMS-MS.

### Co-authors:

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Uwe Karst, University of Münster

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



4D feature detection workflow in MZmine 3.

Poster number: LS-PB-012

## TOPICAL APPLICATION AND INTRATUMORAL INJECTION OF HOST-DEFENCE CAERIN 1.1 AND 1.9 PEPTIDES INDUCE HIGH-LEVEL IMMUNE RESPONSE IN THE TUMOUR MICROENVIRONMENT OF TC-1 TUMOUR BEARING MICE

Abstract ID: 402

Presenting author: Tianfang Wang, Genecology Research Centre, University of the Sunshine Coast

### Introduction

The development of immunotherapies that increase immune activation of tumour-infiltrating lymphocytes against tumour and chronic viral infection-associated lesions is of great immunotherapeutic significance. Isolated from Australian amphibians, *Litoria* genus, host-defence caerin peptides inhibited the proliferation of several different cancer cells, and an additive effect was observed when used together. At concentrations non-toxic to T cells, caerin 1.1 and 1.9 inhibited HIV infecting T cells, as well as the transfer of HIV from dendritic cells to T cells. Moreover, caerin 1.1 and 1.9 inhibited TC-1 tumour growth *in vivo* when injected intratumorally, and the inhibition required an intact adaptive immune system. The stimulation of TNF $\alpha$  mediated apoptosis and the activation of TCR pathways were previously suggested by proteomic analysis.

### Methods

We compared the tumour weight and the survival time of HPV16 E6/E7 transformed TC-1 tumour bearing mice with the treatments including caerin 1.1/1.9 or control peptides. The penetration of caerin 1.9 through the epidermal layer was assessed using the confocal microscopy. The tumour-infiltrating hematopoietic cells were examined by flow cytometry. Single-cell transcriptomics and proteomics were used to quantify changes in cellular activity across different cell types within the TME.

### Preliminary data (results)

The topical application of a temperature-sensitive gel containing caerin 1.1 and 1.9 peptides reduces nearly 50% of the tumour weight. The confocal microscopy confirms the time-dependent penetration of caerin 1.9 through the epidermal layer. Single-cell transcriptomic analysis shows that the caerin 1.1/1.9 gel expands the populations with high immune activation level, largely stimulates the pro-inflammatory activity of NK and dendritic cells. The caerin gel treatment recruits almost two-fold more activated CD8<sup>+</sup> T cells to the TME, relative to the untreated tumour. The TMT10plex-labelling proteomic quantification further demonstrates the activation of interferon-alpha/beta secretion and response to cytokine stimulus by the caerin gel, while the protein contents of several key regulators were elevated by more than 30%, such as *Cd5l*, *Gzma*, *Ifit1*, *Irf9* and *Stat1*. Computational integration of the proteome with the single-cell transcriptome suggested greater activation of NK and T cells with the topical application of caerin peptide gel.

The injection of caerin 1.1/1.9 increased the efficacy of vaccinated TC-1 tumour bearing mice with anti-PD-1 treatment and largely expanded the populations of macrophages and NK cells with higher immune activation level, while reducing immunosuppressive macrophages. More activated CD8<sup>+</sup> T cells were induced with higher populations of memory and effector-memory CD8<sup>+</sup> T subsets. Integrated analysis of the proteome with the single-cell transcriptome supported activation of *Stat1* modulated apoptosis and significant reduction of immune-suppressive B cell function following caerin 1.1 and 1.9 treatment.

### Please explain why your abstract is innovative for mass spectrometry?

The quantitative proteomics revealed the two caerin peptides acted as immunomodulators acting through non-linear signalling pathways of the immune system in the TME, which was consistent with the scRNA-seq observations.

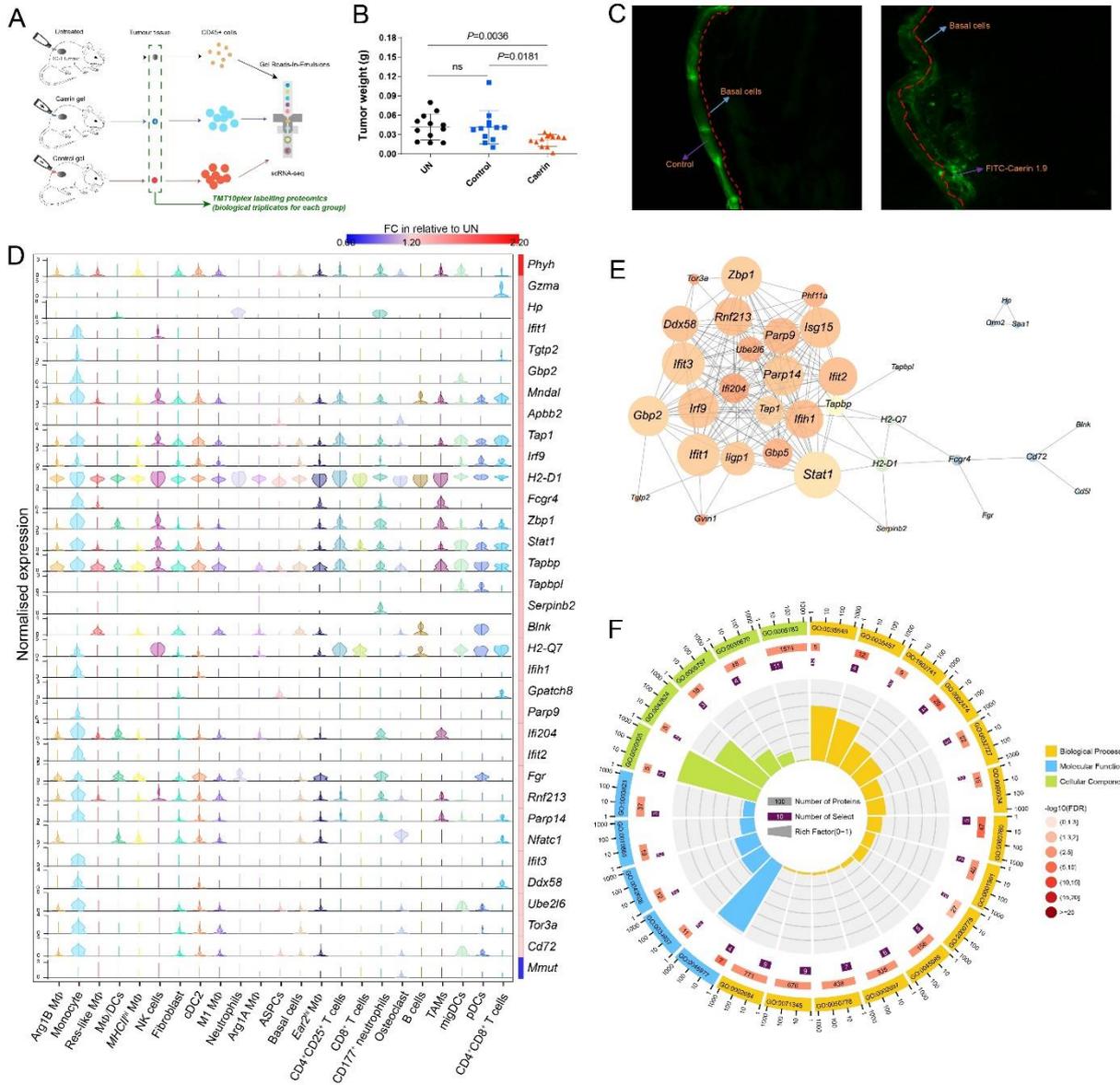
### Co-authors:

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Junjie Li, The First Affiliated Hospital/ Clinical Medical School, Guangdong Pharmaceutical University

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours

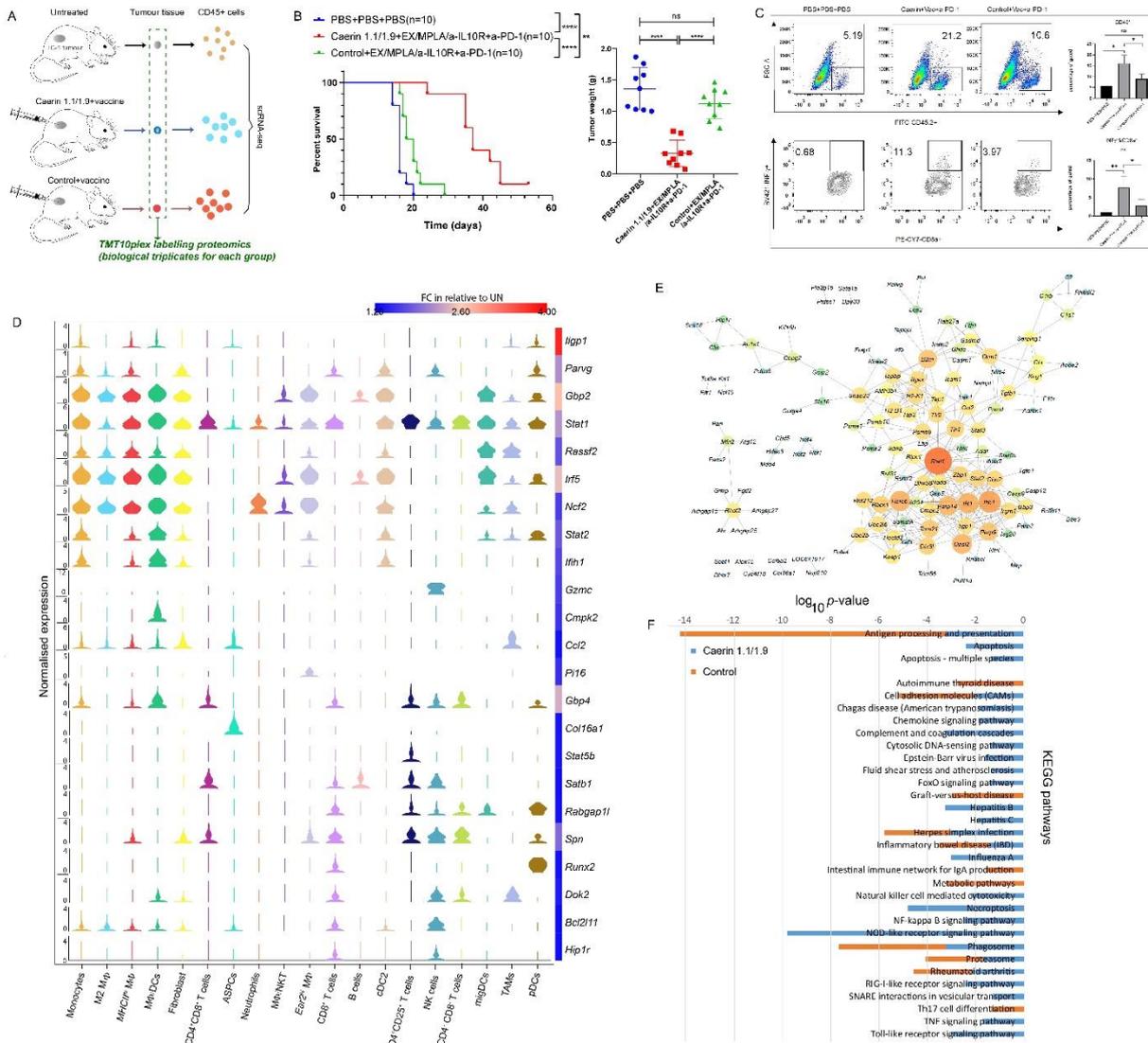
Hejie Li, School of Science, Technology and Engineering, University of the Sunshine Coast  
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Topical application of caerin 1.1/1.9 peptides gel modulates TME

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



Intratumoral administration of caerin 1.1/1.9 peptides enhances cancer immunotherapy

Poster number: **LS-PB-013**

## QUALITY ASSESSMENT TOOLS FOR LC-MS/MS TOP-DOWN PROTEOMICS

Abstract ID: **513**

**Presenting author: David Tabb, Institut Pasteur**

### Introduction

Contemporary top-down mass spectrometry can produce tens of thousands of spectra from a single LC-MS/MS experiment. Matching these MS/MS to proteoforms, however, is quite variable, with many contributing factors. If protein fractionation was employed, what degree of proteoform overlap was observed among fractions? Did the LC-ESI spread proteoforms across the duration of each LC-MS/MS experiment? What charge and mass range do the collected MS/MS represent? Was deconvolution effective in discovering high-mass or high-charge precursors? Researchers may publish proteoform-spectrum matches (PrSMs) with little certainty that identification has been performed optimally. The novel Quality Assessment tools created for this project make these questions far easier to answer, enabling more detailed characterization of top-down proteomics data and setting the stage for rapid technological advances.

### Methods

**TD Auditor:** Assessment of data in mzML format visualizes precursor charge and mass distributions, along with retention time and intensity relationships. The software computes MS/MS scan counts and peak counts from different dissociation types and MS levels.

**Deconvolution Auditor:** The precursor charge and mass range distributions produced through deconvolution techniques such as TopFD, FLASHDeconv, and Bruker DataAnalysis are visualized through Parallel Sets Diagrams.

**MS/MS Refiner:** Summing deconvolved ion masses of complementary fragments enables refinement of precursor mass and charge when this information is unclear in MS scans. Sequence tagging supports prediction of identification probabilities for MS/MS scans.

### Preliminary data (results)

The mzML format enables creation of a census of scans by dissociation type and MS level. For Thermo instruments, “filter strings” reveal the mass analyzer collecting a scan and its dissociation method. ETD methods are reflected in these strings; “@etd15.00” or “@etd10.00@hcd10.00” differ by supplemental activation. The “activation,” “msLevel,” and “selectedIon” mzML tags represent a vendor-agnostic route to this information.

The charge states recorded in raw data may have a ceiling of +25 despite higher-charge precursor ions. Deconvolution via TopFD, FLASHDeconv, or Bruker DataAnalysis (for Q-TOF) is a more reliable guide to the actual charges of massive proteoforms. These algorithms, however, can disagree on charge states, either by isotope spacing disparities or multiplicative errors. The **first image** shows a set of 31 Q-TOF spectra that TopFD declared as +24 precursors. FLASHDeconv agreed that 25 of these 31 spectra were +24, but our DataAnalysis deconvolution script agreed on only 19 precursors (61%), with four +1 charges reflecting that DataAnalysis failed to infer charge.

Deconvolved fragment ions can refine precursor measurement. While deconvolution compresses isotopic packets into single peaks and reduces fragments to neutrality or single charge, it retains distinct masses for complementary fragment ions. Summing these masses re-estimates precursor mass (**second image**). A dynamic programming algorithm will optimize precursor mass to maximize the intensity product of complementary fragment pairs.

Proteoform-Spectrum Matches will be queried for redundancy and Met oxidation / Cys alkylation occupancy rates.

Quality Assessment tools are essential to supporting the next generation of top-down technologies.

**Please explain why your abstract is innovative for mass spectrometry?**

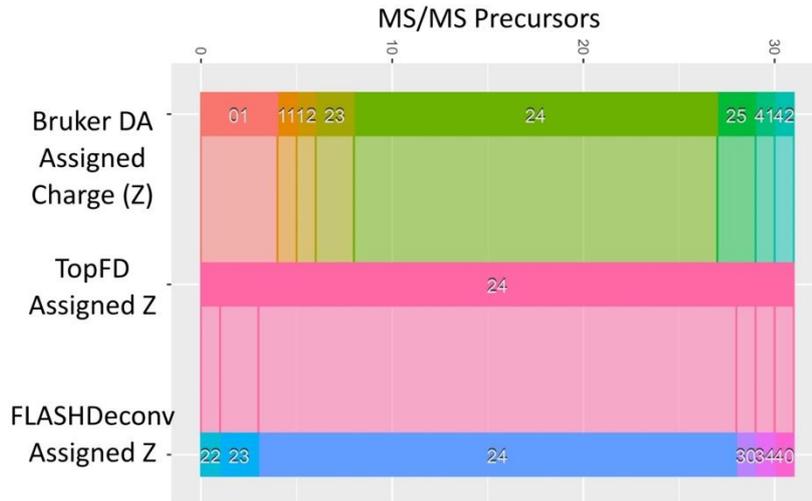
## POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
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Comparing the information yield for different algorithms in top-down proteomics clarifies the state-of-the-art for contemporary software. Both top-down MS/MS production and MS/MS identification stand to benefit.

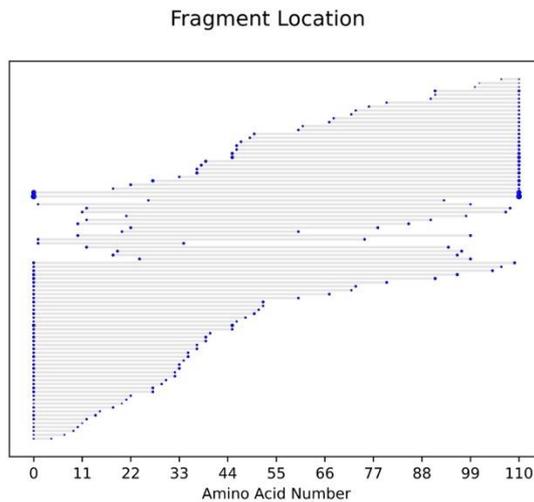
### Co-authors:

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Kyowon Jeong, Universität Tübingen  
Julia Chamot-Rooke, Institut Pasteur



Consensus among assigned precursor charges, visualized in Parallel Sets

c18+z92	12219.81393
c22+z88	12219.81366
c27+z83	12219.80369
c33+z77	12219.77356
c37+z73	12219.78905
c39+z71	12219.80168
c45+z65	12219.80213
c46+z64	12219.82217
c50+z60	12219.81409
c60+z50	12219.77658
c67+z43	12219.81012
c73+z37	12219.80179
c80+z30	12219.75305
c91+z19	12219.82785
average	12219.8011
minus H	1.007825035
yields	12218.79328
actual	12218.77



ClipsMS visualizes MS2 fragments. Complementary pairs sum to proteoform mass.

Poster number: LS-PB-014

## EVALUATION OF PROTEOME ACCESSIBILITY AFTER SIMULTANEOUS PROTEO-METABOLOME LIQUID-LIQUID EXTRACTION (SPE-LLE)

Abstract ID: 626

Presenting author: Anna-Sophia Egger, University of Innsbruck

### Introduction

As our understanding of the complexity of biological organisms increases, so does the need to investigate biologic systems on a comprehensive scale. Technological advances in recent years have also increased the feasibility of “multi-omics” studies, in which different OMICS layer (such as genomics, proteomics or metabolomics) are analyzed and integrated. A challenge in these studies is the large variation that can be amplified when different analytes are extracted from different samples or in different laboratories. To reduce this variance, we have developed a simultaneous extraction method for proteins, lipids and polar metabolites from the same sample (Simultaneous Proteo-Metabolome Liquid-Liquid Extraction, SPM-LLE). In this study, we investigate the accessibility of the proteome with different extraction buffers and compared the performance with a direct proteome extraction.

### Methods

HEK293T cells were either lysed by CHCl<sub>3</sub>-MeOH based SPM-LLE or directly by addition of an extraction buffer containing urea, sodium deoxycholate (SDC) or sodium dodecyl sulfate (SDS). Proteome containing interphase pellets of SPM-LLE was solubilized by addition of an urea, SDC or SDS containing buffer. For LFQ proteomics, same protein amounts were used for tryptic digestion. For SDS-containing samples, single-pot solid-phase-enhanced sample preparation (SP3) procedure was used. Tryptic peptides were desalted by RP-SPE and analyzed by LC-MS/MS using an orbitrap Fusion Lumos. Proteins were identified and quantified by MaxQuant and further data analysis was performed using R.

### Preliminary data (results)

Proteomes from the SPM-LLE interphase pellet were solubilized with an SDS-, SDC- or urea-based buffer. Qualitatively, all three methods (urea: 5,563+/-14, SDS: 4,879+/-129, SDC: 5583+/-63) provide access to a very similar proteome with 75.5% of proteins (n= 4,200) identified in all samples. Quantitatively, considerable differences in extraction efficiency were observed. Generally, urea was the most efficient buffer to extract proteins from the SPM-LLE-interphase including metabolic proteins from glucose, amino acid and lipid metabolism. Examination of physicochemical properties of proteins extracted more efficiently in each buffer revealed that urea and SDC tended to match the distribution of the human reference proteome, whereas the proteins extracted more efficiently with SDS tended to have higher isoelectric points. GO enrichment analysis showed that SDS tended to extract RNA-binding proteins more efficiently than SDC and urea, although urea also extracted RNA-binding proteins more efficiently compared to SDC.

Comparing the extraction of the proteome from SPM-LLE interphase pellet with direct proteome extraction from cells, no differences could be found qualitatively based on the protein identifications. Qualitatively, direct proteome extraction from cells was more efficient than extraction from the interphase pellet for both SDC and SDS. In contrast, urea showed higher extraction efficiency when the proteome was extracted from the interphase pellet including metabolic proteins.

In summary, our results show that urea provides the best proteome accessibility for simultaneous proteo-metabolomics. Interestingly, the performance of urea was better for extraction from the interphase pellet than for direct proteome extraction from cells.

### Please explain why your abstract is innovative for mass spectrometry?

We investigated proteome accessibility for simultaneous proteo-metabolomics.

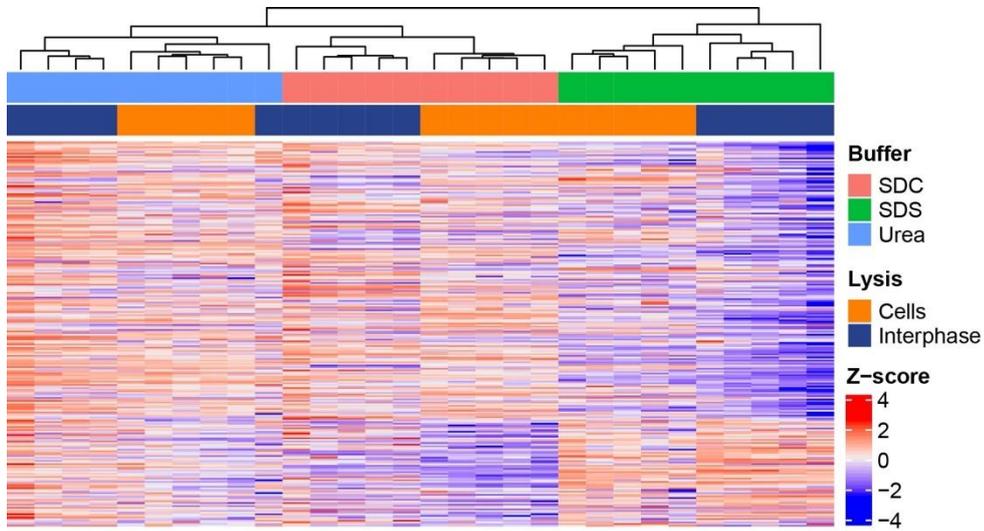
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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Elisabeth Zimmermann, University of Innsbruck*  
*Kathrin Thedieck, University of Innsbruck, University of Groningen, Carl von Ossietzky University Oldenburg*  
*Marcel Kwiatkowski, University of Innsbruck*



Heatmap of protein abundances extracted from different samples.

Poster number: LS-PB-015

## **ANALYTICS FOR MET-ID OF METABOLIC MIXTURES OF NEW PSYCHOACTIVE SUBSTANCES TOWARDS BIOMARKER IDENTIFICATION FOR USE IN ENVIRONMENTAL SETTINGS**

Abstract ID: 644

**Presenting author: Kristina B.M. Still, Amsterdam Institute of Molecular and Life Sciences, Division of BioAnalytical Chemistry, Department of Chemistry and Pharmaceutical Sciences, Faculty of Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081, HV, Amsterdam, the Netherlands, Centre for Analytical Sciences Amsterdam (CASA), 1098 XH Amsterdam, The Netherlands**

### **Introduction**

New-psychoactive-substances (NPS) are compounds with minor structural differences though similar pharmacological effects as illicit drugs, synthesized and sold circumventing prosecution. Their novelty limits information regarding their pharmacological effects and biotransformation and ethical reasons deem traditional drug development clinical studies not feasible. The structural adjustments can alter their mechanism of action and cause harmful side effects. The majority of necessary studies use either pooled urine samples (often from public urinals at festivals) or waste water samples and are thus quite male orientated. NPS intoxications, negative health effects, and emergencies are increasing, indicating the dangers involved with this trend. As the NPS and their metabolites end up in waste waters and possibly also in our drinking water, they also pose a potential threat for the general population.

### **Methods**

The NPS fluoroamphetamine (2,3,4-FA) and fluoromethamphetamine (2,3,4-FMA), derivatives of the illegal drugs amphetamine and methamphetamine, are subjected to female and male microsomal incubations (i.e. mimicking liver oxidation metabolism) to study their metabolic profiles. Pools of metabolite products are thus created. Analyses for Met-ID is then performed using LC-QTOF-MS/MS to characterize and compare the metabolic mixtures. This approach yields structural information of formed metabolites and gives insights in the variation and similarities of metabolism between the different fluoro-isomers of FA and FMA and also between female and male subjects which, in both cases, can show substantial variation.

### **Preliminary data (results)**

With the developed Met-ID methodologies, variation have been seen in efficiency of metabolite formation between the different fluoro-isomers FA and FMA. For both the FA and the FMA, the 2-isomers show the highest in vitro metabolism, followed by the 3-isomers and lastly the 4-isomers. Furthermore, overlap is seen between metabolites formed in FA and FMA, causing potential problems in forensic settings. This, and further variation in the in vitro metabolism between female and male subjects is currently being explored.

To develop an analytical method for monitoring these metabolites in environmental samples, the high abundance metabolites found in the metabolic incubation samples, likely to be found in wastewaters, were pinpointed for incorporation into the analytics workflow. Next, in order to produce standards of some of these metabolites, different straightforward chemical oxidations were performed on the parent compounds in order to investigate which of the oxidation reactions produced oxidation products that were also found as one of the metabolites. For this, the NPS were exposed to different chemical oxidants such as Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) and potassium permanganate (KMnO<sub>4</sub>) in so-called 'chemical incubations' to mimic the metabolic incubations. Oxidation products found to match actual metabolites, for example several mono-hydroxylated derivatives of the FAs and FMAs (i.e. ring-hydroxylated,  $\Delta m/z +16$ ), were then selected for purification to subsequently be used as standards in the development of the analytical method.

### **Please explain why your abstract is innovative for mass spectrometry?**

Hyphenated LC-qTOF-MS/MS methods for studying female and male metabolic profiles of fluoro-FA and fluoro-FMA isomers, and metabolite-like oxidation-products anticipated to be used as standards for monitoring in environmental samples.

### **Co-authors:**

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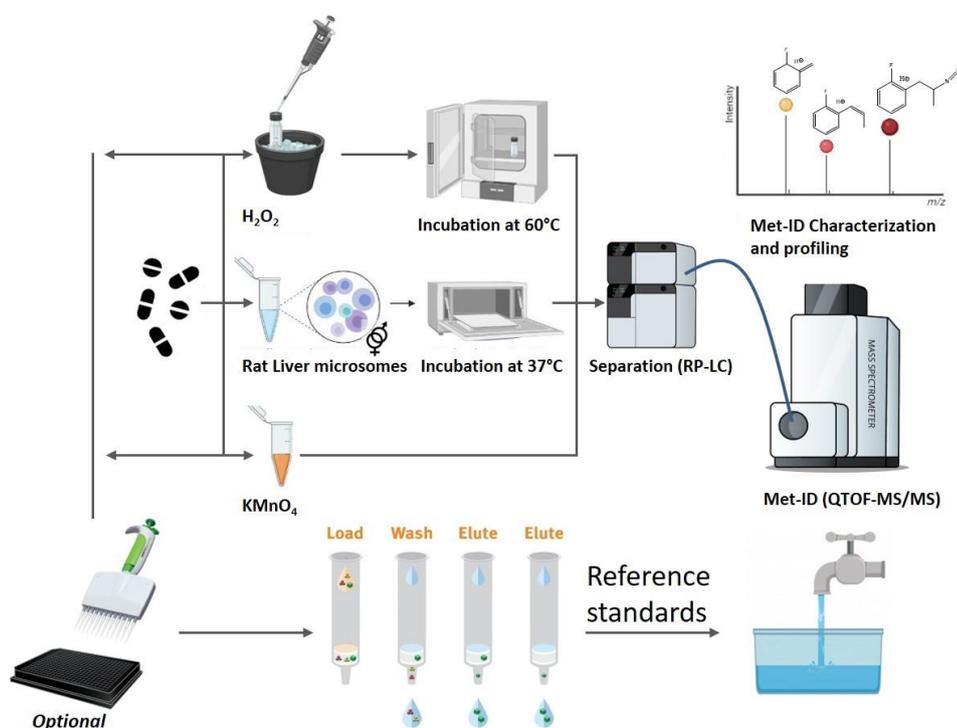
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Workflow of metabolic and chemical incubations for monitoring environmental samples.

Poster number: LS-PB-016

## DATA-INDEPENDENT ACQUISITION PROTEOMICS COMBINED WITH TARGETED LIPIDOMICS REVEALS UNIQUE MOLECULAR SIGNATURES OF THE LUNG IN INSULIN-DEFICIENT DIABETES MELLITUS

Abstract ID: 933

**Presenting author: Bachuki Shashikadze, Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, 81377 Munich, Germany**

### Introduction

Diabetes is a complex metabolic disorder defined by impaired glucose homeostasis due to deficient insulin secretion, impaired insulin sensitivity, or both. Diabetes is one of the biggest global health challenges with a steadily increasing prevalence. In recent years, growing evidence shows the lung as an organ targeted by diabetic injury. However, for the lung, the molecular mechanisms of these complications are not clear. To study the effects of insulin deficiency and hyperglycemia on lung tissue we used a clinically relevant pig model of insulin-deficient diabetes mellitus (MIDY) and wild-type (WT) littermate controls.

### Methods

Mass spectrometry-based proteomics was performed using data-independent acquisition mode. Raw data processing was carried out using DIA-NN. Identification was based on an *in silico* predicted spectral library generated by DIA-NN's built-in deep-learning-based spectra and retention time predictor and further refined by experimental data from a project-specific gas-phase fractionation-based library. For quantitative analysis, an MS-Empire workflow was used which uses peptide-level data for sensitive detection of differentially abundant proteins. Additionally, accurate lipidomics analysis was performed with Multiple Reaction Monitoring (MRM) using internal standards. At least two mass transitions were used for each compound.

### Preliminary data (results)

A total of 49,211 unique peptides from 5465 protein groups could be identified with high confidence (FDR < 0.01). In the diabetic lung, the protein level of pulmonary surfactant-associated protein A – a biomarker of a lung injury was elevated. Additionally, the abundance of key proteins involved in humoral immune response and extracellular matrix organization was changed. Strikingly, we observed a 2.5-fold reduction of polyunsaturated fatty acid lipoxygenase (ALOX15) in the diabetic lung. In line with proteomics data, targeted lipidomics demonstrated reduced levels of lipid products known to be generated by ALOX15 activity. Additionally, correlation analysis of lipidomics data revealed a co-regulation of lipids sharing biological functions and are produced through the lipoxygenase activity. A dysregulated lipoxygenase pathway in the diabetic lung was further confirmed by Co-inertia analysis (CIA) integrating proteomics and lipidomics data. The CIA suggested similar covariations between proteome and oxylipin measurements from MIDY and WT animals and indicated the possible effect of insulin deficiency and hyperglycemia on intrinsic multi-ome communications. In summary, we generated the first comprehensive multi-omics analysis of the lung from a clinically relevant large animal model of insulin-deficient diabetes, providing a valuable resource for future comparative and translational studies. In addition, our dataset and deep chromatogram library of porcine lung represents one of the largest libraries and may be the base for future DIA proteomics studies.

### Please explain why your abstract is innovative for mass spectrometry?

To our knowledge, this is the first proteomics study where peptide level quantification strategy was applied for the analysis of the DIA data.

### Co-authors:

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Elisabeth Kemter, German Center for Diabetes Research (DZD), 85764 Neuherberg, Germany, Chair for Molecular Animal Breeding and Biotechnology, Gene Center and Department of Veterinary Sciences, LMU Munich, 81377 Munich, Germany, Center for Innovative Medical Models (CiMM), LMU Munich, 85764 Oberschleißheim, Germany  
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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Germany*

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Poster number: LS-PB-017

## TEMPLATE-BASED ASSEMBLY OF PROTEOMIC SHORT READS FOR DE NOVO ANTIBODY SEQUENCING AND REPERTOIRE PROFILING

Abstract ID: 937

Presenting author: Joost Snijder, Utrecht University

### Introduction

Antibodies can target a vast molecular diversity of antigens. This is achieved by generating a complementary diversity of antibody sequences through somatic recombination and hypermutation. A full understanding of the antibody repertoire in health and disease therefore requires dedicated *de novo* sequencing methods. Next generation cDNA sequencing methods have laid the foundation of our current understanding of the antibody repertoire, but these methods share one major limitation in that they target the antibody-producing B-cells, rather than the functional secreted product in bodily fluids. Mass spectrometry-based methods offer an opportunity to bridge this gap between antibody repertoire profiling and bulk serological assays, as they can access antibody sequence information straight from the secreted polypeptide products.

### Methods

In a step to meeting the challenge of MS-based antibody sequencing, we present a fast and simple software tool (Stitch) to map proteomic short reads to user-defined templates with dedicated features for both monoclonal antibody sequencing and profiling of polyclonal antibody repertoires.

### Preliminary data (results)

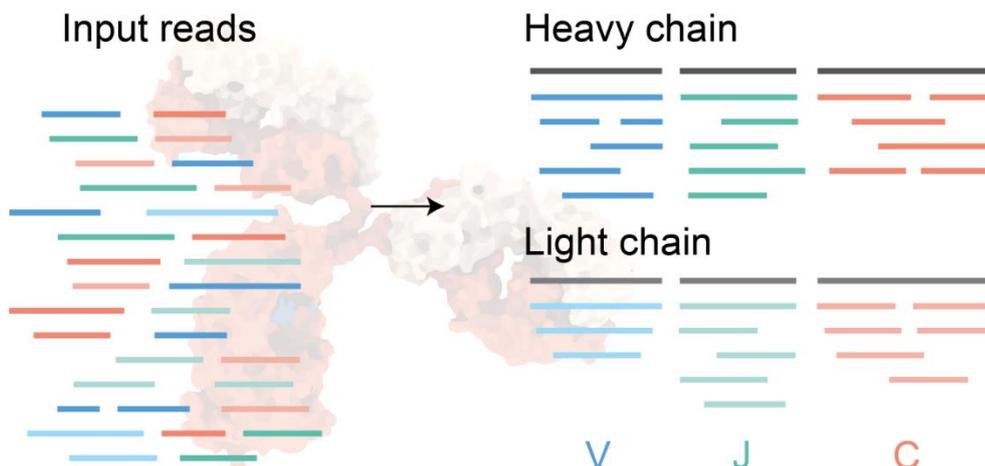
We demonstrate the use of Stitch by fully reconstructing 2 monoclonal antibody sequences with >98% accuracy (including I/L assignment); sequencing a Fab from patient serum isolated by reversed-phase LC fractionation against a high background of homologous antibody sequences; sequencing antibody light chains from urine of multiple-myeloma patients; and profiling the IgG repertoire in sera from patients hospitalized with COVID-19.

### Please explain why your abstract is innovative for mass spectrometry?

Stitch assembles a comprehensive overview of the antibody sequences that are represented in the dataset and provides an important first step towards analyzing polyclonal antibodies and repertoire profiling.

### Co-authors:

Weiwei Peng, Utrecht University  
Douwe Schulte, Utrecht University



Poster number: **LS-PB-018**

## IMPROVED DIA-PASEF BASED QUANTITATIVE PROTEOMICS USING SPECTRONAUT

Abstract ID: **1007**Presenting author: **Sira Echevarria-Zomeno, Biognosys AG**

### Introduction

Ion mobility (IM)-based data independent acquisition (DIA) can bring improvements by an additional dimension of ion separation and a boost in sensitivity. We have previously presented our dia-PASEF analysis pipeline with high-precision IM (Gandhi, ASMS 2020) which automatically defines the optimal extraction window per peptide in IM dimension. Since then, we have greatly improved Spectronaut via a new machine learning framework (Overney, ASMS 2021) and deep learning-based scores. In this work we evaluated the impact of these efforts on the processing of dia-PASEF data with a 4-species controlled quantitative experiment. We found a significant improvement in not only overall identification rate, but also in quantification.

### Methods

We prepared a 4-species mixed proteome sample with two conditions (*H. sapiens* 1:1, *S. cerevisiae* 1:2, *E. coli*, 1:10, *C. elegans* 1.3:1). A 2h gradient was used. For library generation, the pooled sample was fractionated and acquired with a timsTOF Pro in PASEF mode. Using Pulsar, our database search engine, the library resulted in 194,515 precursors and 19,537 proteins across all species. For the quantitative experiment, each condition was acquired in triplicates in dia-PASEF mode. The targeted analysis of dia-PASEF runs using both Spectronaut v15 and v16 with 1% FDR at peptide and protein level.

### Preliminary data (results)

Spectronaut v16 identified ~14,000 protein groups cumulatively across the four species, an improvement of 33% over Spectronaut v15. When considering only identifications with CV < 20%, v16 still identified 17% more protein groups on average for both conditions. Both versions performed similar in accuracy despite having more identifications with v16. The median absolute deviation from the expected log ratio between two conditions across all 4 species was 0.07 in v15 and 0.11 in v16. Furthermore, we performed regulation analysis using an unpaired t-test at protein level (Huang 2019). Upon sorting all the regulated candidate pairs by p-value, v16 recovered 9.3% more true candidate pairs (3046 candidates) with a 5% false positive rate.

Finally, we used additional dia-PASEF datasets covering different sample types and gradient lengths to benchmark the overall identification performance between the two versions. For v16, we saw an average improvement of 44% at precursor and 25% at protein group level with ultra-short gradient ( $\leq 20$  mins) and of 16% at precursor and 5% at protein group level for longer gradient ( $\geq 60$  mins) dia-PASEF datasets over v15. We speculate that the reason for a relatively stronger improvement in the four species experiment and the samples acquired with ultra-short gradient is due to the higher complexity of the data which stand to benefit more from improved scoring.

### Please explain why your abstract is innovative for mass spectrometry?

We present an improved dia-PASEF analysis pipeline which delivers high performance in identification and quantification, to an important extend thanks to the implementation of artificial intelligence technology into Spectronaut

### Co-authors:

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Normand Overney, Biognosys AG  
Dariush Mollet, Biognosys AG  
Anna Susmelj, Biognosys AG  
Lukas Reiter, Biognosys AG

## Session: Glycomics & Glycoproteomics

Poster number: LS-PB-020

### **TUMOR ASSOCIATED CARBOHYDRATE ANTIGENS (TACAS) AS PROMISING TARGETS FOR THE DEVELOPMENT OF IMMUNOTHERAPY FOR COLORECTAL CANCER**

Abstract ID: 30

**Presenting author: Katarina Madunić, Leiden University Medical Center**

#### **Introduction**

Colorectal cancer (CRC) is one of the most prevalent malignancies worldwide with over 900,000 deaths in 2020. New molecular targets are needed for the development of targeted therapies to provide a better treatment for a larger group of CRC patients. Heavily glycosylated proteins play a very important role in the gut homeostasis, however, little attention was given to their glycosylation. Expression of aberrant carbohydrate antigens in cancer, also known as tumor-associated carbohydrate antigens (TACAs) provides a great potential for the development of new targeted immunotherapeutics. Here we present an in-depth study decoding the tumor specific *N*- and *O*-linked glycan signatures of CRC derived from epithelial regions of both primary tumors and metastatic sites compared to healthy colon mucosa from the same patients.

#### **Methods**

We established a workflow that sequentially releases the *N*- and *O*-glycans from approximately 20,000 cells from laser capture microdissected regions of formalin fixed paraffin embedded (FFPE) tissues. The released glycans are analyzed with porous graphitized carbon (PGC)-LC-MS/MS in negative ion mode. This platform enables a powerful separation of isomeric species as well as in-depth structural characterization of potential TACAs.

#### **Preliminary data (results)**

Distinctive *N*- and *O*-glycosylation features were found in cancer, cancer stroma as well as in normal colon mucosa. Over 100 *O*-linked glycans were detected in the cancer regions which did not show any expression in normal mucosa. From those, seven core 2 *O*-glycans were exclusively found in more than 33% of the cancers which carried the terminal sialyl-LewisX/A antigen or terminal  $\alpha$ 2-3-linked sialylation. Of these, two glycans were found in 72% of the analyzed cancers and 94% of the investigated cancers expressed either one of the two *O*-glycans with the composition H<sub>2</sub>N<sub>2</sub>F<sub>1</sub>S<sub>1</sub> and H<sub>2</sub>N<sub>2</sub>S<sub>1</sub> demonstrating great specificity (Figure 1). In contrast, normal colon mucosa predominantly expressed core 3 *O*-glycans, carrying  $\alpha$ 2-6-linked sialylation, (sulpho-)LewisX/A and Sda antigens (Figure 2). Additionally, preliminary results revealed that 26 distinct *N*-linked glycosylation signatures were found only in the cancer samples, and 11 individual *N*-glycans showed statistically significant upregulation in cancer compared to normal colon mucosa.

#### **Please explain why your abstract is innovative for mass spectrometry?**

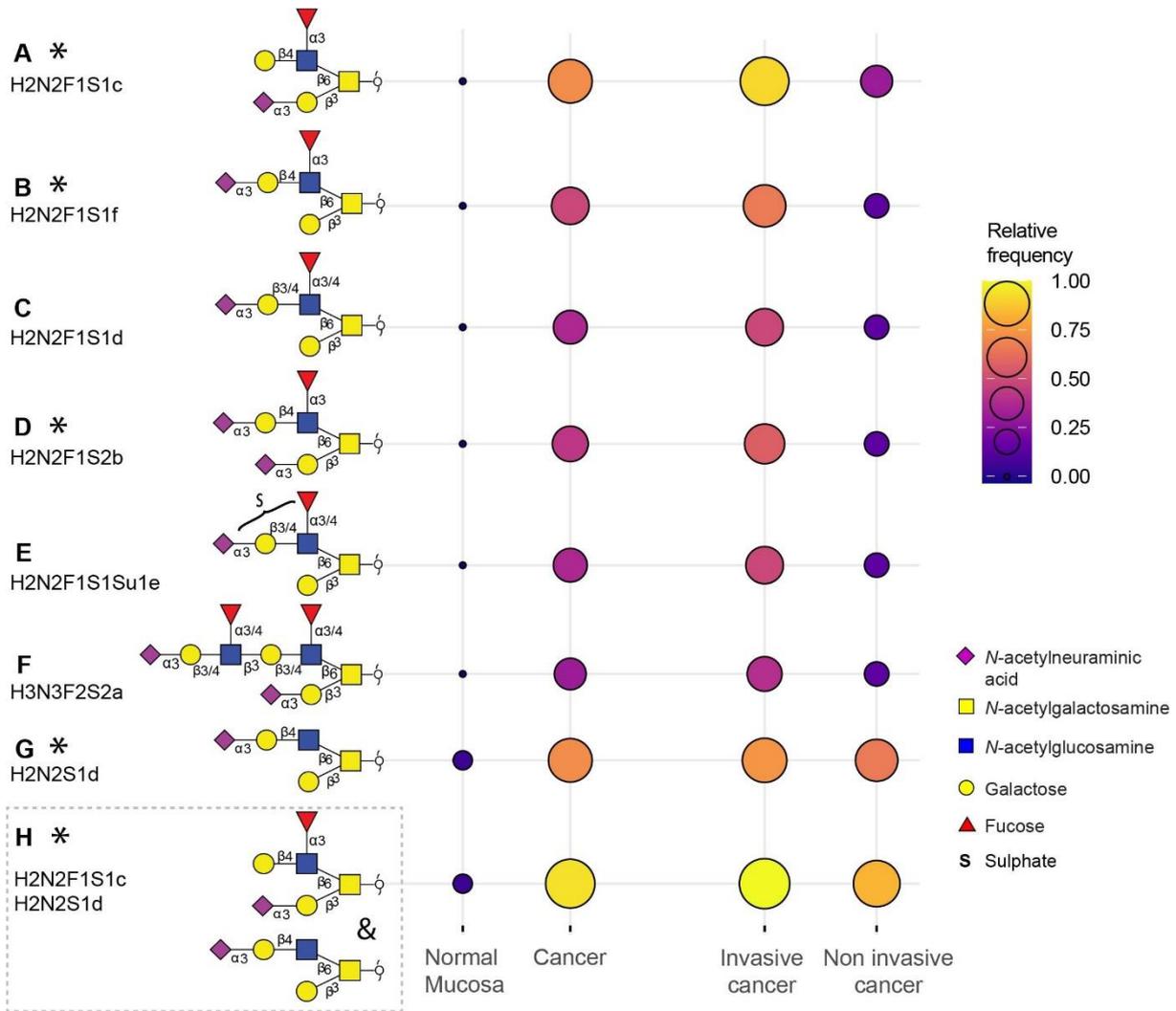
The released glycans are analyzed with porous graphitized carbon (PGC)-LC-MS/MS in negative ion mode enabling powerful separation of isomeric species as well as in-depth structural characterization of TACAs.

#### **Co-authors:**

*Oleg A. Mayboroda, Leiden University Medical Center*  
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*Julia Weber, Utrecht University*  
*Hans Morreau, Leiden University Medical Center*  
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*Guinevere S.M. Lageveen-Kammeijer, Leiden University Medical Center*  
*Manfred Wuhrer, Leiden University Medical Center*

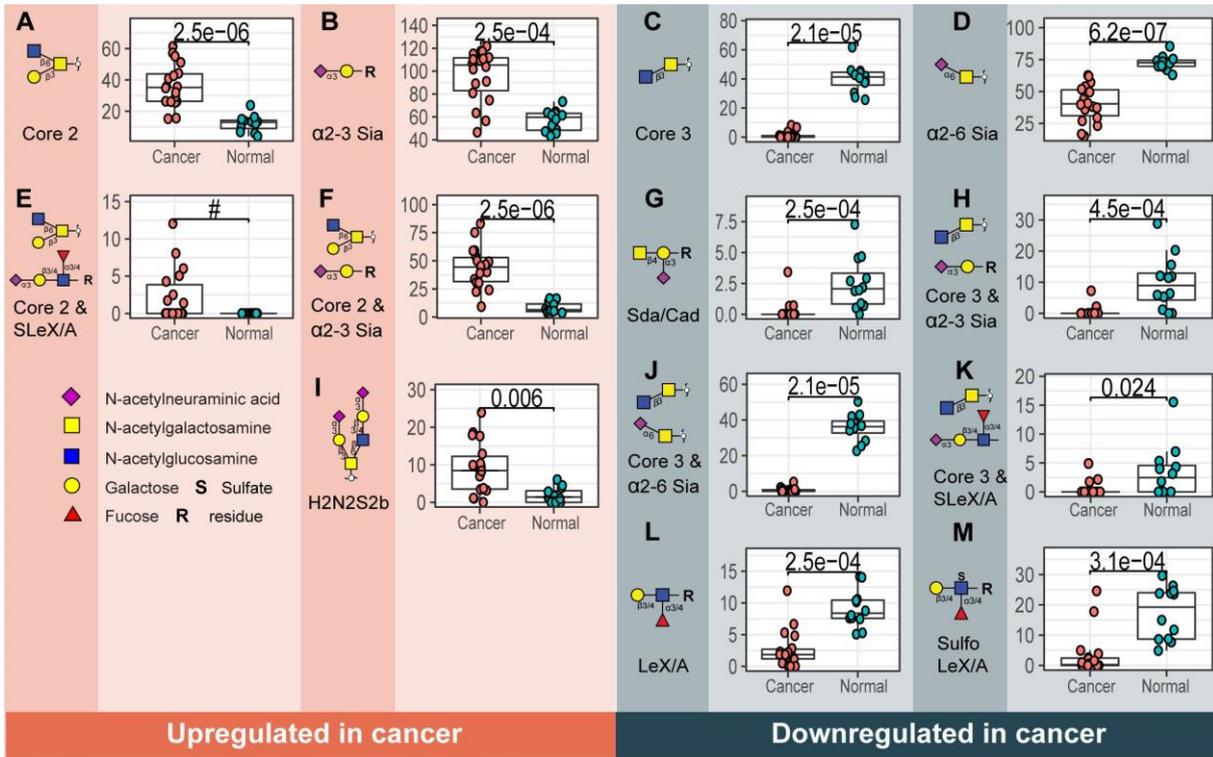
POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



Poster number: LS-PB-021

## SOFTWARE TOOLS FOR SITE-SPECIFIC N/O-GLYCOSYLATION AND GLYCOPEPTIDE ABUNDANCE DISTRIBUTION ANALYSES OF HUMAN GLYCOPROTEINS

Abstract ID: 35

**Presenting author: Concepcion Remoroza, Mass Spectrometry Data Center, Biomolecular Measurement Division, National Institute of Standards and Technology**

### Introduction

This paper presents methods for automated identification, display, and comparison of site-specific glycopeptide results using mass-spectral library-based methods for handling the difficulties involved in data analysis. The abundance distributions of glycans for a single sequence termed Glycopeptide Abundance Distribution Spectra (GADS)<sup>1</sup> and Tandem MS spectra. GADS represents distributions that vary with differences in protein expression, while actual tandem mass spectra depend on glycopeptide fragmentation energies. These tandem mass spectra can be used to confirm the accuracy of each peak in a GADS and enable direct identifications of these glycopeptides. Peaks in this library are annotated using the MS Piano<sup>2</sup> program. This present work focuses on N/O-linked glycopeptides. The generated GADS and MS<sup>2</sup> libraries will be applied and compared to datasets from other laboratories.

### Methods

The major human glycoproteins, lactoferrin, tenascin and immunoglobulins A1, A2, D, E, J, M, Polymeric Ig Receptor were denatured, reduced, alkylated and then digested by trypsin, chymotrypsin, GluC, and alpha-lytic. Human milk Standard Reference Material was also digested and enriched by HILIC-SPE. The separation was performed on a nano-UHPLC system-Orbitrap MS using HCD & ion-trap MS fragmentation methods. Data analysis tools, Byonic, Biologic, pGlyco, MS Fragger and newly developed NIST annotation MS\_Piano and Glycopeptide Abundance Distribution Spectrum (GADS) software were used. We monitor the spectra and abundances using well-characterized databases of MS<sup>2</sup> and MS<sup>1</sup> of various glycopeptides to verify the identifications.

### Preliminary data (results)

MS\_Piano is a tool that provides thorough annotation of glycopeptide spectrum for automated measures of quality and aids researchers in examining spectrum correctness. Its utility is demonstrated for the glycopeptides of various human glycoproteins. To our knowledge, this is the first study to systematically illustrate the annotation of a glycopeptide spectrum in lactoferrin digests that contain four fucose residues in sialofucosylated glycans, high-mannose occupancy at Asp 1018 of tenascin glycoprotein, and varied positions of fucose residues on the same glycosylation site in the MS<sup>2</sup> spectrum of polymeric Ig receptor glycopeptide.

The MS<sup>2</sup> spectra from IgA1 digests documented that MS\_Piano can be used for O-linked glycopeptide annotation. At Ser 111 of antibody IgA1, glycan G3H2S2 (Figure 1) was identified and annotated. The identified O-linked glycan is a mucin O-glycosylation core structure.

The established approach was used for data sets generated from the reference milk at NIST, Utrecht and Wuhan laboratories.

GADS directly displays and compares relative ion abundance values for each distinct peptide sequence carrying site-specific glycosylation (Figure 2). The investigation of lactoferrin from human milk in other labs shows the high similarity of the reference milk with healthy donors at early lactation stages (week 8-12). Also, GADS generated by HCD/CID matched well those acquired using EThcD.

We note that glycopeptide search methods, namely pGlyco and MSfragger-glyco, whose results are in good agreement with those of the present method. These strategies are extended in the analysis of viral, influenza-vaccine, and human glycoproteins as well as Covid positive plasma.

**Please explain why your abstract is innovative for mass spectrometry?**

We present novel methods for analyzing N/O-linked glycosylation of glycoproteins that involve reference library building and searching.

**Co-authors:**

Meghan Burke, National Institute of Standards and Technology  
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 Yuri Mirokhin, National Institute of Standards and Technology  
 Xiaoyu Yang, National Institute of Standards and Technology  
 Dmitrii Tchekhovskoi, National Institute of Standards and Technology  
 Stephen Stein, National Institute of Standards and Technology

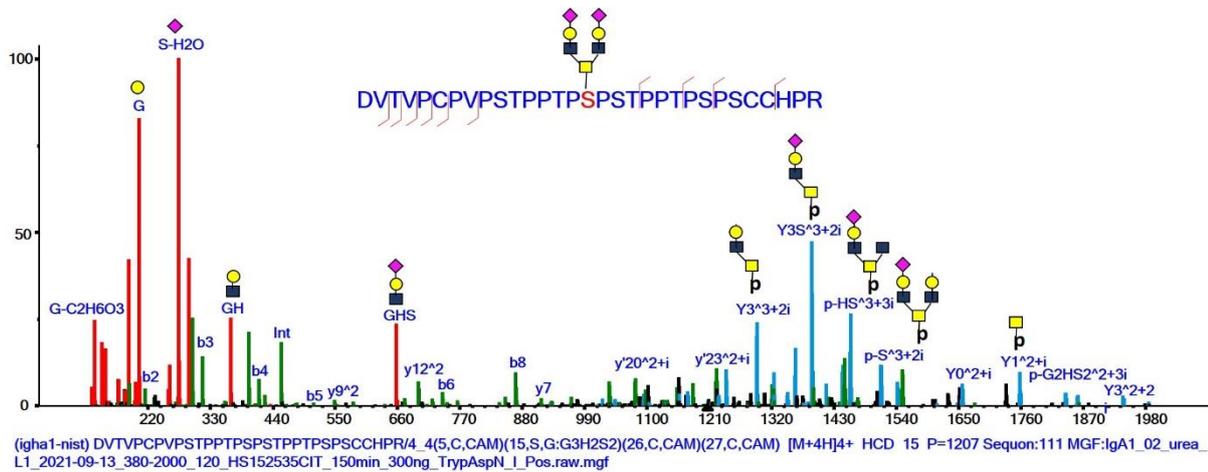


Figure-1. MS<sup>2</sup> spectrum annotation for O-linked IgA1 glycopeptide.

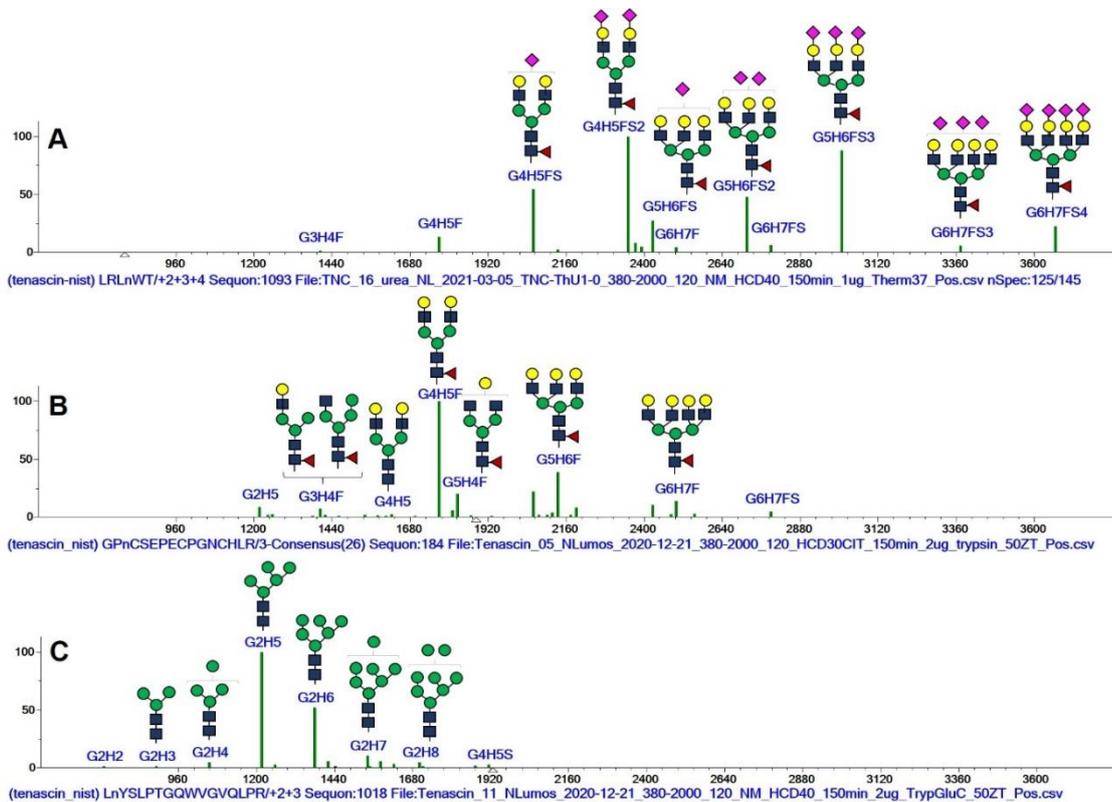


Figure 2. Graphical representation of the glycosylation of tenascin glycopeptides.

Poster number: LS-PB-022

## HIGH DIVERSITY OF GLYCOSPHINGOLIPID GLYCANS OF COLORECTAL CANCER CELL LINES REFLECTS THE CELLULAR DIFFERENTIATION PHENOTYPE

Abstract ID: 73

Presenting author: Di Wang, Leiden University Medical Center

### Introduction

Colorectal cancer (CRC) was reported to be the fourth most commonly diagnosed cancer (6%) and the second leading cause of cancer death (9%) worldwide in 2018. Currently, therapeutic solutions for individual CRC patients lack efficiency due to the heterogeneity of the disease, its asymptomatic clinical course as well as its diagnosis at a late stage. To allow early diagnosis and treatment of CRC, and to gain a better understanding of the progression of CRC, biomarker discovery is essential. Cancer-associated changes of protein glycosylation have been widely studied in CRC. However, glycosphingolipids (GSLs), which are the major carriers of cell surface carbohydrates, and their relation to CRC have rarely been investigated due to their complexity and analytical challenges.

### Methods

In this study, GSL glycans were enzymatically released from GSLs extracted from cell lysates using specific endoglycoceramidase I (EGCase I) followed by reduction and purification. An in-depth analysis of GSL glycans of 22 CRC cell lines was conducted using porous graphitized carbon nano-liquid chromatography coupled with electrospray ionization-tandem mass spectrometry (PGC-nanoLC-ESI-MS/MS) in negative ion mode. A separation between colon-like and undifferentiated cell lines driven by the expression of glycosylation features was revealed by unsupervised principal component analysis. A Spearman correlation was performed to investigate the correlation between the expression of GSL glycans and published transcriptomics data of corresponding genes.

### Preliminary data (results)

Striking differences in the expression of GSL glycans were found between the various CRC cell lines which could be related to different cell classifications. Where undifferentiated cell lines showed a higher expression of GSL glycans with blood group A, B and H antigens, the colon-like cell lines showed a higher abundance of (sialyl)-Lewis<sup>A/X</sup> and Lewis<sup>B/Y</sup> antigens. Besides, GSL glycans expression correlated with relevant glycosyltransferases (GTs) involved in their biosynthesis and transcription factors (TFs) implicated in colon differentiation. For instance, FUT3, encoding an essential GT for the biosynthesis of (sialyl)-Lewis antigens, presented a significant positive correlation with (sialyl)-Lewis<sup>A/X</sup> as well as with Lewis<sup>B/Y</sup> antigens. Additionally, ST6GAL1, encoding the GT which adds a sialic acid in  $\alpha$ 2,6-linkage, showed a trend towards a positive correlation with  $\alpha$ 2,6-sialylation. Moreover, correlations between GTs and TFs were revealed. Specifically, FUT3 correlated with CDX1, ETS2, HNF1A, HNF4A, MECOM and MYB which are upregulated in colon-like cell lines pointing to a potential role of these TFs in the expression of Lewis type glycosylation features via the regulation of FUT3 during colon differentiation. Moreover, the comparison of the GSL- and O-glycome in CRC cell lines revealed significant associations including Lewis<sup>A/X</sup>, sialyl-Lewis<sup>A/X</sup>, Lewis<sup>B/Y</sup>, blood group B, blood group H and  $\alpha$ 2,6-sialylation.

In conclusion, our work shows specific glycosylation features of CRC cell lines and reveals potential novel layers of GSL regulation relevant for future research in colon differentiation and CRC.

### Please explain why your abstract is innovative for mass spectrometry?

Negative-mode LC-MS allowed the in-depth structural elucidation and isomer differentiation of 94 unique GSL-glycans from 22 CRC cell lines.

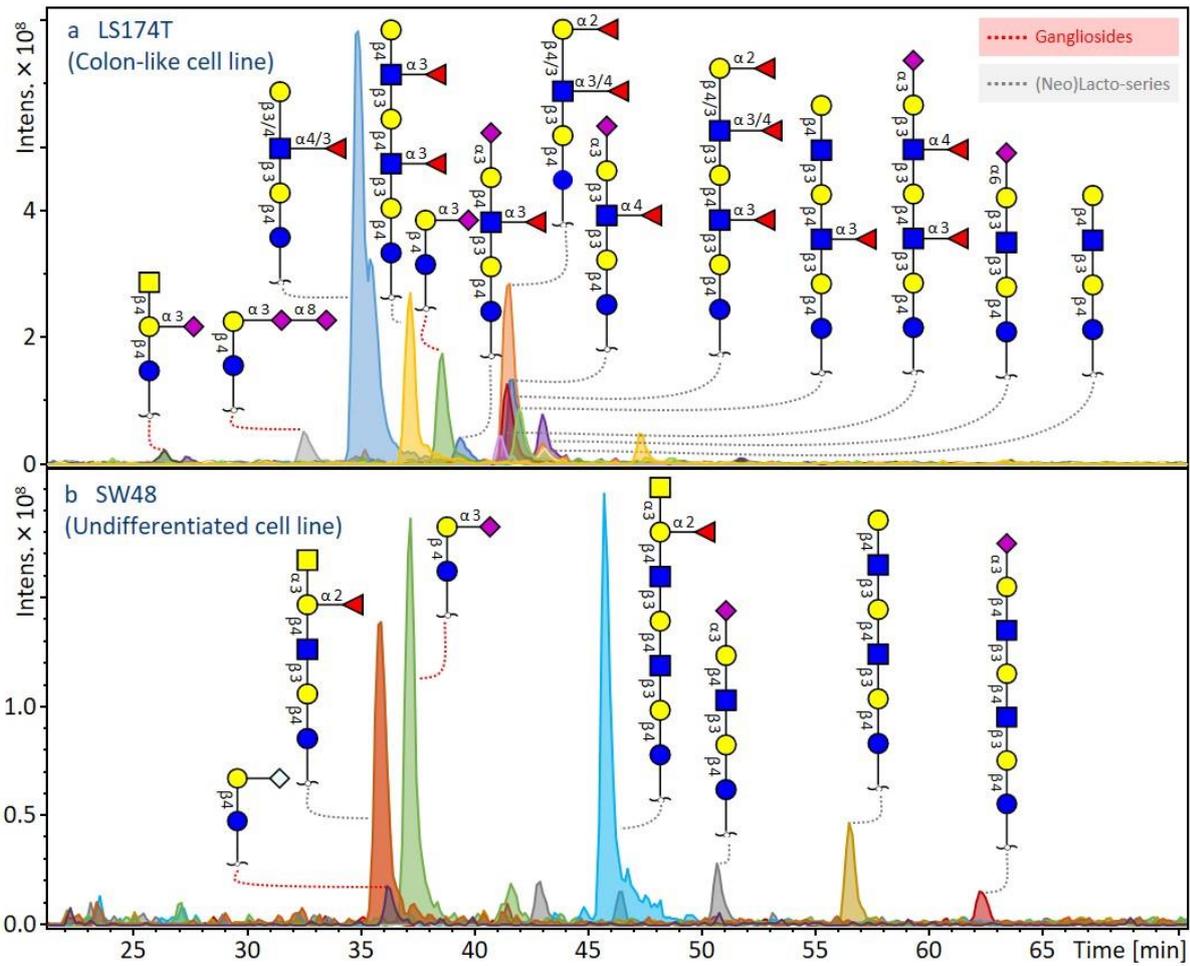
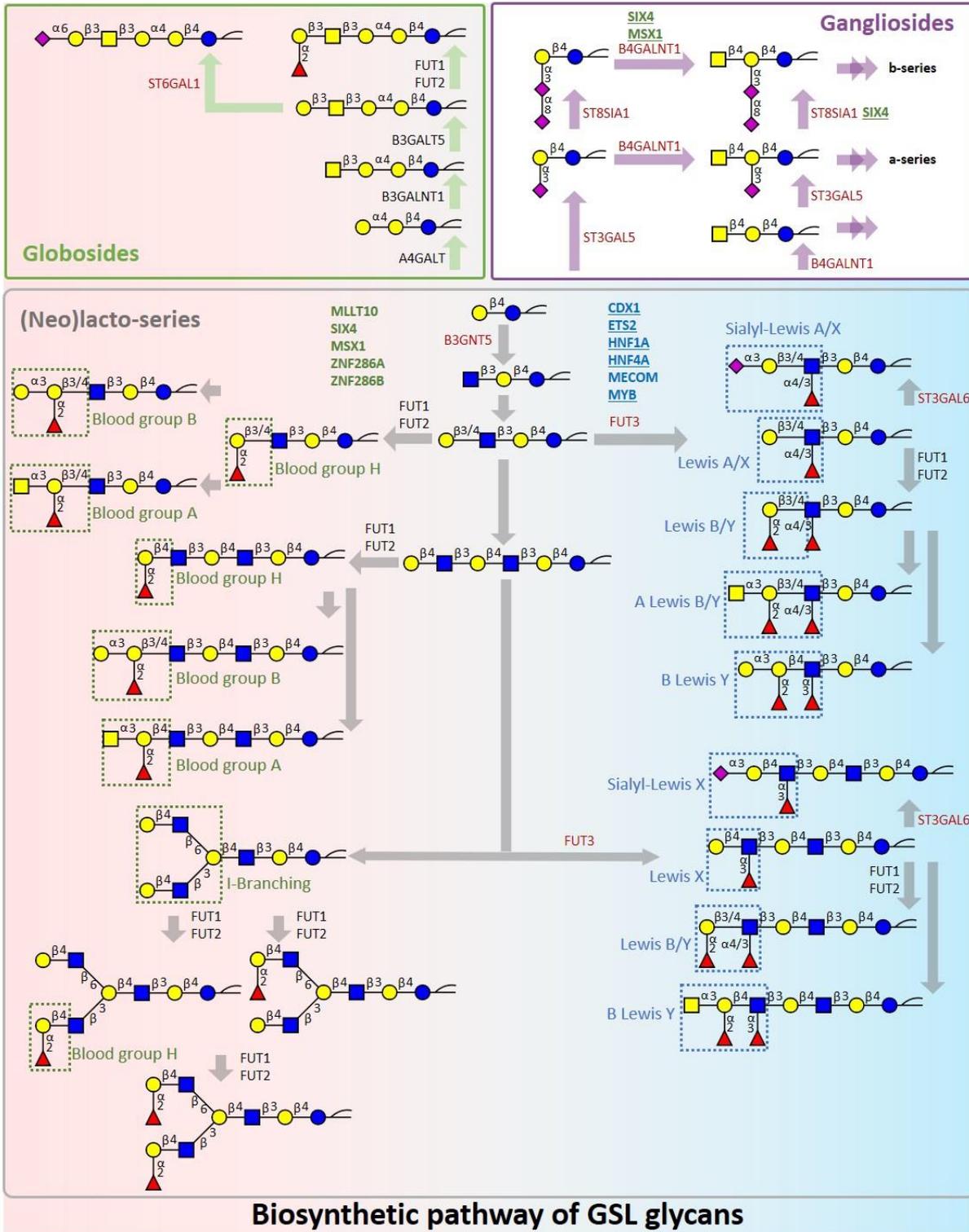


Fig. 1 GSL glycans profile of two CRC cell lines.

Undifferentiated cell lines

Colon-like cell lines



Differences in the expression of GSL glycans between CRC classification

Poster number: LS-PB-023

## STRUCTURE-ACTIVITY RELATIONSHIPS OF HEPARAN SULFATE OLIGOSACCHARIDES STUDIED IN AN IN-VITRO CELL MODEL AND BY MASS SPECTROMETRY

Abstract ID: 91

**Presenting author: Charlotte Rambaud, Université Paris Saclay, Université d'Evry Val d'Essonne, CNRS, Laboratoire analyse, Modélisation et Matériaux pour la Biologie et l'Environnement (LAMBE)**

### Introduction

Mucopolysaccharidosis (MPS) are rare genetic diseases that can have lethal effects. MPS patients lack one of the glycosaminoglycans (GAGs) catabolism enzymes, which results in the accumulation of partially catabolized GAGs which, in the case of MPS III, leads to neurodegeneration when accumulated in the brain. Consequently, GAGs have been identified as potential biomarkers of these pathologies. Our project focuses on studying the GAG heparan sulfate (HS) and its derived oligosaccharides associated with the MPS IIIB and IIIC disease to bring new insights on MPS neuropathological effects and help the clinical research for MPS therapies. HS is highly heterogeneous regarding its sulfation pattern, and thus, our principal objective is to establish structure-activity relationships between structural features of HS oligosaccharides and neurodegenerative mechanisms observed in MPS.

### Methods

A library of various HS oligosaccharides derivatives has been produced by exploiting the specificities of different depolymerization enzymes from the lyase and hydrolase classes. The structural identity of each HS oligosaccharide has been established by MALDI-TOF / MS using ionic liquid matrix and HILIC-MS coupling. Afterward, the *in-vitro* exposition of different cerebral cell types to HS oligosaccharides has been carried out to establish the relationship between structural features of the oligosaccharides and their *in-vitro* neuropathological effects such as oxidative stress or neuroinflammation.

### Preliminary data (results)

HS oligosaccharides exhibit various structural key points on their [uronic acid-glucosamine] disaccharide units, such as sulfation sites, epimerization of uronic acid, and saturation on the non-reducing end. Considering the physiological catabolism and the biological context of MPS III, we studied the inflammatory impact of sulfation, saturation, and polymerization degree of HS oligosaccharides on microglia cells. Oligosaccharides were prepared from two HS derivatives; heparin (HP) and heparosan (HN), the first being most completely sulfated, corresponding to the S-domain of HS, and the second devoid of sulfate groups, corresponding to the unsulfated domain of HS. A primary depolymerization was carried out with heparinases, lyases forming an unsaturated uronic acid as the non-reducing end, and the resulting unsaturated even oligosaccharides, from tetrasaccharides to deltasaccharides, were purified by preparative size exclusion liquid chromatography. To prepare saturated oligosaccharides, we implemented a secondary depolymerization using the combination of the two exo-enzymes  $\Delta$ 4,5-glycuronidase and 2-O-sulfatase which yielded uneven oligosaccharides with a saturated glucosamine as the non-reducing end. The sequences of the enzymatically produced oligosaccharides were established by MALDI-TOF / MS analysis using an ionic liquid matrix and ZIC-HILIC-MS coupling. The biological effects of the well structurally defined oligosaccharides were studied in a cell culture model of MPS III, in which microglial cells were exposed to the different oligosaccharides. Recording of neuroinflammation and oxidative stress provided insight into structure-activity relationships and valuable clues for cellular processes involved in neurodegeneration.

### Please explain why your abstract is innovative for mass spectrometry?

NA

### Co-authors:

Jérôme Ausseil, Institut Toulousain des maladies infectieuses et Inflammatoires (Infinity), INSERM, CNRS, Université Toulouse III

Nissrine Ballout, Institut Toulousain des maladies infectieuses et Inflammatoires (Infinity), INSERM, CNRS, Université Toulouse III

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

*Bernard Priem, Centre de Recherche des Macromolécules végétales (CERMAV), CNRS*

*Florence Gonnet, Université Paris Saclay, Université d'Evry Val d'Essonne, CNRS, Laboratoire analyse, Modélisation et Matériaux pour la Biologie et l'Environnement (LAMBE)*

*Régis Daniel, Université Paris Saclay, Université d'Evry Val d'Essonne, CNRS, Laboratoire analyse, Modélisation et Matériaux pour la Biologie et l'Environnement (LAMBE)*

*Salomé Poyer, Université Paris Saclay, Université d'Evry Val d'Essonne, CNRS, Laboratoire analyse, Modélisation et Matériaux pour la Biologie et l'Environnement (LAMBE)*

Poster number: **LS-PB-024**

## **IN-DEPTH DISSECTION OF N-, O- AND GLYCOSPHINGOLIPID GLYCOSYLATION IN PANCREATIC ADENOCARCINOMA CELLS UPON TGF- $\beta$ CHALLENGE USING PGC LC-MS/MS**

Abstract ID: **208****Presenting author: Tao Zhang, Leiden University Medical Center**

### **Introduction**

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal tumors in the world. It is characterized by a poor prognosis and a failure to respond to therapy. Glycosylation of cellular proteins and lipids is a common post-translational modification in cells, which affects many cellular processes such as cell adhesion, proliferation, migration, and invasion. Transforming growth factor- $\beta$  (TGF- $\beta$ ) plays a key role in tumor progression, which is often associated with aberrant glycosylation. How PDAC cells respond to TGF- $\beta$  and the role of glycosylation therein is, however, not well known. In part, this can be attributed to the insufficient sensitivity and analytical depth of methodologies for comprehensive analysis used in previous studies.

### **Methods**

Here, a human epithelial-like PDAC cell line PaTu-S was employed to investigate TGF- $\beta$  responses and resulting glycosylation changes. Exploiting the benefits of porous graphitized carbon chromatography coupled to tandem mass spectrometry, we qualitatively and quantitatively investigated N- and O-linked glycans relying on PVDF-assisted glycan release in a 96-well format and glycosphingolipid-glycans based on an established protocol using liquid-liquid extraction and enzymatic digestion in PaTu-S upon TGF- $\beta$  treatment. Next, by combining transcriptomic analysis of glycosylation-associated genes with mass spectrometry glycomics, we systematically assessed the TGF- $\beta$ -induced alterations in the three major classes of cell surface glycans: N-, O- and GSL-glycans.

### **Preliminary data (results)**

Upon TGF- $\beta$  treatment, PaTu-S cells were first analyzed for any effects on gene expression, morphological changes, loss of epithelial traits, and gain of mesenchymal markers. PaTu-S cells responded to TGF- $\beta$  by upregulating SMAD2 phosphorylation and target gene expression. TGF- $\beta$  induced expression of the mesenchymal marker N-cadherin, but did not significantly affect epithelial marker E-cadherin expression. The differences of N-glycans, O-glycans and glycosphingolipid glycans in PaTu-S cells with TGF- $\beta$  stimulation were examined. TGF- $\beta$  treatment primarily induced N-glycome aberrations involving elevated levels of branching, core fucosylation, and sialylation in PaTu-S cells, in line with TGF- $\beta$ -induced changes in the expression of glycosylation-associated genes. In addition, we observed differences in O- and GSL-glycosylation profiles after TGF- $\beta$  treatment, including lower levels of sialylated Tn antigen and neo-expression of globosides. Furthermore, we investigated the critical role of SOX4 in TGF- $\beta$  signaling and TGF- $\beta$ -induced glycosylation in PaTu-S cells. The expression of transcription factor SOX4 was upregulated upon TGF- $\beta$  stimulation, and its depletion blocked the TGF- $\beta$ -induced N-glycomic changes. Thus, TGF- $\beta$ -induced N-glycosylation changes can occur in a SOX4-dependent and SMAD4-independent manner in the pancreatic PaTu-S cancer cell line.

In summary, we report a broad-scale analysis of N-, O-, GSL-glycosylation changes after TGF- $\beta$  treatment in PaTu-S pancreatic adenocarcinoma cells which revealed the essential role of the transcription factor, SOX4, in TGF- $\beta$ -induced upregulation of N-glycans independent of SMAD4 expression.

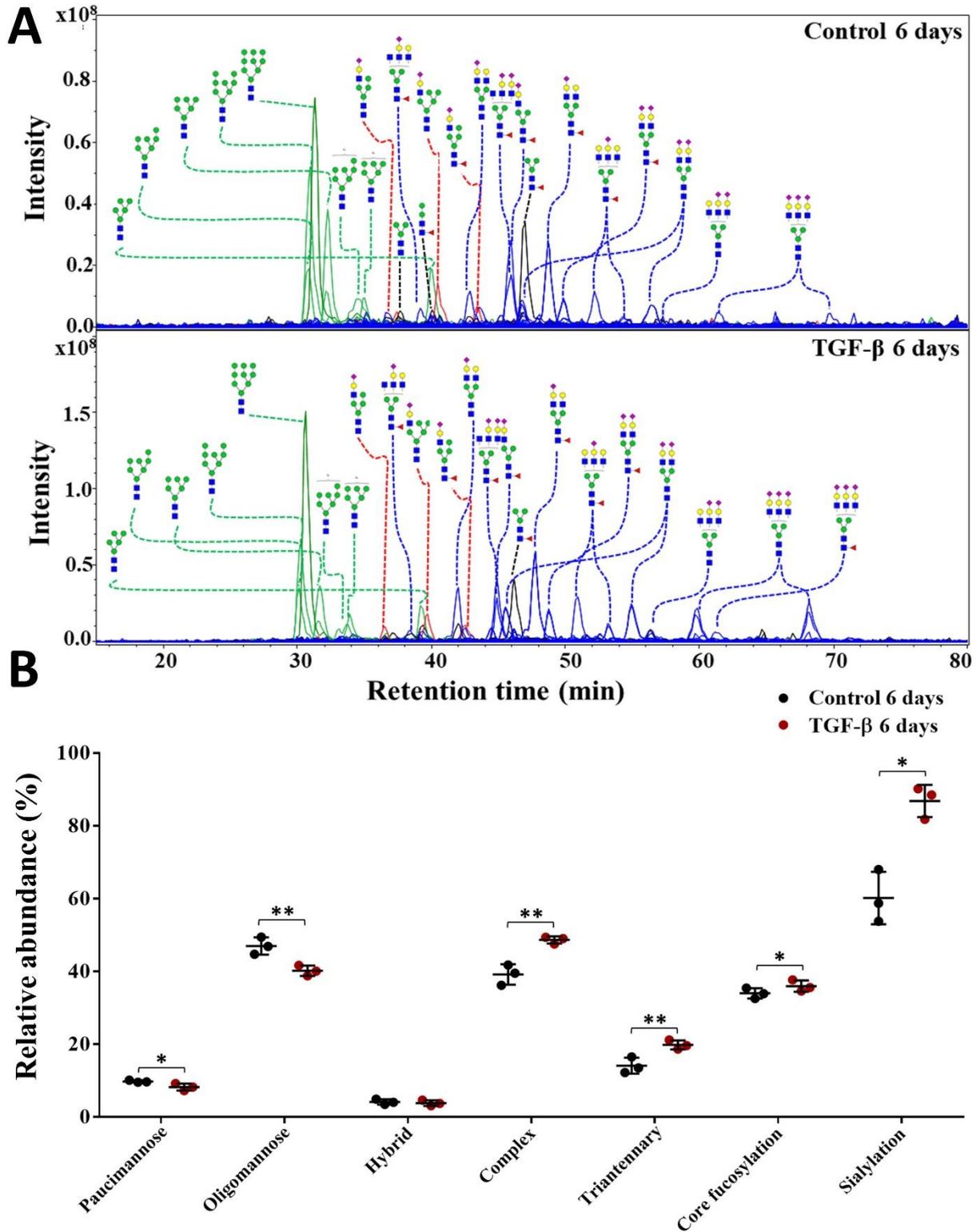
### **Please explain why your abstract is innovative for mass spectrometry?**

The combination of porous graphitized carbon chromatography and mass spectrometry in negative ion mode allowed the in-depth structural elucidation and isomer differentiation.

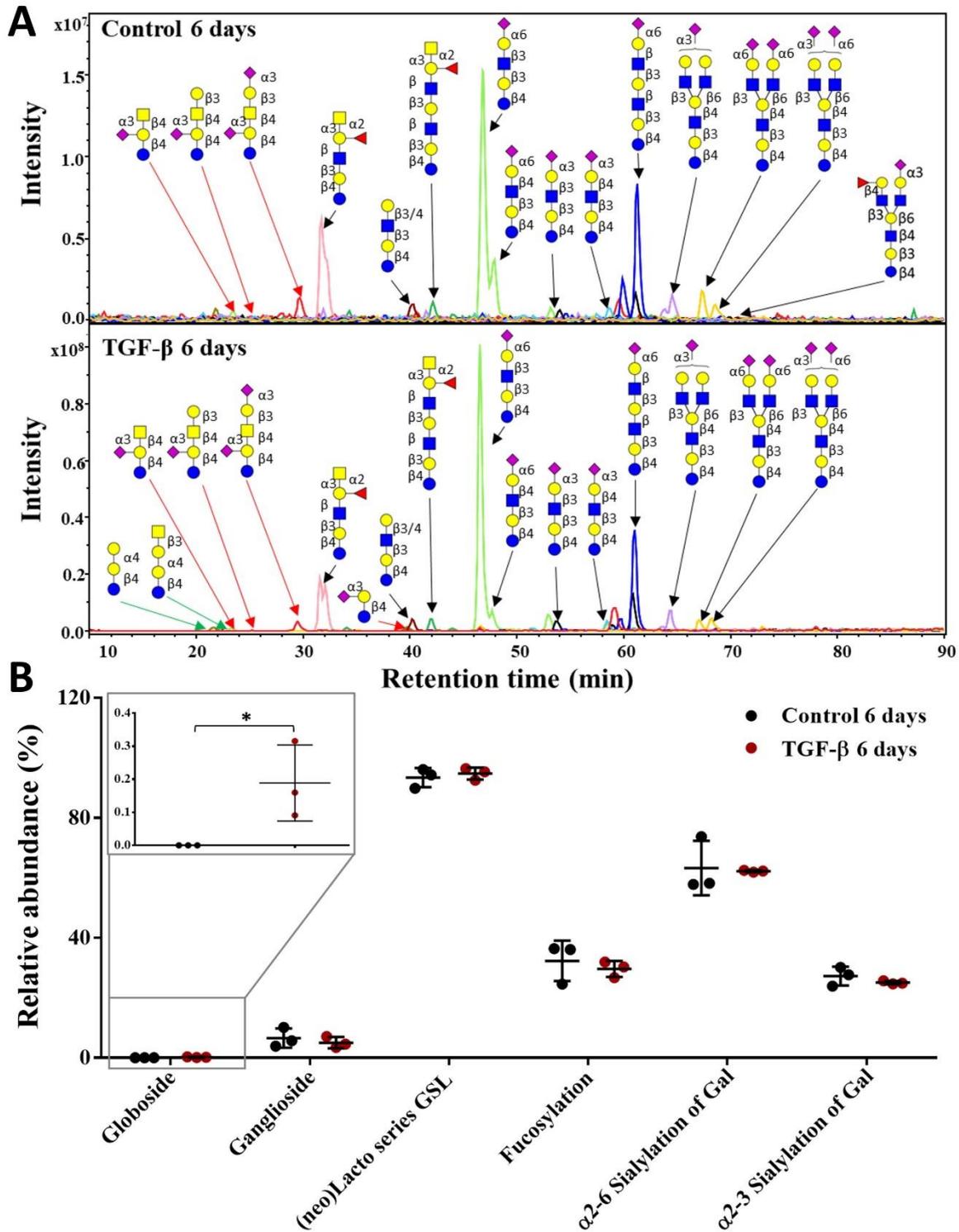
### **Co-authors:**

*Jing Zhang, Leiden University Medical Center*  
*Zejian Zhang, Leiden University Medical Center*  
*Stephanie Holst, Leiden University Medical Center*

Constantin Blöchl, Leiden University Medical Center  
 Katarina Madunic, Leiden University Medical Center  
 Peter ten Dijke, Leiden University Medical Center  
 Manfred Wuhrer, Leiden University Medical Center



Differences of *N*-glycosylation in PaTu-S cell line upon TGF- $\beta$  treatment.



Differences of glycosphingolipid-glycans in PaTu-S cell line upon TGF-β treatment.

Poster number: LS-PB-025

## O-METHYLATED N-GLYCANS DISTINGUISH MOSSES FROM VASCULAR PLANTS

Abstract ID: 243

Presenting author: David Stenitzer, University of Natural Resources and Life Sciences

### Introduction

In the animal kingdom, a stunning variety of N-glycan structures has emerged with phylogenetic specificities of various kinds. In the plant kingdom, however, N-glycosylation appears as strictly conservative and uniform. From mosses to all kinds of gymno- and angiosperms, land plants mainly express structures with the common pentasaccharide core substituted with xylose, core  $\alpha$ 1,3-fucose, maybe terminal GlcNAc residues and Lewis A determinants. In contrast, green algae biosynthesize unique and unusual N-glycan structures with uncommon monosaccharides, a plethora of different structures and various kinds of O-methylation. Mosses, a group of plants that are separated by at least 400 million years of evolution from vascular plants, were hitherto seen as harbouring an N-glycosylation machinery identical to that of vascular plants.

### Methods

To challenge this view, we have analysed the N-glycomes of several moss species using MALDI-TOF/TOF, PGC-MS/MS and GC-MS. With MALDI-TOF a general overview of the glycosylation pattern in the analysed mosses was achieved. Whereas negative mode PGC-MS/MS and GC-MS was used for structural analysis of the methylated N-glycans.

### Preliminary data (results)

While all species contained the plant-typical heptasaccharide with no, one or two terminal GlcNAc residues (MMXF, MGnXF and GnGnXF, respectively), many species exhibited MS signals with 14.02 Da increments as characteristic for O-methylation. Throughout all analysed moss N-glycans the level of methylation differed strongly even in the same family. In some species, methylated glycans dominated, while others had no methylation at all. To learn more about the structure of the methylated glycans as well as the position of the methyl groups, PGC-ESI-MS/MS and GC-MS was performed. Negative-mode PGC-ESI-MS/MS pointed towards methylation only on the upper arm of the common plant glycans (Figure 1B). GC-MS revealed the main glycan from *Funaria hygrometrica* to contain 2,6-O-methylated terminal mannose (Figure 1A). The main glycans of *Funaria hygrometrica* and *Plagiomnium undulatum* show a terminal 2,6-O-methyl mannose (Figure 2). Some mosses additionally presented very large, likewise methylated complex-type N-glycans. This first finding of methylation of N-glycans in land plants mirrors the presumable phylogenetic relation of mosses to green algae, where O-methylation of mannose and many other monosaccharides is a common trait.

### Please explain why your abstract is innovative for mass spectrometry?

Negative-mode MS/MS reveals structural data about methylated N-glycans.

### Co-authors:

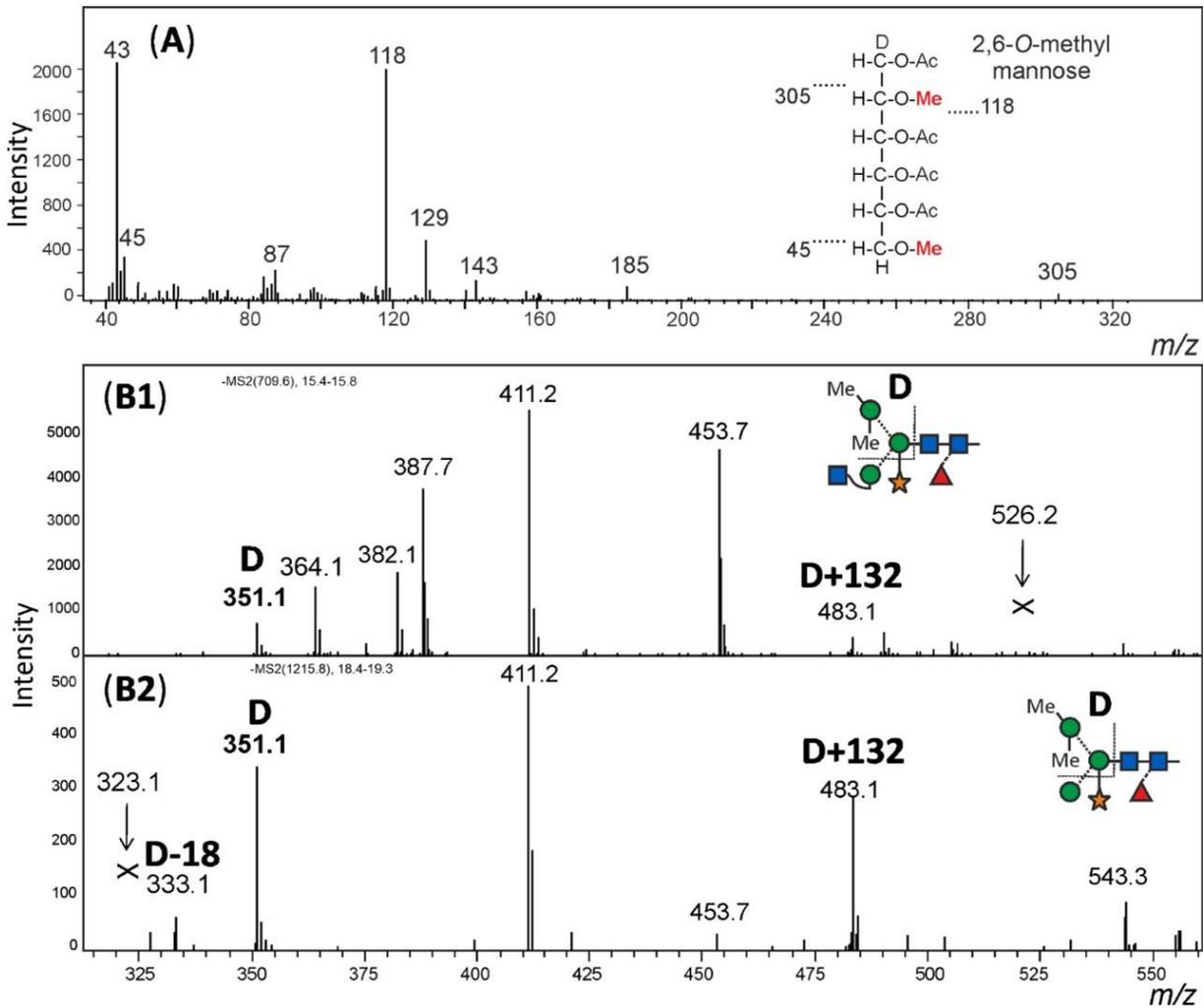
Reka Mocsai, University of Natural Resources and Life Sciences

Harald Zechmeister, University of Vienna

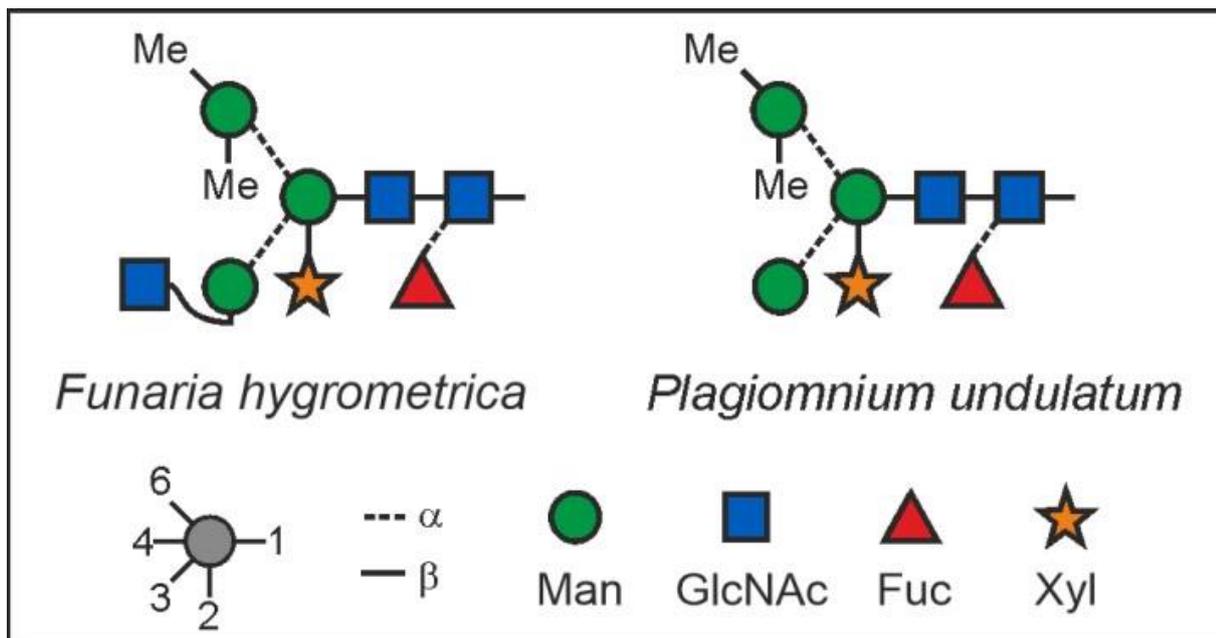
Ralf Reski, University of Freiburg

Eva L. Decker, University of Freiburg

Friedrich Altmann, University of Natural Resources and Life Sciences



PGC-ESI-MS/MS and GC-MS of glycans from the analysed mosses.



Main glycan structures of *Funaria hygrometrica* and *Plagiomnium undulatum*.

Poster number: **LS-PB-026**

## **IMMUNOGLOBULIN G1 FC GLYCOSYLATION AS AN EARLY HALLMARK OF SEVERE COVID-19**

Abstract ID: **245**

**Presenting author: Tamas Pongracz, Center for Proteomics and Metabolomics, Leiden University Medical Center**

### **Introduction**

Immunoglobulin G1 (IgG1) effector functions are impacted by the structure of fragment crystallizable (Fc) tail-linked *N*-glycans. Low fucosylation levels on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein specific (anti-S) IgG1 has been described as a hallmark of severe coronavirus disease 2019 (COVID-19) and may lead to activation of macrophages via immune complexes thereby promoting inflammatory responses, altogether suggesting involvement of IgG1 Fc glycosylation modulated immune mechanisms in COVID-19.

### **Methods**

In this prospective, observational single center cohort study, IgG1 Fc glycosylation was analyzed by nano liquid chromatography – electrospray ionization – time-of-flight mass spectrometry following affinity capturing from 1300 serial plasma samples of 159 PCR-confirmed SARS-CoV-2 infected patients.

### **Preliminary data (results)**

At baseline close to disease onset, anti-S IgG1 glycosylation was highly skewed when compared to total plasma IgG1. A rapid, general reduction in glycosylation skewing was observed during the disease course. Low anti-S IgG1 galactosylation and sialylation as well as high bisection were early hallmarks of disease severity, whilst high galactosylation and sialylation and low bisection were found in patients with low disease severity. In line with these observations, anti-S IgG1 glycosylation correlated with various inflammatory markers.

Association of low galactosylation, sialylation as well as high bisection with disease severity suggests that Fc-glycan modulated interactions contribute to disease mechanism. Further studies are needed to understand how anti-S IgG1 glycosylation may contribute to disease mechanism and to evaluate its biomarker potential.

The above described methodological approach – besides functional and cellular assays – is currently used to assess glycosylation dynamics of naïve and antigen-experienced vaccinees.

To gain insight into systemic changes of glycosylation in patients of the SARS-CoV-2 infection cohort, we analyzed their total plasma *N*-glycosylation with matrix-assisted laser desorption ionization – Fourier transform ion cyclotron resonance – mass spectrometry following linkage-specific sialic acid derivatization, of which data awaits investigation.

### **Please explain why your abstract is innovative for mass spectrometry?**

Ultra-high-throughput clinical glycoproteomics has been achieved by a conventional electrospray ionization – time-of-flight mass spectrometry approach.

### **Co-authors:**

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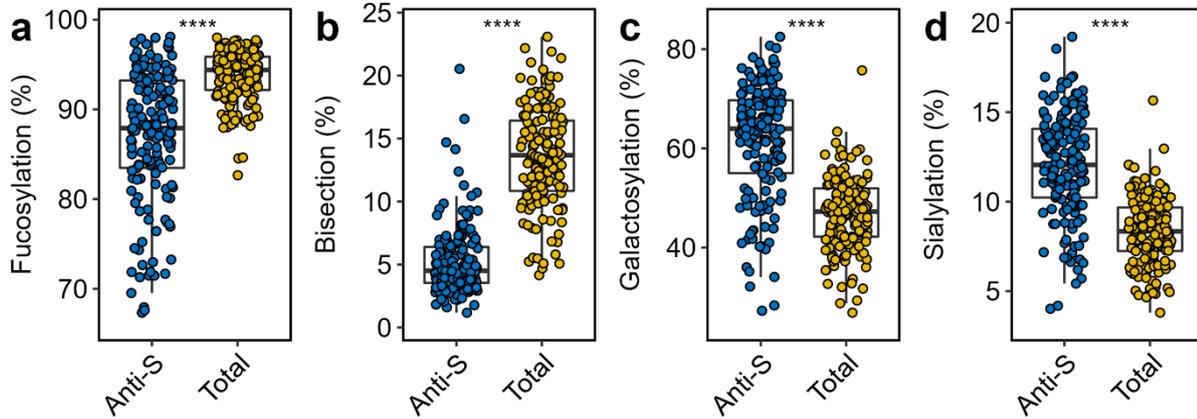
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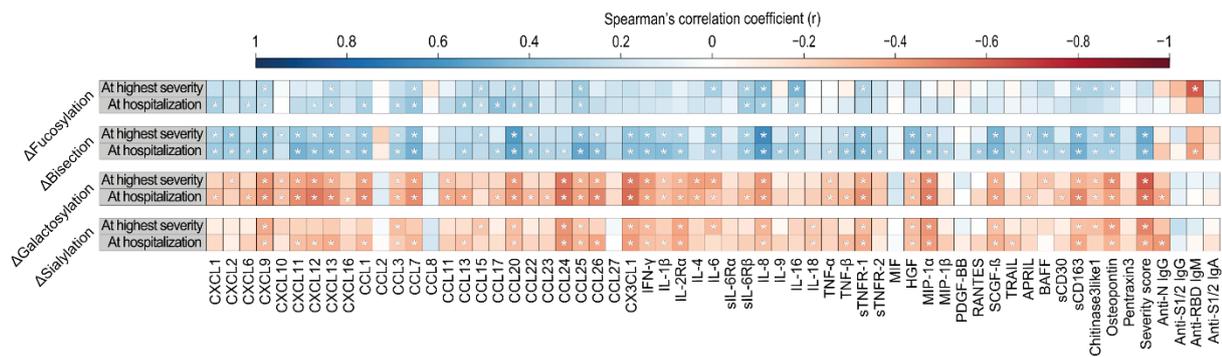
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Comparison of  $\Delta$ glycosylation traits of patients admitted to ICU/non-ICU.



Heatmap visualizing Spearman's correlations between  $\Delta$ glycosylation traits and inflammatory markers.

Poster number: LS-PB-027

## GLYCOSAMINOGLYCAN ALTERATIONS IN PROSTATE CANCER AND BENIGN PROSTATE HYPERPLASIA FFPE TISSUE SAMPLES

Abstract ID: 278

**Presenting author:** Lilla Turiák, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network

### Introduction

Mass spectrometry-based analysis of tissue specimen offers crucial information and insight on biological processes taking place at the origin of a disease. Identifying structural alterations in glycosaminoglycan (GAG) chains among cancerous and non-cancerous tissues can be an attractive, however, challenging approach in biomarker research. Our aim was to analyze and compare the chondroitin sulfate (CS) and heparan sulfate (HS) content and sulfation pattern among different pathological grades of prostate cancer (PCa, n=52) and benign prostate hyperplasia (BPH, n=16) samples.

### Methods

PCa tissues were removed by either radical prostatectomy or transurethral resection and were categorized into low (n=20), intermediate (n=16) and high (n=16) risk groups according to Gleason grading and these groups were balanced for age and overall survival. CS and HS chains were degraded into disaccharide constituents following on surface bacterial lyase digestion and purified using a graphite-based pipet tip SPE system. Recently published salt gradient nanoHPLC-MS/(MS) methods were used for separating and quantifying HS and CS disaccharides. Statistical analysis was carried out using R in RStudio.

### Preliminary data (results)

We found that overall quantity of CS and HS was 1.8, and 1.4-times larger in the PCa samples than in BPH, respectively. Significant changes were identified in the sulfation motifs of both CS and HS chains. Regarding CS, a decreasing trend was observed during cancer progression for the non-sulfated and 4-*O*-sulfated disaccharide relative intensities, while the 6-*O*-sulfated disaccharide showed a steep and significant increase. Thus, a significant increase in the 6S/4S ratio was observed: it differed significantly in all pairwise comparisons except between low and intermediate risk, the median values increasing by 2.4-fold between BPH and the high risk PCa group. Regarding HS, smaller differences were observed. The relative intensities of the non-sulfated and monosulfated disaccharides differed significantly between BPH and the low risk PCa group, a comparison being the most important clinically. A slight decrease in the rate of sulfation and an increase in *N*-sulfation compared to *O*-sulfation was observed.

Finally, our Kaplan-Meier survival analyses revealed that high total quantity of HS, high 6S/4S ratio of CS, and the low relative abundance of doubly and triply sulfated HS disaccharides were significantly associated with shorter disease-specific survival.

Our preliminary findings provide a good basis for future potential classification of prostate malfunctions based on changes in their GAG chains.

### Acknowledgments

Projects no. PD 121187 and FK 131603 have been implemented with the support provided from the NRD Fund of Hungary, financed under the NRD PD<sub>16</sub> and FK<sub>19</sub> funding schemes.

### Please explain why your abstract is innovative for mass spectrometry?

Sensitive nano-LC-MS(MS) method and workflow for glycosaminoglycan structural characterization of limited FFPE tissue samples

### Co-authors:

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



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*Domonkos Pál, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics*  
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*László Drahos, MS Proteomics Research Group, Research Centre for Natural Sciences, Eötvös Loránd Research Network*  
*Ilona Kovalszky, 1st Department of Pathology and Experimental Cancer Research, Semmelweis University*

Poster number: **LS-PB-028**

## STRUCTURAL ANALYSIS OF IMMUNOGLOBULIN G EPI TOPE BY MS-IR

Abstract ID: **286****Presenting author: Alicia Infantino, Institut Lumière Matière, Université Lyon 1, CNRS**

### Introduction

N-glycans present in glycoprotein are involved in many biological processes including autoimmune diseases like rheumatoid arthritis (RA). It has been highlighted that the extremities of these N-glycans called epitope play a key role in these diseases. Unfortunately, the structure of the epitope is currently not well understood. Indeed, classical approaches give us much information about the global structure of carbohydrates but only few about the epitope because it may carry a number of functional modifications, which introduces a whole new level of heterogeneity and analytical complexity.

### Methods

We propose an original approach based on the coupling of mass spectrometry and infrared ion spectroscopy<sup>1</sup> to study epitope and their regiochemistry. This strategy is composed of three steps: N-glycans isolation thanks to an enzymatic deglycosylation, a MS-MS labelling of interest fragment and finally a spectroscopic identification with the MS-IR coupling.

(1) Schindler, B.; Barnes, L.; Renois, G.; Gray, C.; Chambert, S.; Fort, S.; Flitsch, S.; Loison, C.; Allouche, A.-R.; Compagnon, I. Anomeric Memory of the Glycosidic Bond upon Fragmentation and Its Consequences for Carbohydrate Sequencing. *Nat Commun* **2017**, *8* (1), 973. <https://doi.org/10.1038/s41467-017-01179-y>.

### Preliminary data (results)

Considering the importance of sialylated extremities in RA, we currently focused our research on this epitope present at the surface of immunoglobulin G (IgG). Sialylated extremities can be present in IgG in two forms: 3'-sialyl-N-acetyllactosamine isomer or 6'-a-sialyl-N-acetyllactosamine isomer. Our strategy allows to determine the ratio between the two isomers but also reduce limit of detection thanks to a concentration effect. Therefore, our approach could be a helpful tool to do an early diagnostic of RA if glycosylation differences are found between a healthy person and a patient with RA.

### Please explain why your abstract is innovative for mass spectrometry?

The innovation of this work is the use of the mass spectrometry and infrared ion spectroscopy to study a not well understood part of the N-glycans : the epitope.

### Co-authors:

Oznur YENI, Institut Lumière Matière, Université Lyon 1, CNRS  
Baptiste MOGE, Institut Lumière Matière, Université Lyon 1, CNRS  
Isabelle COMPAGNON, Institut Lumière Matière, Université Lyon 1, CNRS

Poster number: LS-PB-029

## COMPREHENSIVE SITE-SPECIFIC N-GLYCAN ANALYSIS OF ACE2 AND SARS-COV-2 PROTEINS

Abstract ID: 317

**Presenting author: Rudolf Figl, Core Facility Mass Spectrometry, University of Natural Resources and Life Sciences (BOKU)**

### Introduction

The viral entry of SARS-CoV-2 is dependent on the binding of its Spike protein to angiotensin converting enzyme 2 (ACE2) of the host cell. Both proteins are heavily glycosylated and the type and site occupancy of the glycosylation is crucial for the interaction. The comprehensive evaluation of *N*-glycan profiles of several biotechnological produced proteins (spike, nucleocapsid, RBD, ACE2) is presented in this study to shed light on the differences of the individual platforms.

### Methods

Recombinant expressed and purified proteins were carbamidomethylated and digested with various proteases to obtain site specific (glyco-)peptides, which were analysed using a QTOF MS system (Bruker maXis 4G) in the positive DDA mode.

Glycopeptides were identified as sets of peaks consisting of the peptide moiety and the attached *N*-glycan varying in the number of HexNAc units, hexose, deoxyhexose and pentose residues. Verification of putative glycopeptides was done by MS/MS.

### Preliminary data (results)

#### Analysis of ACE2:

ACE2 produced in CHO cells and plants was analysed. The eight individual glycosylation sites were identified site specific as glycopeptides performing a combined chymotryptic/tryptic digest. Both expression systems showed the expected glycoforms and additionally differed in the site occupancy of some glycosites, which affected the binding properties of the spike protein to ACE2.

#### Analysis of Spike:

The 22 *N*-glycan sites of Spike remain highly conserved among all SARS-CoV-2 variants identified, showing the importance of the glycosylation. In our study we were able to identify all 22 sites in three different production systems, allowing an interesting comparison of the biotechnological platforms used. To achieve a complete glycosite coverage, five different proteases were used (trypsin, pepsin, Chyotrypsin, GluC, LysC).

### Please explain why your abstract is innovative for mass spectrometry?

No innovations

### Co-authors:

*Clemens Grünwald-Gruber, Core Facility Mass Spectrometry, University of Natural Resources and Life Sciences (BOKU)*  
*Daniel Maresch, Core Facility Mass Spectrometry, University of Natural Resources and Life Sciences (BOKU)*  
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*Department of Chemistry, University of Natural Resources and Life Sciences (BOKU)*

Poster number: LS-PB-030

## INTEGRATED N- AND O-GLYCOMICS OF ACUTE MYELOID LEUKEMIA CELL LINES.

Abstract ID: 355

**Presenting author: Constantin Blöchl, Center for Proteomics and Metabolomics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands, Department of Biosciences, University of Salzburg, Hellbrunner Strasse 34, 5020 Salzburg, Austria**

### Introduction

Increasingly recognized as a hallmark of cancer formation and progression, aberrant protein glycosylation is profoundly shaping the activity of oncogenic pathways and the interactions of cancer cells with their microenvironment. In addition to several types of cancer where this interplay is already established, severe implications of altered glycosylation signatures have recently been identified in the pathogenesis of acute myeloid leukemia (AML), the most common form of acute leukemia. Although first mechanistic studies could identify malignant glycan epitopes and accordingly correlations of glycosyltransferase (GT) expression and the outcome of disease were found, a global glycomics study of AML cells was hitherto not conducted.

### Methods

To contribute to the current knowledge in this field, we performed an exploratory analysis of N- and O-glycans of 21 widely used AML cell lines appertaining to a wide range of subtypes. Relying on the benefits of porous graphitized carbon chromatography coupled to tandem mass spectrometry (PGC nanoLC-MS2), we performed a structural characterization of released glycans followed by their relative quantification (Figure 1). In addition, publicly available transcriptomics data was introduced to bioinformatically explore associations between the observed protein glycosylation and the expression of specific GTs and transcription factors.

### Preliminary data (results)

Overall, the studied AML cell lines exhibited distinct glycan signatures differing in relevant glycan traits including highly relevant epitopes such as the sialyl-Lewis A/X antigen. This motif has been recognized as a driver of chemoresistance in AML and the inhibition of its functions is currently investigated in clinical trials. Furthermore, principal component analysis of integrated AML glycosylation fingerprints visualized the clustering of AML subtypes as classified by the French-American-British (FAB) system. This is intriguing as the FAB classification is predominantly based on the phenotypic appearance of AML blasts and is linked to their cellular differentiation. In order to identify upstream regulators of the observed glycosylation phenotypes, we implemented transcriptomics data of selected GTs and hematopoietic transcription factors, e.g., *TAL1*, *GATA1/2*, and *CEBPA*. To this end, correlation analyses identified several candidate axes of transcription factors and downstream GTs that may govern the observed glycan phenotype of AML cells. Taken together, we characterized and quantified a high number of N- and O-glycan structures throughout a broad set of AML cell lines and subtypes. We report varying expression of derived traits and glycan epitopes including such that are associated with poor prognosis, present evidence for underlying glycosyltransferases and transcription factors and therefore provide insights into the regulation of AML protein glycosylation as a stepping stone for further research.

### Please explain why your abstract is innovative for mass spectrometry?

Dopant-enriched nitrogen gas supported electrospray ionization followed by tandem mass spectrometry in negative ion mode facilitated the exact structural characterization of AML cell glycans enabling clear identification of glycan epitopes.

### Co-authors:

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*Katarina Madunić, Center for Proteomics and Metabolomics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands*

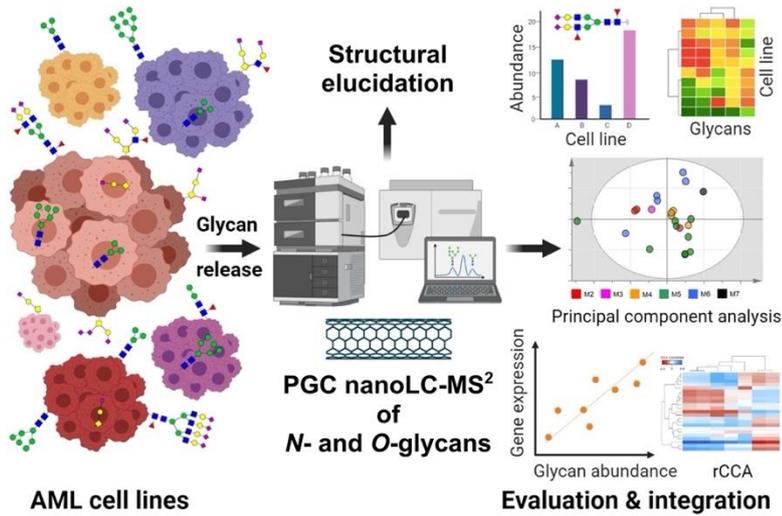
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Tao Zhang, Center for Proteomics and Metabolomics, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands



Overview of the conducted study. Created with biorender.com.

Poster number: **LS-PB-031**

## **GLYCOPROTEOMIC ANALYSIS OF ISOBARIC LABELED PLASMA PSA BY RP-LC-MS**

Abstract ID: **385****Presenting author: Wei Wang, LUMC**

### **Introduction**

Altered glycosylation of prostate-specific antigen (PSA) in serum has been correlated to prostate cancer (PCa). Its sialylation and fucosylation are important features to distinguish PCa patients from non-PCa individuals. However, most of the studies were done by means of lectin affinity binding which is limited in providing in-depth information. To understand the biology behind the altered glycosylation, a workflow is needed that allows to analyze the PSA glycosylation in an in-depth and high sensitive manner. The primary objective of this study was to establish such platform that can identify and characterize PSA glycopeptides derived from the circulation (PSA > 3 ng/mL).

### **Methods**

PSA was captured from 8 mL of plasma using a VHH antiPSA antibody followed by tryptic digestion. To distinguish differently linked sialylated isomers, sialic acids were derivatized in a linkage specific manner. As the tryptic peptide backbone consist of only two amino acids, PSA glycopeptides do not retain on a C18 column. Therefore, tandem mass tag (TMT) labeling was introduced to increase the hydrophobicity of the glycopeptides. In addition, reverse phase liquid chromatography hyphenated with mass spectrometry (RPLC-MS) was used for the analysis.

### **Preliminary data (results)**

PSA was successfully isolated from plasma by immunoaffinity capture. The capture procedure was optimized, resulting in a capturing efficiency of approximately 57%. Tryptic PSA glycopeptides were modified on the sialic acids with a derivatization efficiency of 94%. The peptide backbone was labeled with TMT, showing a labeling efficiency of 84%. Due to the TMT label, PSA glycopeptides can be analyzed with RP-LC-MS. Moreover, LC parameters were optimized to allow optimal separation of PSA glycopeptides, including isomeric separation. Preliminary results revealed that a concentration of 3 ng/mL PSA (4 mL plasma) resulted in the identification of 10 PSA glycopeptides including the distinction of differently linked sialylated isomers and diverse glycosylation features (mono-/di-sialylation, (non-)fucosylation, with/without LacdiNAc). Further improvement is envisioned to enable high-throughput analysis by multiplexing several samples in a single analysis. Finally, we intend to investigate the diagnostic and prognostic potential of PSA glycosylation derived from plasma for the early diagnosis of PCa as well as for the differentiation between aggressive and indolent PCa.

### **Please explain why your abstract is innovative for mass spectrometry?**

The established highly sensitive PSA glycoproteomics assay enables in-depth analysis of PSA derived from plasma (>3 ng/mL).

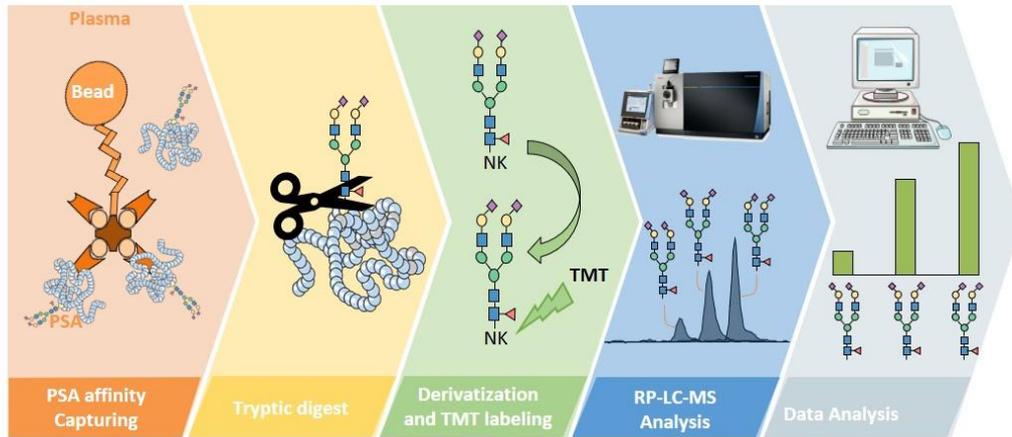


Figure 1. Workflow. MS picture source: <https://www.thermofisher.com/order/catalog/product/IQLAAEGAAPFADBMBHQ>.

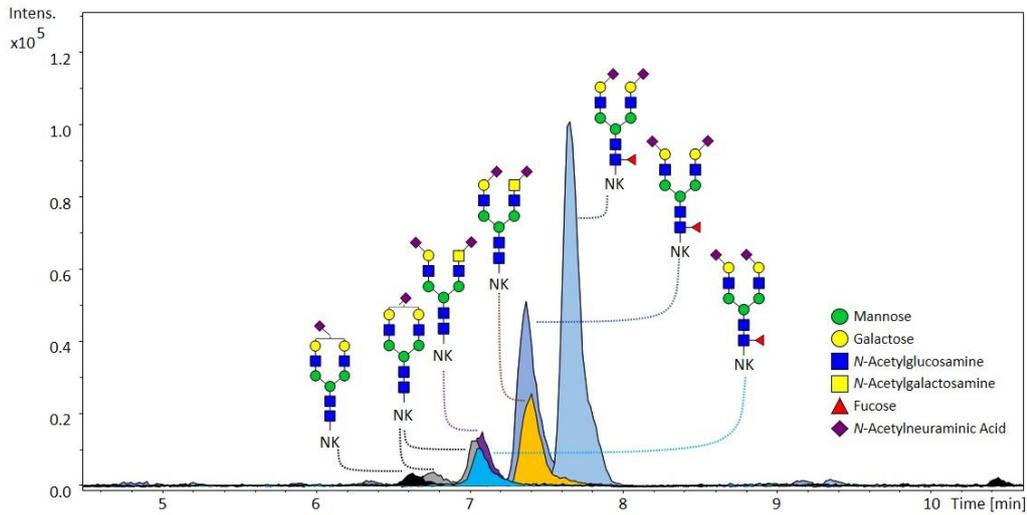


Figure 2. PSA glycopeptides presenting isomers and diverse features.

Poster number: **LS-PB-032**

## **IGG N-GLYCANS WORKFLOW IN TYPE 2 DIABETES COHORTS WITH ULTRAHIGH RESOLUTION MS AND UPLC.**

Abstract ID: **412**

**Presenting author: Elham Memarian, Center for Proteomics and Metabolomics, Leiden University Medical Center, the Netherlands, Genos Glycoscience Research Laboratory, Zagreb, Croatia**

### **Introduction**

Type 2 diabetes (T2D) is an increasing worldwide health care problem and a leading cause of many complications like cardiovascular disease, kidney failure, blindness, and amputations. All immunoglobulin G (IgG) molecules carry N-Glycans, and their changes have been linked to clinical risk factors for T2D, such as age, BMI, smoking, and dyslipidemia. Our group and others recently reported on associations between total plasma N-glycome and T2D and showed significant changes. However, total N-glycomic changes do not inform us on the exact underlying proteins and processes involved and results are influenced by the relative abundance of proteins in the circulation. Here, we applied our new automated ultrahigh resolution MS method and UPLC method to assess associations of IgG, one of the most common type of antibody, with T2D complications.

### **Methods**

We have developed an automated 96-well plate method in a large subsample of DCS cohort (n=1519 T2D cases with complication) using our ultrahigh-resolution Fourier-transform ion cyclotron resonance mass spectrometry (MALDI-FTICR-MS). Also, IgG N-glycosylation was measured in two other independent T2D cohorts, IgG N-glycosylation was measured by UPLC (DiaGene n=1815, GenodiabMar n=640). Blood plasma samples were randomized over some 96-well plates, together with technical replicates. IgG was isolated from plasma using 96-well filter plates including protein G beads, eluted with 0.1 M formic acid and dried by speedvac. From that, N-glycans were released with the addition of PNGase F.

### **Preliminary data (results)**

In DCS cohort, for stabilization and linkage differentiation, sialic acids were derivatized. Then DCS released glycans were purified and spotted on MALDI target plate using an automated liquid handling platform. Also released glycans from DiaGene and GenoDiabMar was injected in UPLC. To investigate the association of IgG N-glycosylation with T2D complications, we meta-analysed data from these three large independent samples.

IgG galactosylation was negatively associated with prevalent and incident nephropathy. A negative association of galactosylation and sialylation were also demonstrated for prevalent macrovascular complications. On the other hand, the recently developed method on DCS using MALDI-FTICR-MS improved resolution, mass accuracy, dynamic range and the signal-to-noise ratio, especially for larger glycans. Moreover, in comparison to another workflows, stabilization and neutralization of sialic acids had enabled us to discriminate between  $\alpha$ 2,3- and  $\alpha$ 2,6-linked sialic acids.

It was shown that there is an association between IgG N-glycosylation traits and T2D complications. Moreover, our new method for DCS improved the detection of several glycan species, thus providing possible new insights into the association between IgG and T2D complications.

### **Please explain why your abstract is innovative for mass spectrometry?**

- Discriminate between  $\alpha$ 2,3- and  $\alpha$ 2,6-linked sialic acids.
- Automated ultrahigh resolution MS

### **Co-authors:**

*Ralph Heijmans, Department of Internal Medicine, Erasmus MC - University Medical Center, Rotterdam, the Netherlands*

*Roderick C. Sliker, Department of Cell and Chemical Biology, Leiden University Medical Center, the Netherlands,*

## POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

*Department of Epidemiology and Data Science, Amsterdam University Medical Center, location VUMC, Amsterdam Public Health institute, Amsterdam, The Netherlands*

*Adriana Sierra , Department of Nephrology, Hospital del Mar, Institut Mar d'Investigacions Mediques, Barcelona, Spain*

*Olga Gornik , Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia*

*Joline WJ Beulens , Department of Epidemiology and Data Science, Amsterdam University Medical Center, location VUMC, Amsterdam Public Health institute, Amsterdam, The Netherlands, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands*

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*Clara Barrios , Department of Nephrology, Hospital del Mar, Institut Mar d'Investigacions Mediques, Barcelona, Spain*

*Leen M 't Hart , Department of Cell and Chemical Biology, Leiden University Medical Center, the Netherlands,*

*Department of Epidemiology and Data Science, Amsterdam University Medical Center, location VUMC, Amsterdam Public Health institute, Amsterdam, The Netherlands, Department of Biomedical Data Sciences, Section Molecular Epidemiology, Leiden University Medical Center, the Netherlands*

*Manfred Wuhrer , Center for Proteomics and Metabolomics, Leiden University Medical Center, the Netherlands*

*Mandy van Hoek , Department of Internal Medicine, Erasmus MC - University Medical Center, Rotterdam, the Netherlands*

Poster number: **LS-PB-033**

## HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF HUMAN BRAIN HEMANGIOMA GANGLIOSIDOME

Abstract ID: **419**

**Presenting author: Ica Raluca, National Institute for Research and Development in Electrochemistry and Condensed Matter, Timisoara, Romania, Faculty of Physics, West University of Timisoara, Romania**

### Introduction

Hemangiomas are the most common vascular tumors, in general benign, characterized by dilation of blood vessels. These tumors occur frequently in childhood, sometimes being harmless and asymptomatic. However, symptomatic lesions can cause bleeding, ulceration, or disturbance of the functions of the affected organs. The exact cause of the occurrence is not known yet, but the way these tumors develop is currently under investigation. Since the research in the field of glycomics has demonstrated that sialylated glycosphingolipids, known as gangliosides, represent valuable biomarkers of the central nervous system diseases, here, we have investigated human brain hemangioma gangliosides using an approach based on high-resolution mass spectrometry (HR-MS) on an Orbitrap mass spectrometer with nanoESI tuned for operating in the negative ion mode.

### Methods

The native ganglioside mixture was extracted and purified from cavernous hemangioma specimen. The tumor, located in the right hemisphere of the frontal cortex in an adult patient, was removed by surgery. For MS analysis the sample were dissolved in methanol up to final concentration of 5 pmol/ $\mu$ L and subjected to nanoESI Orbitrap MS and MS<sup>2</sup> in the negative ion mode for screening and sequencing. For HCD MS/MS the precursor ions were selected within an isolation width of 1 m/z unit. The product ion spectra were combined over scans acquired at variable collision energy within 35-65 eV range.

### Preliminary data (results)

The optimized HR-MS platform, allowed the discrimination of 62 ions, corresponding to 52 different ganglioside species, which represents roughly twice the number of species existing in the current inventory of gangliosides associated with cavernous hemangioma in adult human brain. The screening mass spectra revealed a ganglioside pattern dominated by GD-type of structures as well as an elevated incidence of species characterized by a low degree of sialylation and short glycan chains, including asialo GA1 (d18:1/18:0), which offer a new perspective upon the ganglioside composition in this benign tumor. Many of the structures are characteristic for this type of tumor only and are to be considered in further investigations for their potential use in early brain hemangioma diagnosis based on molecular markers. Remarkably, HR-MS also revealed for the first time that gangliosides expressed in hemangioma present a variety of fatty acid compositions, with some species characterized by long fatty acids chains, having from 22 to 27 carbon atoms, while others by shorter chains, from C13 to C18 fatty acids. The detailed fragmentation analysis performed by HCD tandem MS provided information on the structural elements related to the glycan core and ceramide moiety, which confirmed the molecular configuration of GD3 (d18:1/24:1) and GD3 (d18:1/24:2) species with potential biomarker role.

### Please explain why your abstract is innovative for mass spectrometry?

HR MS and HCD MS/MS, introduced here for the determination of human hemangioma gangliosidome, allowed the discovery of novel species and the structural characterization of potential biomarkers.

### Co-authors:

*Sarbu Mirela, National Institute for Research and Development in Electrochemistry and Condensed Matter, Timisoara, Romania*

*Munteanu Cristian, Institute of Biochemistry of the Romanian Academy, Bucharest, Romania*

*Zamfir D. Alina, "Aurel Vlaicu" University of Arad, Arad, Romania;*

Poster number: LS-PB-034

## COMBINING SIZE-EXCLUSION CHROMATOGRAPHY AND ION MOBILITY MASS SPECTROMETRY FOR THE IDENTIFICATION OF CHONDROITIN/DERMATAN SULFATE OCTAMERS IN DECORIN

Abstract ID: 459

**Presenting author:** Alina D. Zamfir, Department of Condensed Matter, National Institute for Research and Development in Electrochemistry and Condensed Matter, Department of Technical and Natural Sciences, “Aurel Vlaicu” University of Arad

### Introduction

Chondroitin (CS) and dermatan sulfates (DS) represent linear polysaccharides organized macroscopically by segregation into alternating blocks of specific sulfation patterns and microscopically by formation of sequences with specific binding functions. The structural determination of CS/DS domains is a crucial step in the evaluation of their biological role. Due to the high sensitivity and versatility, in recent years, nanoESI MS became a valuable technique in CS/DS investigation. Since in ESI MS the difficulties in discriminating isobaric structures still persist, in this study we have introduced a methodology combining offline size exclusion chromatography (SEC) with ion mobility separation (IMS) MS for the structural analysis of hybrid CS/DS. The potential of SEC and IMS MS is demonstrated here on CS/DS octamers from human decorin.

### Methods

The hybrid CS/DS chain, released by  $\beta$ -elimination from DCN prepared from conditioned media of cultured human skin fibroblasts, was depolymerized using 5 mU chondroitin B lyase. SEC was performed on a Superdex Peptide HR10/30 column. The collected octasaccharides, purified and dissolved in methanol were infused by (-)nanoESI into a Synapt G2S and subjected to IMS MS and CID MS<sup>2</sup>. The signal was acquired at 0.8-0.9 kV ESI and 30-40 V cone voltage. IMS wave velocity was set at 650 m/s and IMS wave height at 40 V. CID was performed using collision energies in the 30-50 eV range.

### Preliminary data (results)

The released CS/DS chain was depolymerized with chondroitin B lyase, which cleaves the 3GalNAc $\beta$ 1-4IdoA $\alpha$ 1 glycosidic bond irrespective of the sulfation code *i.e.* the number of sulfate groups and their sites. Chondroitin B lyase mechanism involves the neutralization of the negative charge of the carboxylic acid group followed by abstraction of the C-5 proton and elimination of the C4 hydroxyl group by introducing a C4-C5 double bond. Following SEC, the GlcA-rich octasaccharide fraction was collected and subjected to (-)nanoESI IMS MS and MS<sup>2</sup>. The IMS MS 2D data revealed the separation into mobility families based on sulfation. Hence, by SEC/IMS MS, the mixture of variable length chains, resulted following the digestion, was separated according to: i) the carbohydrate chain length by SEC and ii) the sulfation by IMS. The IMS MS separation and screening evidenced one saturated and seven unsaturated domains differing in the number of the sulfates decorating the chains. Of these, one species was found non-sulfated, three undersulfated, and one regularly sulfated bearing a single sulfate group per disaccharide repeat. Among the oversulfated domains, highly interesting are the newly discovered hexasulfated-[4,5- $\Delta$ -IdoAGalNAc(GlcAGalNAc)<sub>3</sub>] and the saturated hexasulfated-[HexAGalNAc(GlcAGalNAc)<sub>3</sub>]. The latter species documents that the original CS/DS chain is characterized by an oversulfated non-reducing end. The sequencing data on the hexasulfated-[4,5- $\Delta$ -IdoAGalNAc(GlcAGalNAc)<sub>3</sub>], collected by IMS CID MS<sup>2</sup>, disclosed one mobility feature and ions diagnostic for the sulfation code of this structural motif.

### Please explain why your abstract is innovative for mass spectrometry?

The hybrid DCN-derived CS/DS octasaccharide fraction was investigated by the offline combination of SEC and IMS MS<sup>2</sup>, which allowed the discrimination of novel structures with unusual sulfation pattern.

### Co-authors:

Raluca Ica, Department of Condensed Matter, National Institute for Research and Development in Electrochemistry and Condensed Matter, Faculty of Physics, West University of Timisoara  
Edie Sharon, Department of Chemistry, The College of Arts & Science, Indiana University  
David E. Clemmer, Department of Chemistry, The College of Arts & Science, Indiana University  
Mirela Sarbu, Department of Condensed Matter, National Institute for Research and Development in Electrochemistry and Condensed Matter

Poster number: LS-PB-035

## O-GLYCAN PROFILING USING HPAE-PAD HYPHENATED WITH A HIGH-RESOLUTION ACCURATE MASS (HRAM) MASS SPECTROMETER

Abstract ID: 462

Presenting author: Wai-Chi Man, ThermoFisher Scientifics

### Introduction

O-glycosylation is often quite challenging to study, not only because of the lack of a universal enzyme for O-glycan release from proteins, but also because of the absence of a common glycan core. Furthermore, the heterogeneity of glycans, both in composition and in linkage, complicates their analysis and structural elucidation. Good O-linked glycan profiling requires high-resolution separation and reliable identification of released glycans. Therefore, high resolution chromatographic separations are important for subsequent MS characterization. High-performance anion-exchange chromatography coupled with pulsed amperometric detection and mass spectrometric detection (HPAE-PAD/MS) is a well-established and powerful technique for glycan analysis. Here, we demonstrate a workflow combining chemical release of O-glycans using reductive  $\beta$ -elimination under reducing conditions, followed by sample cleanup.

### Methods

We applied HPAE-PAD/MS to analyze O-glycan structures released from four different glycoproteins. The glycoproteins used were porcine gastric mucin type III, bovine fetuin, bovine fibrinogen, and bovine thyroglobulin. The column effluent containing separated glycans passed through desalter to remove sodium ions prior to MS. Q Exactive™ MS was employed in negative mode ESI. Full mass scan: m/z 400-2000, resolution: 120,000 (FWHM) at m/z 200, AGC:  $3 \times 10^6$ , maximum IT: 100 ms. Data dependent MS2 using Top 5. The possible glycan structures were identified by SimGlycan™ software (PREMIER Biosoft, Palo Alto, CA) high-throughput search and score function.

### Preliminary data (results)

The mass accuracy of all the detected structures was less than 5 ppm, ensuring high confidence peak annotation. The structures were confirmed by annotating diagnostic fragmentation patterns observed in MS2 spectra. The approach described here identified O-glycan structures that were in good agreement with structures previously reported for the four glycoproteins.

### Please explain why your abstract is innovative for mass spectrometry?

For thyroglobulin, this is the first instance of direct experimental demonstration of the presence of an O-glycan structure using HPAE-PAD/MS with high resolution accurate mass.

### Co-authors:

Sachin Patel, ThermoFisher Scientifics  
Manali Aggrawal, ThermoFisher Scientifics  
Jeff Rohrer, ThermoFisher Scientifics

Poster number: LS-PB-036

## PROBING THE STRUCTURE OF GLYCOSYL CATIONS USING CRYOGENIC INFRARED SPECTROSCOPY

Abstract ID: 489

Presenting author: Chun-Wei Chang, Institut für Chemie und Biochemie, Freie Universität Berlin, 14195 Berlin

### Introduction

The controlled formation of glycosidic bonds is crucial in virtually all branches of glycoscience. However, controlling the stereoselectivity in this reaction remains a great challenge due to its complexity and unclear mechanism. A better understanding of reaction mechanism requires a detailed understanding of the underlying glycosyl cation intermediate. However, due to its instability and extremely short lifetime detailed structural knowledge on glycosyl cations is sparse. Herein, we applied an integrated strategy of cold-ion infrared spectroscopy and computational calculations to unravel the structure of glycosyl cations. High-resolution gas-phase IR spectra are highly diagnostic and in combination with theory can provide structural model with atomic resolution. This structural information greatly improves the knowledge of reaction mechanism and helps to develop novel synthetic routes for carbohydrate synthesis.

### Methods

The utilized setup is based on the encapsulation of glycosyl cations ultra-cold helium nanodroplets and subsequent irradiation with tunable IR laser. Glycosyl cations are produced by nESI and in-source release of a leaving group at the anomeric center. A quadrupole mass analyzer is used to select the cation, which is subsequently transferred into an ion trap where it is cooled and encapsulated in superfluid helium nanodroplet. In this cryogenic environment, the conformational freedom of the sugar ring is highly restricted. After laser irradiation, highly-resolved vibrational spectra are obtained. Comparison between experimental spectra and density functional theory provides highly-resolved structural models.

### Preliminary data (results)

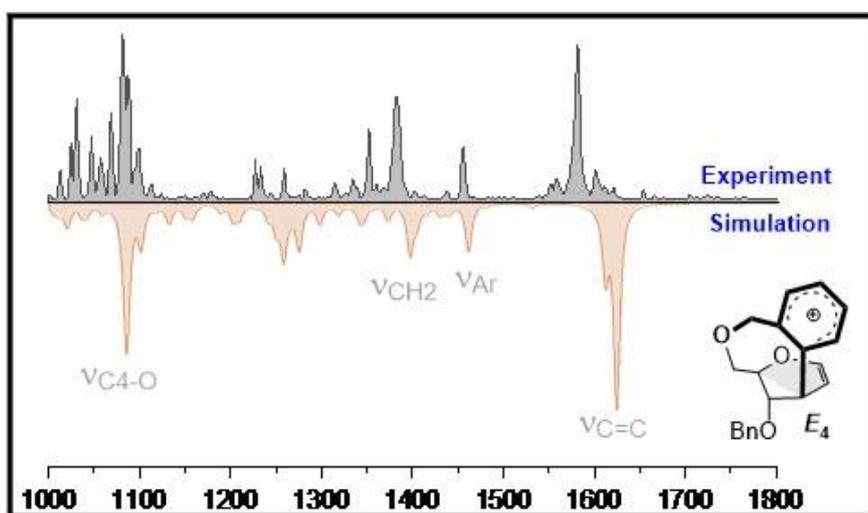
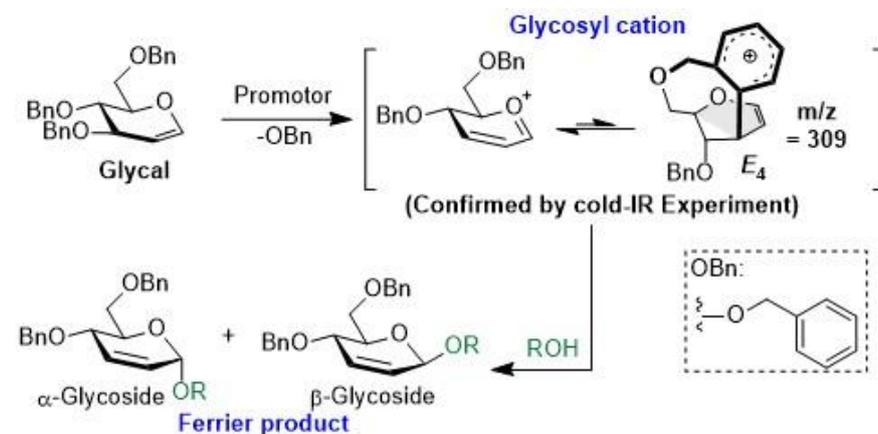
The Ferrier glycosylation reaction is a chemical transformation from a glycol to a 2,3-unsaturated glycoside (Ferrier glycoside). The activation of the glycan involves the cleavage of a C3-benzyl (OBn) group to form a Ferrier cation intermediate (Figure 1), which directly determines the anomeric stereoselectivity of the reaction. These short-lived intermediates were successfully produced in gas phase by nESI and subsequent in-source activation. The obtained cryo-IR spectra exhibited well-resolved absorption bands between 900-1800  $\text{cm}^{-1}$ . Interestingly, although the phenyl (Ph) in benzoyl groups has been recognized non-participating by synthetic chemists for decades, our experimental and theoretical data clearly provide evidence for an intramolecular participation of the phenyl group and the C3 position of sugar ring (Figure 1). This interesting result shows that the aromatic ring system is quite electron rich and thus makes Friedel-Crafts type reactions highly possible. During the transformation, the formation of the phenyl-bridged intermediate destroys the conjugated  $\pi$  system, however, the aromatic ring can be reconstructed through nucleophilic addition of an acceptor. The phenyl participation further significantly twists the sugar conformation into an  $E_4$  envelope which further shapes the stereoselective outcome of the reaction. The here-obtained advances in mechanistic understanding provide several new concepts for stereo-directing participation and thus assist the reaction optimization in oligosaccharide synthesis.

### Please explain why your abstract is innovative for mass spectrometry?

Ultra-Cold Helium nanoDroplet Mass Spectrometry (He-droplet-MS), coupled with Infrared (IR) Spectroscopy, was introduced to unravel the structure of glycosyl cation intermediates to unravel the mechanism of glycosylation reactions.

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



Experimental cold-IR spectrum of glycosyl cations with calculated spectra.

Poster number: **LS-PB-037**

## **MALDI GLYCAN IMAGING REVEALS SPECIFIC N-GLYCAN MODIFICATIONS IN CHOLANGIOCARCINOMA COMPARED TO HEPATOCELLULAR CARCINOMA IN SERUM AND TISSUE**

Abstract ID: **562**

**Presenting author: Shaaron Ochoa Rios, Medical University of South Carolina**

### **Introduction**

Cholangiocarcinoma (CCA) is a cancer of the bile duct cells, accounts for 15% of all primary liver cancers worldwide and about 8,000 people in the United States are diagnosed with CCA each year. CCA incidence is increasing due to the lack of effective serum biomarkers for early detection and treatment strategies. Tumor masses are often not identifiable by imaging modalities because of the location of the tumor in the hilum of the liver or within the bile ducts. Alterations in N-glycosylation with different types of cancer have demonstrated an immense potential to be used as diagnostic and therapeutic strategies. Here we identify N-linked glycan modifications in CCA tissue and serum that could be used exploited as strategies for CCA diagnosis.

### **Methods**

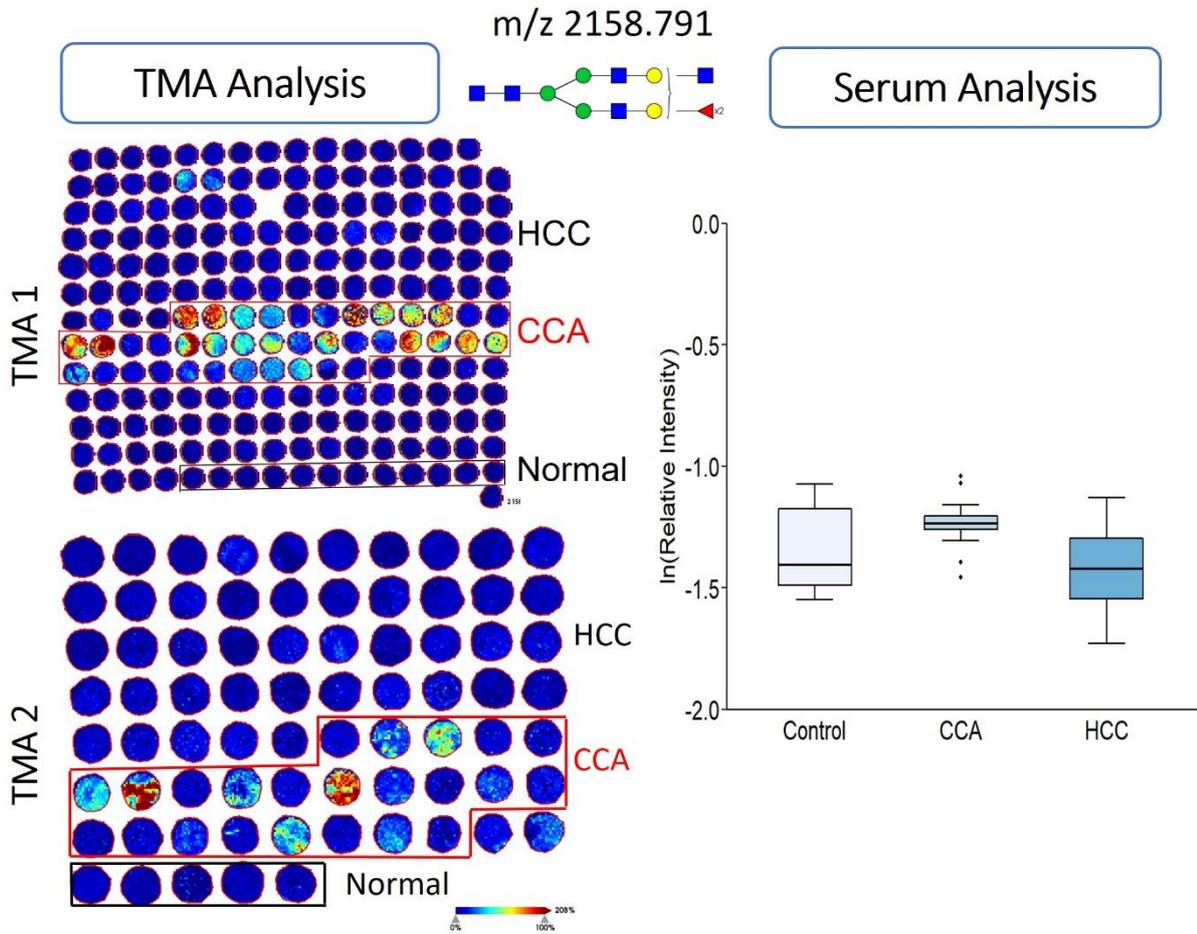
Our laboratory has developed methods that allow for in situ tissue and serum or plasma-based N-linked glycan analysis using matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS). We used this methodology to quantitatively and qualitative study the N-linked glycan modifications in tissue and serum. Two Tissue Microarrays (TMA) and a cohort of serum samples were used in this study. CCA samples from tissue and serum were compared to the N-linked glycan alterations with either samples with liver disease (not cancer) or samples from patients with hepatocellular carcinoma (HCC).

### **Preliminary data (results)**

We characterized N-glycan modifications in tissue and serum from patients with CCA, HCC, and other type of liver damage. Bisecting and fucosylated glycans were the main N-glycan modifications found significantly elevated in CCA tissues as compared to normal tissue ( $p < 0.0001$ ) or HCC ( $p < 0.0001$ ). Specifically, these glycans fell into two major classes, those with increased levels of fucosylation and those with increased levels of bisecting with or without any fucose modifications. To validate the N-glycan alterations observed in the TMA tissue, a serum cohort including HCC and control serum was analyzed. As in tissue, bisecting fucosylated glycans were significantly altered in serum from patients with CCA. When compared to serum from patients with HCC, this N-glycan modification was able to distinguish between the two types of cancers.

### **Please explain why your abstract is innovative for mass spectrometry?**

We identify specific N-glycan modifications to be significantly altered in tissue and serum of CCA patients that could be exploited as a biomarker for this deadly cancer.



N-glycan modification in CCA tissue (left) and serum (right)

Poster number: LS-PB-038

## GLYCOPROTEOMIC CHARACTERIZATION OF SACCHAROMYCES CEREVISIAE YEAST CELL WALL MANNOPROTEINS GROWN IN FED-BATCH CULTURE

Abstract ID: 649

**Presenting author: Marie Yammine, Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille., Lesaffre International, 137 rue Gabriel Péri**

### Introduction

*Saccharomyces cerevisiae* yeast is a unicellular eukaryotic fungus used in a wide range of applications. The yeast cell is protected by a rigid external shell called the yeast cell wall (YCW). The YCW has a cross-linked multi-molecular structure arranged in a double-layered aspect. While the internal layer is rich in polysaccharides, mainly  $\beta$ -glucans with a minority of chitin, the external layer consists of mannoproteins that can be bound either covalently or non-covalently to the internal layer. Being the YCW second most abundant component, the mannoproteins are densely mannosylated by both simple O-glycans and complex N-glycans. Their structural based-functional properties favors their growing implementation in nutrition and health, but have been little investigated. This work aims to characterize YCW mannoproteins using mass spectrometry (MS) glycoproteomics workflows.

### Methods

YCW isolation was performed by mechanical lysis of S288c yeast cells grown in YPD medium (30 °C, pH 5) for 48 h and purified by ultracentrifugation. YCW isolates O-deglycosylation was carried out chemically using 0.1 M ammonia or enzymatically with mannosidase (20 U), while N-deglycosylation was realized by PNGase F/Endo H (20 U) through eFASP method adaptation. The resulting tryptic peptides were analyzed by nanoESI-LC-MS/MS. Proteins identification and label-free quantification were obtained using Proteome Discoverer™ 2.2 queried against *Saccharomyces* Genome Database (S288C) dataset. Proteins subcellular localization was determined by Gene ontology. Deglycosylations' efficiency was checked using Byonic™.

### Preliminary data (results)

Bottom-up proteomics showed that the preceding combination of various enzymatic and chemical strategies for O- and N-deglycosylation have different impacts on mannoproteins identification and quantification compared to the control case. When deglycosylations were applied, non-YCW identified proteins were diminished compared to the control case. This might be due to the adsorption of these contaminants to mannoproteins' glycans. We showed a significant increase in the number of identified cell wall proteins (CWPs) when enzymatic O-deglycosylation with Jack bean  $\alpha$ -mannosidase was performed, in combination (37 CWPs) or not (34 CWPs) to Endo H N-deglycosylation. This was also quantitatively reflected by the relative abundance of CWPs that exceeded 40% when Endo H and  $\alpha$ -mannosidase were applied. However, chemical O-deglycosylation with ammonia has its drawbacks by decreasing the number of identified peptides, thus identified mannoproteins (from 32 to 20) and their amount (from 16% to 11%), due to a degradative effect. Besides, the mild enzymatic O- and N-deglycosylations increased advantageously the sequence coverage of mannoproteins and allowed O- and N-glycosylation sites mapping. The enzymatic deglycosylation with mannosidase interestingly enriched integral PIR family mannoproteins known to be extensively O-glycosylated, supporting the hypothesis of O-glycans' implication in mannoproteins linkage to YCW glucans. In addition, through its action on mannose residues of GPI anchors, important mannoproteins previously unidentified by standard proteomics were found, such as Hpf1 of biotechnological relevance.

Mannoproteins O- and N-glycans were isolated simultaneously and efficiently as demonstrated *in silico* by Byonic™ analysis and *in vitro* permitting their analysis by MS following their chemical derivatization.

### Please explain why your abstract is innovative for mass spectrometry?

This work reports the first one-pot glycoproteomic preparation workflow for YCW mannoproteins' structural characterization based on stepwise deglycosylations adapted to eFASP and applied to the same sample of YCW.

### Co-authors:

Fabrice Bray, *Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille.*  
 Stéphanie Flament, *Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille.*  
 Antoine Picavet, *Lesaffre International, 137 rue Gabriel Péri*  
 Emmanuel Poilpré, *Lesaffre International, 137 rue Gabriel Péri*  
 Juliette Caron, *Lesaffre International, 137 rue Gabriel Péri*  
 Isabelle Mouly, *Lesaffre International, 137 rue Gabriel Péri*  
 Christian Rolando, *Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille., Shrieking Sixties, 1-3 Allée Lavoisier*

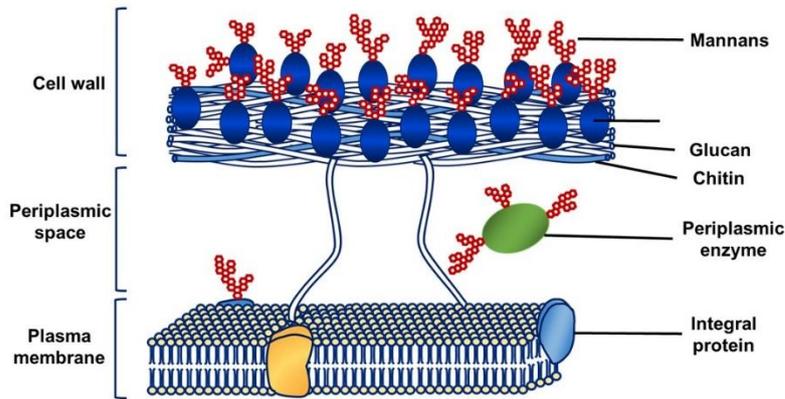


Figure 1 – *Saccharomyces cerevisiae* yeast cell wall molecular structure

*Saccharomyces cerevisiae* yeast cell wall molecular structure

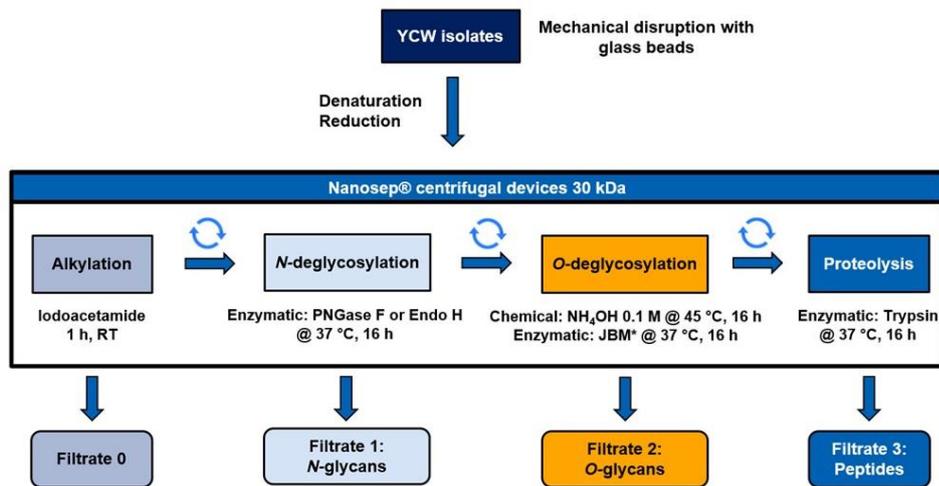


Figure 2 – Glycoproteomic workflow for *Saccharomyces cerevisiae* YCW mannoproteins characterization

Glycoproteomic workflow for *Saccharomyces cerevisiae* YCW mannoproteins characterization

Poster number: **LS-PB-039**

## **LC-MS/MS BASED ANALYSIS OF N-GLYCANS REVEALS SPECIFIC GLYCAN PATTERNS FOR NEWLY DEFINED PROTEOMIC CANCER SUBTYPES**

Abstract ID: 651

**Presenting author: Hannah Voß, University Medical Center Hamburg Eppendorf**

### **Introduction**

Investigating the proteome can add a significant layer of information to manifold existing methylation, mutation, and transcriptome data on tumors, as proteins represent the pharmacologically addressable phenotype of a disease. Post-translational protein modifications (PTMs)-resulting in different proteoforms for individual proteins-significantly increase the functional diversity of the proteome. The analysis of highly diverse N-Glycans, who play important functions in cell-adhesion, immune surveillance, cellular signaling, migration and cell-cell communication, is crucial for the understanding of cancer progression but highly limited by the lack of availability of biological background knowledge due to the non-template-based biosynthesis of these structures and the absence of proper algorithmic strategies.

### **Methods**

Here we defined proteomic cancer subtypes, combining in-house generated and publicly available proteome data on human tumors of a defined cancer type applying a newly defined framework for data harmonization across independent proteomic subtypes. Based on the evident identification of differentially regulated asparagine linked N-glycan synthesis and transport linked proteins, we performed a differential N-glycan analysis by solid phase permethylation coupled reversed phase LC-MS/MS. Using in house scripting, we quantified more than 300 different glycan species.

### **Preliminary data (results)**

Subsequent statistical analysis confirmed the representation of proteomic subtypes at the N-Glycan level and revealed tumor subtype specific N-Glycan signatures, correlating with relevant clinical parameters. These findings significantly underline the importance of PTM analysis in cancer research and provide new insights into potential immunological mechanism of different cancer subgroups at the proteome and glycan level.

### **Please explain why your abstract is innovative for mass spectrometry?**

Integration of independent in-house generated and publically available datasets, definition of cancer subtypes, differential cancer subtype specific glycomic signatures from optimized LC-MS/MS analysis of permethylated glycans.

### **Co-authors:**

*Boija Peng, University Medical Center Hamburg Eppendorf*  
*Yudon Guan, University Medical Center Hamburg Eppendorf*  
*Julia Neumann, University Medical Center Hamburg Eppendorf*  
*Hartmut Schlüter, University Medical Center Hamburg Eppendorf*

Poster number: LS-PB-040

## DIFFERENTIAL QUANTITATIVE O-GLYCOMICS OF SACCHAROMYCES CEREVISIAE YEAST CELL WALL MANNOPROTEINS IN DISTINCT CULTURE CONDITIONS

Abstract ID: 665

**Presenting author: Marie Yammine, Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille., Lesaffre International, 137 rue Gabriel Péri**

### Introduction

The parietal cell wall of *Saccharomyces cerevisiae* yeast cell shield it from various extrinsic stresses. This complex bilayered lattice comprises external mannoproteins linked to internal  $\beta$ -glucans and chitin. These mannoproteins are characterized by the rich mannosylated decoration, which can be subdivided into two forms depending on their degree of polymerization as well as their binding to the peptide backbone. These mannosylation forms are namely O-glycans and N-glycans. The YCW composition is suggested to dynamically fluctuate depending on growth conditions. However, little is known about YCW mannoproteins O-glycans' diversity. In this investigation, we are interested to decipher the quantitative and structural fluctuation of YCW mannoproteins O-glycans during the course of a discontinuously nourished batch culture as well as at the end of a continuously fed-batch culture.

### Methods

Batch and fed-batch cultures of S288C strain were carried out in YPD medium (30 °C, pH 5). YCW were isolated from different harvest times of batch and fed-batch cultures following mechanical disruption of yeast cells and delipidation by hot SDS extraction. Chemical O-deglycosylation with 0.1 M ammonia was performed. O-glycans were subsequently derivatized using the chromophore 4-aminobenzoate butyl ester (ABBE) through chemical aminative reduction reaction. Labeled O-glycans were separated by RPLC and identified by MS/MS analysis through CID fragmentation. Two different linear gradients were applied. For absolute quantitation, 0.5 pmol/ $\mu$ L of derivatized chitobiose was spiked.

### Preliminary data (results)

0.1 M ammonia chemical O-deglycosylation allowed isolating efficiently O-glycans from YCW mannoproteins. Subsequently to their isolation, O-glycans were chemically derivatized and analyzed by MS for structural investigation. Through implementation of an 80 min-acetonitrile linear gradient, different O-glycans' structures were detected upon their ABBE derivatization. Thanks to this analysis, we were able to elute the largest O-glycan containing five mannoses at 27 min in the case of batch-cultured cells. Then, eluted successively smaller O-glycans with successive elution time difference of 1 min. Unlikely, fed-batch cultured cells showed a supplementary O-glycan of six mannose residues according to the application of the same analysis. This feature has never been reported to date. Besides, acetonitrile-based gradient permitted the separation of two isomers per O-glycan structure, probably being  $\alpha$ - and  $\beta$ -anomers. Nevertheless, when switching to another methanol-based gradient, further complex chromatographic profiles were revealed: indeed, we were able to resolve at least three isomers per O-glycan structure. These structural isomers were proved according to their contrasting CID fragmentation patterns. Beyond the structural variation in O-glycans, the absolute quantification indicated the dynamic disparity of O-glycans amounts related to growth phase and culture mode. The quantification demonstrated the predominance of the small O-glycans structures composed of two and three mannose residues (34.4% and 28.8% respectively). Furthermore, when comparing their amounts from different harvests and cultures, we were able to see that the diauxic shift phase from glucose to ethanol consumption (9 h from batch culture launching) yielded the maximal amount of O-glycans (1.5 nmol per mg YCW).

### Please explain why your abstract is innovative for mass spectrometry?

This investigation originally displays a full structural and quantitative characterization revealing YCW mannoproteins O-glycans' dynamic change in S288C yeast strain depending on culture modes and conditions.

### Co-authors:

*Fabrice Bray, Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille.*  
*Stéphanie Flament, Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille.*  
*Antoine Picavet, Lesaffre International, 137 rue Gabriel Péri*

Emmanuel Poilpré, Lesaffre International, 137 rue Gabriel Péri

Juliette Caron, Lesaffre International, 137 rue Gabriel Péri

Isabelle Mouly, Lesaffre International, 137 rue Gabriel Péri

Christian Rolando, Miniaturization for Synthesis, Analysis & Proteomics, USR 3290, CNRS, University of Lille., Shrieking Sixties, 1-3 Allée Lavoisier

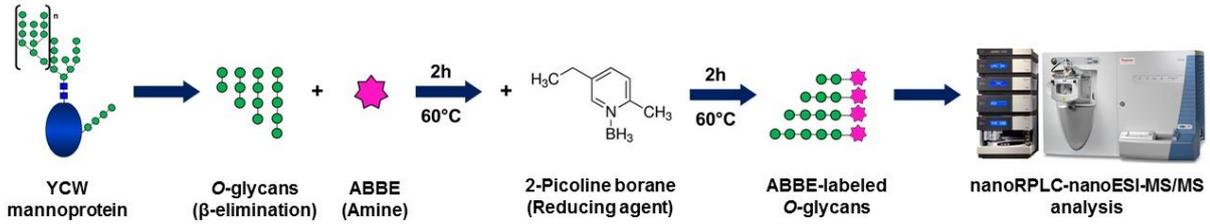


Figure 1 – Applied workflow for *Saccharomyces cerevisiae* YCW mannoproteins' O-glycomics characterization

Applied workflow for *Saccharomyces cerevisiae* YCW mannoproteins' O-glycomics characterization

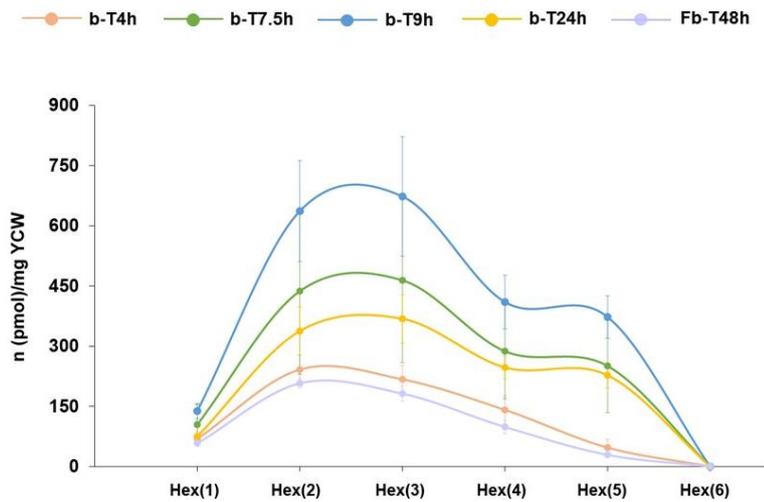


Figure 2 – Quantitative variability of O-glycans structures depending on culture conditions

Quantitative variability of O-glycans structures depending on culture conditions

Poster number: LS-PB-041

## GLYCAN BINDING PROPERTIES OF SARS-COV-2 RECEPTOR BINDING DOMAIN VARIANTS OF CONCERN

Abstract ID: 761

**Presenting author: Linh Nguyen, Department of Chemistry, University of Alberta**

### Introduction

The ongoing COVID-19 global pandemic is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 spike (S) glycoprotein is responsible for binding to the primary host receptor (angiotensin-converting enzyme 2, ACE2) and mediates viral entry. Certain classes of host glycans such as heparan sulfates and sialic acid-containing glycolipids have been shown to serve as attachment factors and enhance cell binding and facilitate infection. Over the past two years, multiple emerging variants of concern, with increased transmissibility have emerged. The goal of the present work is to identify host glycans that are recognized by these variants.

### Methods

The catch-and-release electrospray ionization mass spectrometry (CaR-ESI-MS) assay was used to screen both a defined library of 139 purified glycans (mostly mammalian), and natural N-glycan libraries produced from various human tissues against the SARS-CoV-2 receptor binding domain (RBD) of the S protein of Alpha, Beta, Gamma, Delta and Omicron variants. Next, we performed direct ESI-MS assay to quantify binding strengths of those RBDs with a small subset of glycan ligands. CaR-ESI-MS and direct binding measurements were performed using an Ultra-High Mass Range and Classic Orbitrap mass spectrometers, respectively, equipped with a nanoflow ESI source.

### Preliminary data (results)

CaR-ESI-MS screening of a defined library identified the ganglioside GM1 as the top hit for RBD in wild type, Alpha, Gamma and Delta strains. No hits were found for the Beta variant while Delta exhibits the most promiscuous glycan binding of all strains tested. Quantitative ESI-MS binding measurements performed on a subset of glycans revealed that GM1 is the highest affinity ligand for wild type, Alpha, Gamma, Delta and Omicron strains. Interestingly, blood group A type antigens had same binding strength to Delta variant as GM1. The enhancement in the neutral glycans binding to Delta variant might correlate with its increased transmissibility. Notably, binding of neutral glycans to Omicron variant substantially decreased compared with wild type. Future efforts will be directed to elucidating the specificities of the N-glycans ligands produced from human lung, intestine and brain tissues which can be recognized by these five strains.

### Please explain why your abstract is innovative for mass spectrometry?

Application of MS-based shotgun glycomic approach into examining roles of glycans in infection of SARS-CoV-2 variants

### Co-authors:

*Ilhan Tomris, Department of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University*

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*John S. Klassen, Department of Chemistry, University of Alberta*

Poster number: LS-PB-042

## MASS SPECTROMETRY IMAGING SPATIALLY IDENTIFIES COMPLEX-TYPE N-GLYCANS AS PUTATIVE CARTILAGE DEGRADATION MARKERS IN HUMAN KNEE OSTEOARTHRITIS TISSUE

Abstract ID: 861

**Presenting author:** Yea Rin Olivia Lee, Clinical and Health Sciences, University of South Australia, Adelaide, South Australia, Australia

### Introduction

The majority of human proteins are being modified by covalent attachment of complex oligosaccharides, called *N*-glycans. They are involved in virtually all physiological and pathological processes. However, the involvement of *N*-glycans in knee osteoarthritis (KOA) progression at the tissue level, especially within articular cartilage, is still poorly understood. Thus, the aim of this study was to spatially map and identify KOA-specific *N*-glycans from formalin-fixed paraffin-embedded (FFPE) osteochondral tissue of the tibial plateau relative to cadaveric control (CTL) tissues.

### Methods

Human FFPE osteochondral tissue from end-stage KOA patients ( $n=3$ ) and CTL individuals ( $n=3$ ), aged >55 years old, were analyzed by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

### Preliminary data (results)

Overall, it was revealed that 22 *N*-glycans were found in the cartilage region of KOA and CTL tissue. Of those, 15 *N*-glycans were more prominent in KOA cartilage than CTL cartilage. We then compared sub-regions of KOA and CTL tissues based on the Osteoarthritis Research Society International (OARSI) histopathological grade (1 to 6), where 1 is an intact cartilage surface and 6 is cartilage surface deformation. Interestingly, three specific complex-type *N*-glycans,  $(\text{Hex})_4(\text{HexNAc})_3$ ,  $(\text{Hex})_4(\text{HexNAc})_4$ , and  $(\text{Hex})_5(\text{HexNAc})_4$ , were found to be localized to the superficial fibrillated zone of degraded cartilage (KOA OARSI 2.5-4), compared to adjacent cartilage with less degradation (KOA OARSI 1-2) or relatively healthy cartilage (CTL OARSI 1-2).

### Please explain why your abstract is innovative for mass spectrometry?

Our results demonstrate that using MALDI-MSI approaches, *N*-glycans specific to degraded cartilage in KOA patients have been identified at the tissue level for the first time.

Poster number: LS-PB-043

## IDENTIFICATION OF HUMAN MILK OLIGOSACCHARIDES BY IMS2 COUPLED CRYOGENIC IR SPECTROSCOPY

Abstract ID: 887

Presenting author: Ali H. Abikhodr, EPFL

### Introduction

While oligosaccharides, or glycans, play a fundamental role in many biological processes, their intrinsic isomeric heterogeneity presents a significant challenge to their analysis. The scarcity of isomerically pure analytical standards further complicates this analysis. In this work we demonstrate how the combination of ion mobility spectrometry (IMS) with mass spectrometry (MS) and cryogenic infrared (IR) spectroscopy can be used to identify isomers with a reduced need for standards. This is made possible through an IMS<sup>2</sup> technique, where fragments of mobility-separated parent oligosaccharides are identified using both IMS and IR spectroscopy, allowing us to reconstruct the isomeric form of the parent molecule in a bottom-up approach.

### Methods

We begin with mobility separation of oligosaccharide isomers using structures for lossless ion manipulations (SLIM). After separation during one or more cycles along a 10 m labyrinthine path, we fragment the parent molecules by collision-induced dissociation (CID) directly on our SLIM device. We then send the fragments through a second stage of mobility separation before directing them to an ion trap, where we perform cryogenic IR spectroscopy to identify them. By identification of fragments diagnostic of the isomeric form of the parent molecule, we identify the latter using just a few standards to construct a fragment IR database.

### Preliminary data (results)

We demonstrate our approach by following the described method for the identification of isomeric human milk oligosaccharides (HMOs). Structurally diagnostic fragments of the hexasaccharide Lacto-N-difucohexaose (LNDFH) were first generated on the SLIM device and then assigned and identified through their IR spectra. The fragment reference spectra used for this analysis were either fingerprints that we had previously measured for isomers of the pentasaccharide LNFP or of its fragments. This small database of IR spectra allows us to determine uniquely the location of the fucose residues on LNDFH.

We then repeated the procedure for the larger oligosaccharides MFLNH (7 residues) and DFLNH (8 residues) found in human milk for which we do not have standards. In this case, the fucose positions can be determined using the same set of IR spectra as above, and the backbone can be determined using the IR fingerprint of the hexasaccharide LNH. As we grow the database by adding isomer-selective IR fingerprints of the parent molecules, still larger human milk oligosaccharides can be identified.

### Please explain why your abstract is innovative for mass spectrometry?

Cryogenic IR spectroscopy of mobility separated CID fragments provides the means to unambiguously identify isomers of HMOs.

### Co-authors:

Ahmed Ben Faleh, EPFL  
Stephan Warnke, EPFL  
Vasyl Yatsyna, EPFL, University of Gothenburg  
Thomas R. Rizzo, EPFL

Poster number: **LS-PB-044**

## ION MOBILITY-MASS SPECTROMETRY BASED GLYCOSAMINOGLYCAN DISACCHARIDE ANALYSIS

Abstract ID: **912****Presenting author: Lukasz Polewski, Department of Biology, Chemistry, Pharmacy, Institute of Chemistry and Biochemistry, Freie Universität Berlin**

### Introduction

Glycosaminoglycans (GAGs) are linear and highly negatively charged polysaccharides. They are ubiquitous molecules which exhibit a wide range of biological functions and are involved in the tissue integrity, cell recognition, migration, proliferation and protein folding. Although structurally simple at first glance, with a repeating backbone of alternating hexuronic acid and hexosamine dimers, they display a highly complex structure, which results from their heterogeneous sulfation pattern.

The characterization of GAGs is usually performed using "disaccharide analysis." Here the polymeric GAG chains are enzymatically digested into disaccharides, labeled with a fluorescence marker, and analyzed using liquid chromatography which takes up to an hour.

### Methods

The TimsTOF Pro, one of the new generation ion mobility (IM) instruments, uses trapped ion mobility spectrometry (TIMS) to separate ions based on their size and shape. Here, the ions are dragged into the spectrometer by a constant nitrogen gas flow, while an electric field in the opposite direction is used to decelerate the ions. This allows trapping of the ions and subsequent release, depending on their respective ion mobility, by gradually decreasing the electric field. Measurements were performed on a home-built 3D-printed direct infusion nESI source.

### Preliminary data (results)

The limiting factor in the high throughput GAG disaccharide analysis is the chromatography run at the end of the workflow. To accelerate the acquisition time, we here aim to develop an analysis workflow, which utilizes IMS instead of chromatography for the separation of isomeric GAG disaccharides. Using direct infusion nESI with disposable capillaries potentially cuts the measurement time to a few minutes instead of up to an hour.

A full set of disaccharides consists of 12 structures from which 8 possess isomers. In their native form, most disaccharides cannot be readily distinguished using IMS. To reduce sample complexity and induce intramolecular interactions that aid isomer separation, chemical modifications have been carried out. In the first step a propionylation is performed, which reduces the number of isomeric species from 8 to 6. Second, a reducing end label is attached to the disaccharides, which is able to interact with sulfate and carboxylic acid groups via hydrogen bridge bonding and therefore increases differentiability of the isomers with TIMS. Furthermore, quantification is facilitated by spiking stable isotope labeled standards, which are easy to obtain in the second derivatization step using labelled reagents.

### Please explain why your abstract is innovative for mass spectrometry?

Direct infusion nESI of complex glycan mixture with a home-built nESI source on the Bruker TimsTOF Pro.

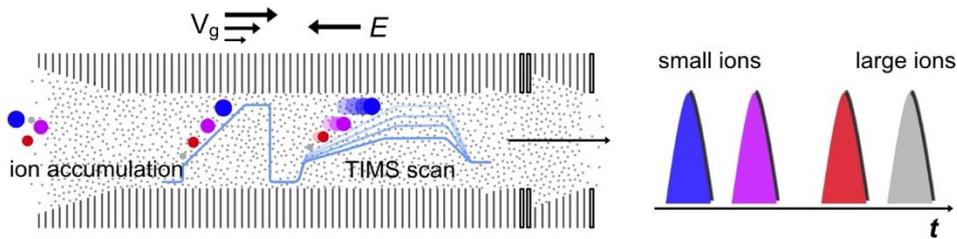
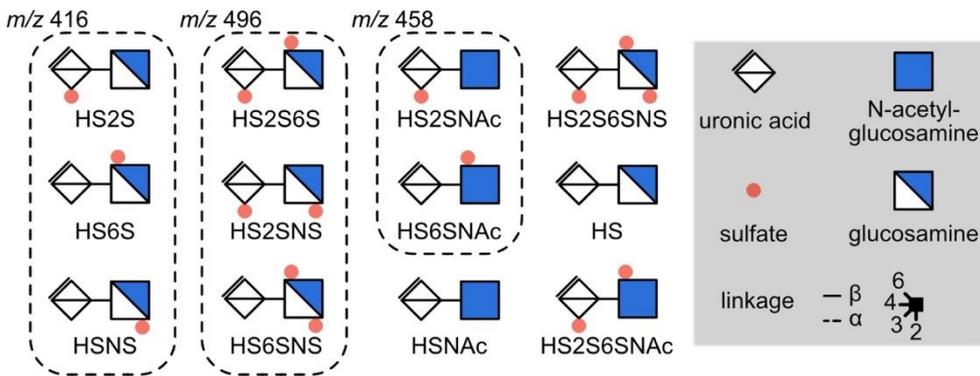
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POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



Heparin/Heparan-sulfate disaccharides resulting from digestion and TIMS separation principle.

Poster number: **LS-PB-045**

## **NOVEL FRAGMENTATION TECHNOLOGY FURTHER ENABLES IN-DEPTH GLYCOPEPTIDE CHARACTERIZATION IN GLYCOPROTEINS**

Abstract ID: **960**

**Presenting author: Jenny Albanese, SCIEX**

### **Introduction**

A newly developed electron activated dissociation (EAD) approach allows for tunability of electron energy to produce different fragmentation patterns of the electron-based dissociation (ExD) family.

The fragmentation mode, collision induced dissociation (CID) is used traditionally for peptide mapping. However, traditional approaches do not allow consistent identification and localization of glycans on peptides

By combining increased detection of fragments using a Zeno trap on a QTOF instrument for increased duty cycle, enables higher confidence in data assignment. The data presented here demonstrates how Zeno EAD combination is ideal for in-depth glycopeptide analysis.

### **Methods**

Therapeutic monoclonal antibody, Herceptin, was denatured with 7.2M guanidine hydrochloride, 100mM Tris buffer pH 7.2, followed by reduction with 10mM DL-dithiothreitol and alkylation with 30mM iodoacetamide. Digestion was performed with trypsin/lys-C at 37°C for 16h. 10µl (4µg) of the trypsin/Lys-C digest was separated with a CSH C18 column (1.7µm particle size, 130Å, 2.1×100mm). Column temperature was maintained at 50°C. Data was acquired in DDA mode using a ZenoTOF 7600 system. Zeno trap was enabled for this analysis and used either CID or EAD fragmentation. Data was processed using commercially available software.

### **Preliminary data (results)**

Data interpretation from several digests and sample sets resulted in the identification of peptide fragments, oxonium ions, peptide glycan fragments and overall sequence coverage. Comparing CID and EAD sequence coverages resulted in identification of major differences for glycosylated species. While CID MS/MS spectra were dominated by oxonium ions, they showed low abundant b- and y-ions. However, EAD MS/MS spectra showed a rich abundance of c- and z-ions with much higher intensity and better, -more complete coverage of the peptide backbone for various glycosylated peptides. The EAD data provided fragment ions with the intact glycosylation attached, allowing for an unambiguous localization of the glycan portion. Furthermore, the use of EAD did not reduce the backbone coverage of non-glycosylated peptides but improved the information gain for glycosylated peptides drastically.

Glycopeptides compete with many non-glycosylated high abundance peptides in a typical DDA acquisition. The glycans studied here included the abundant G0F, G1F and G2F species and a few of the lower abundant glycans. One example us the high-mannose species, known to be present at low levels, could be identified using EAD fragmentation In comparison, it was difficult to identify in the CID data.

The dynamic range for low abundance glycopeptide with mannose 5 for example, has a 10x lower abundance than G0F-containing peptide, but could still be identified with high quality fragment ion spectra.

### **Please explain why your abstract is innovative for mass spectrometry?**

EAD technology with Zeno trapping allows for more accurate and in-depth characterization and localization of N-linked glycopeptides, including those of low abundance.

### **Co-authors:**

*Zoe Zhang, SCIEX*

Poster number: **LS-PB-046**

## UNRAVELLING THE FUCOSE MIGRATION PRODUCT IN BLOOD GROUP EPITOPES LEWIS X AND BLOOD GROUP H TYPE 2

Abstract ID: **1026**Presenting author: **Maike Lettow, Fritz-Haber-Institut der Max-Planck-Gesellschaft**

### Introduction

Fucosylation is a prominent structural feature in mammalian glycans. Almost exclusively at ultimate or penultimate site at the non-reducing end of a glycan's chain, fucose is involved in many biological processes, such as recognition processes during fertilization. The lack of a hydroxy group at the C6 position and the naturally occurring l-configuration, render fucose an unusual monosaccharide. In tandem mass spectrometry, the rearrangement of a fucose monosaccharide to adjacent or remote sites has been known for more than 25 years. Most recently, fucose migration in protonated ions has also been described for intact glycan ions without explicit activation. Yet, the final product of the rearrangement and the mechanism have not been fully understood to date.

### Methods

High-resolution infrared spectra of the trisaccharides were measured using cryogenic IR spectroscopy in helium droplets with prior cooling of the ions to 90 K, to potentially slow the reaction. Using ion mobility-mass spectrometry, arrival time distributions were recorded with and without pre-IMS activation. The injection voltage is varied below the dissociation threshold (sub-CID) to mimic harsh source conditions. Radical directed-dissociation mass spectra are recorded with and without sub-CID activation prior fragmentation again to mimic harsh source conditions on a different mass spectrometer. The experimental results are combined with high-throughput density-functional theory modeling to elucidate the molecular structure of the product.

### Preliminary data (results)

The three mass spectrometry-based experiments, cryogenic IR spectroscopy, IM-MS and RDD MS, indicate that the blood group antigens Lewis x and blood group H type 2 are either (a) migrating to a third chemical structure or (b) interconverting to each other. The extensive theoretical calculations were matched to the experimental CCS and IR spectra and aided to identify the rearrangement product as a third chemical structure, in which the fucose moiety has an  $\alpha(1 \rightarrow 6)$  glycosidic bond to the terminal galactose. Especially for Lewis x, the barrier for migration must be considerably low compared to blood group H type 2, which highlights that similar structural motifs can still behave differently.

### Please explain why your abstract is innovative for mass spectrometry?

The fucose migration product in the blood group antigens Lewis x and blood group H type 2 has a different chemical structure than any naturally occurring structural motif.

### Co-authors:

*Eike Mucha, Fritz-Haber-Institut der Max-Planck-Gesellschaft*

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*Kim Greis, Fritz-Haber-Institut der Max-Planck-Gesellschaft, Institute of Chemistry and Biochemistry, Freie Universität Berlin*

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## Session: Imaging MS - applications in Life Science & Health

Poster number: LS-PB-019

### **AUTOMATED PIPELINE FOR RELIABLE AND HIGH THROUGHPUT ANALYSES OF LOW INPUT PROXIMITY-LABELLING SAMPLES**

Abstract ID: 978

**Presenting author: Therese Dau, The Leibniz Institute on Aging – Fritz Lipmann Institute**

#### **Introduction**

Proximity-labelling has emerged as an important tool to study protein-protein interactions directly in-cell. Labelling time could be considerably reduced by optimisation of biotin ligases and has opened up the use for a wide variety of experiment, e.g., time-course analyses. Despite these advances sample preparation is still very labour intensive and limits experimental size. Here, we optimised the workflow for high throughput purposes by combining our low sample input pipeline with automated sample preparation on a liquid handler.

#### **Methods**

We implemented an optimized version of our sample preparation workflow for enrichment and digestion of biotinylated proteins (Mackmull, MT et al. MSB 2017) on a liquid handler. In short, biotinylated cells were lysed and bound to streptavidin cartridges. Samples were digested on the beads and eluted in two steps. The second elution step breaks the biotin-streptavidin bond with a mixture of acetonitrile and trifluoroacetic acid. Samples were analysed on an Orbitrap Exploris 480 coupled to an EvoSep One System and run with a pre-programmed EvoSep gradient of 21 minutes (60SPD).

#### **Preliminary data (results)**

The enrichment of the biotinylated proteins via streptavidin and subsequent on-bead digest has been implemented on the automated liquid handling platform Bravo. The first advantage of this setup is the increase in the number of samples (up to 96 samples) that can be processed in parallel. More importantly, this procedure improves the reproducibility, especially the harsher elution with the acetonitrile/methanol mixture. This elution step is very sensitive to exposure time and has shown greater variation between experiments. Using our automated workflow, we were able to reduce the sample input from 20 to 4 Mio cells. By employing EvoSep LC system, we reduced measurement time to one sixth (21 vs. 120 min) of our previous analyses. Taken together we were able to implement a new workflow that improved reproducibility and speeds up sample processing and measuring time for proximity labelling experiments considerably.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Establishment of an automated high throughput pipeline for proximity labelled sample processing.

Poster number: LS-PB-047

## INVESTIGATION OF FUNCTIONAL CONSEQUENCES OF EPILEPSY-CAUSING MUTATIONS IN A MOUSE MODEL OF FOCAL CORTICAL DYSPLASIA

Abstract ID: 89

Presenting author: Isabeau Vermeulen, M4I, Maastricht University

### Introduction

Focal cortical dysplasias (FCD) are a group of highly epileptogenic localized cortical lesions responsible for focal epileptic seizures. FCD is the most common cause of refractory epilepsy in the paediatric population and second most common aetiology in adults. A large portion of FCD results from abnormal brain development (*in utero*) due to genetic mutations. We have developed a relevant mouse model of FCD to investigate mutations of interest. These somatic mutations lead to mosaicism in the affected brain, which leads to difficulties in unravelling the functional consequences of these mutations. In this work we have used mass spectrometry imaging (MSI), to get insight in how the mutations affect both the brain lipidome and proteome while maintaining the spatial information.

### Methods

Fresh-frozen mouse brain sections were sprayed with norharman matrix (HTX-TM sprayer) and analyzed at 20  $\mu$ m raster size on a tims-ToF (Bruker). Data dependent acquisition (DDA) was performed on Orbitrap Elite hybrid ion trap for lipid identification. Lipid data analysis was performed in Scils and Lipostar. Fluorescence images were taken with a Leica SP8 STED. Laser capture microdissection of the FCD region was performed using a Leica LMD 7000 instrument. LMD samples were trypsin-digested and further processed for LC-MS/MS peptide analysis on a Ultimate 3000 UHPLC system coupled to a Q-Exactive (Thermo). Protein identification was performed in Proteome Discover.

### Preliminary data (results)

The MSI lipidomics data clearly showed distinct features in the lipid profile of the FCD area compared to the control and naïve brains. Segmentation and pLSA were able to define the FCD area in the affected brain. This information was combined with the DDA data where we could identify phosphocholines, phosphoethanolamines, phosphoinositols, phosphoserines, sphingomyelins, triacylglycerols, prostaglandins and glycerophosphates. Several sulfatides showed a decrease in the FCD affected brain. In order to define where the FCD affected neurons were most present, we used fluorescence imaging on consecutive tissue sections. The development of FCD is an *in vivo* process which results in different presentations of the affected region. Injection of the plasmid tagged with a fluorescent reporter, GFP for FCD, makes visualization of the FCD region possible. The fluorescence data and MSI segmentation data were overlaid using common features in the sections. This multimodal overlay was used to define the regions to cut out for further proteomic analysis. A complete overview of the workflow is given in figure 1. Our results clearly show differences in both lipidomics and proteomics of the affected brain in the FCD area compared to control and naïve brains. This can help to understand the functional consequences of the somatic mutation in FCD. This work demonstrates that MSI and MSI-guided proteomics in a multimodal set-up can be applied in the drug discovery pipeline and more specific in the early target identification steps.

### Please explain why your abstract is innovative for mass spectrometry?

Multimodal spatial omics approach combining MSI, fluorescence imaging and LC-MS/MS-based proteomics to understand etiology of FCD, epileptogenesis and unravel how the mutations are affecting the brain lipidome and proteome

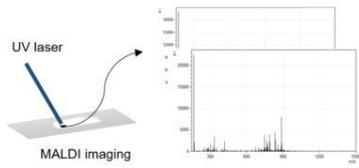
### Co-authors:

Berta Cillero Pastor, M4I, Maastricht University  
Natalia Rodriguez, UCB Pharma  
Ron M.A. Heeren, M4I, Maastricht University

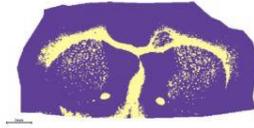
**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

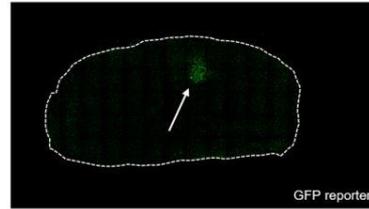
**A** Mass Spectrometry Imaging of Lipids



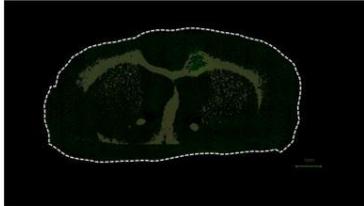
**B** Segmentation of MSI data



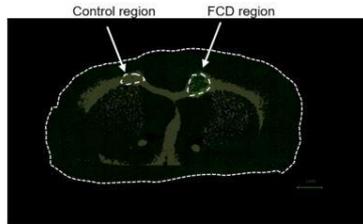
**C** Fluorescence Imaging



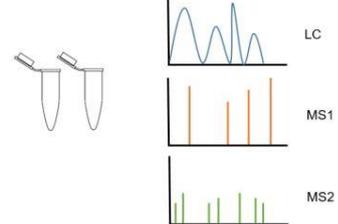
**D** Overlay of MSI segmentation and fluorescence



**E** Laser Capture Microdissection



**F** LC-MS/MS of peptides



Schematic overview of the workflow

Poster number: **LS-PB-048**

## COMPUTATIONAL ANALYSIS OF ALZHEIMER AMYLOID PLAQUE COMPOSITION IN 2D- AND ELASTICALLY RECONSTRUCTED 3D-MALDI MS IMAGES

Abstract ID: 106

**Presenting author: Thomas Enzlein, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, Paul-Wittsack Str. 10, 68163, Mannheim, Germany., KU Leuven-VIB Center for Brain & Disease Research, VIB, 3000 Leuven, Belgium., Department of Neurosciences, Leuven Institute for Neuroscience and Disease, KU Leuven, 3000 Leuven, Belgium.**

### Introduction

Alzheimer's disease (AD) is the leading cause of dementia. AD is characterized by deposition of amyloid peptides called plaques. Analysis of MSI datasets is typically performed on single pixels or manually assigned regions of interest (ROIs). However, many sparse, small objects such as AD plaques are neither single pixels nor a manageable number of ROIs. We propose a new approach, *PlaquePicker*, to facilitate the comparative evaluation of plaque-like objects by MSI and statistical evaluation of heterogeneous amyloid peptide composition. Furthermore, we reconstructed a 3D-model of plaques using *M2aia*, a novel, interactive, fast and memory efficient application for multi-modal and 3D reconstruction and manipulation of MSI data.

### Methods

The proposed method was applied on two AD mouse models; APP NL-G-F and APP PS1. APP PS1 is based on overexpression of amyloid precursor protein (APP) whereas APP NL-G-F is using a knock-in approach leading to expression of APP on wild-type level. The two models were compared on the basis of their A $\beta$ -ratios and plaque sizes. Each plaque was considered individually enabling population statistics and computing measures of statistical dispersion using *PlaquePicker* (<https://github.com/CeMOS-Mannheim/PlaquePicker>). The 3D reconstruction of the data was performed using a combination of rigid and elastic MSI image registration using *M2aia* (<https://m2aia.de/>) enabling 3D analysis of plaques.

### Preliminary data (results)

Using the proposed method we observed distinct heterogeneous plaque populations in the NL-G-F model but only one class of plaques in the PS1 model. Furthermore, statistical single-plaque analysis in reconstructed 3D-MSI objects revealed the A $\beta$ <sub>1-42Arc</sub> peptide to be located either in the core of larger plaques or in small plaques without co-localization of other A $\beta$  isoforms. In 3D, a substantially larger number of small plaques were observed than that indicated by the 2D-MSI data, suggesting that quantitative analysis of molecularly diverse sparsely-distributed features may benefit from 3D-reconstruction.

### Please explain why your abstract is innovative for mass spectrometry?

We introduce a platform for the analysis of sparsely-distributed features in MSI that enables single-object-based statistics. We highlight how *M2aia* can be used for 3D-reconstruction and interactive evaluation of 3D-MSI-data.

### Co-authors:

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

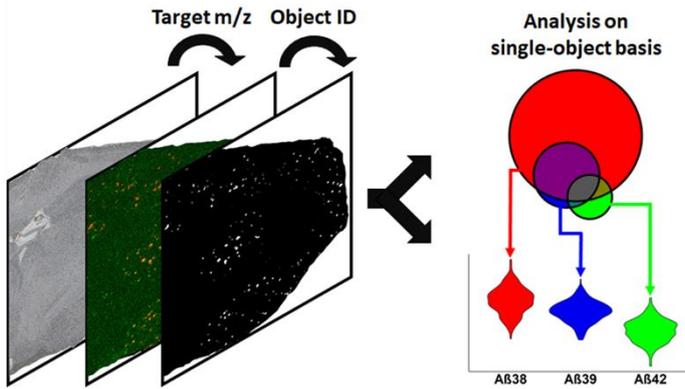
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Carsten Hopf, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, Paul-Wittsack Str. 10, 68163, Mannheim, Germany.



Graphical abstract of PlaquePicker workflow

Poster number: **LS-PB-049**

## MAPPING GLUCOSYLSPHINGOSINE ACCUMULATION IN A PARKINSON'S DISEASE MOUSE MODEL CARRYING A GBA-1 MUTATION

Abstract ID: 116

**Presenting author: Shane Ellis, Molecular Horizons and School of Chemistry and Molecular Bioscience, University of Wollongong, Illawarra Health and Medical Research Institute**

### Introduction

Genetic deficiency in the glucocerebrosidase gene (GBA1) is a major risk factor for Parkinson's disease (PD) development. Glucocerebrosidase (GCase) catalyses the breakdown of glucosylceramide (GluCer) and glucosylsphingosine (GlcSph) substrates, and its deficiency leads to the accumulation of glucose-carrying glycosphingolipids, yet the spatial distribution of these species is largely unknown. This provides a strong rationale for mass spectrometry imaging of GSL content in the brain to detect changes in region-specific distribution accompanying neurodegeneration. However, this is challenging with conventional matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) due to poor ionisation efficiencies of many GSLs making detection of low abundance GluSph and GalSph species difficult. We have overcome this by laser post-ionisation (MALDI-2) that significantly improves the sensitivity of GSL detection

### Methods

Data was acquired on mouse brain tissue obtained from control or mutant mouse model carrying either heterozygous (HET) or homozygous (HOM) D409V GBA mutations. Samples were collected on ITO coated slides and coated with 2,5-dihydroxybenzoic acid and internal standards using a TM-sprayer (HTX Technologies, USA). MALDI-2 data was acquired using an Orbitrap Elite mass spectrometer (Thermo Scientific, Germany) coupled to a reduced pressure MALDI interface (Spectrograph LLC, USA). MALDI-2 was achieved using a 266 nm laser (Litron Lasers, UK, 500  $\mu$ J/pulse) that intersects plume of desorbed neutrals  $\sim$ 400  $\mu$ m above the sample surface.

### Preliminary data (results)

Consistent with previous studies MALDI-2 was shown to increase the signal intensities for many GSL-containing lipids. This enabled detection of endogenous [HexSph(d18:1;2)+H]<sup>+</sup> throughout mouse brain tissue (Hex collectively referring to the species carrying either galactose or glucose). Isotopically labelled internal standards of GluCer and GluSph were used for both signal normalisation and to evaluate possible formation of HexSph by in-source fragmentation in HexCer during MALDI-2-MSI experiments.

Rich spectra enabling the imaging of many neutral GSLs species such as HexCer, Hex2Cer and Cer1P and HexSph species were produced in the positive-ion mode. HexSph signal was localised primarily within the white matter fibre tracts and brain stem regions, similar to other neutral GSL species. A significant increase in HexSph signal was observed across D409V mice compared to matched WT) mice with the highest observed in the mice carrying the HOM mutation. This is amongst the first detections of HexSph lipids from brain tissue using MSI. These data were supported by LC-MS/MS measurements showing this increase in signal to arise almost exclusively from GluSph and is in agreement with the expected metabolic effects of the GBA mutation. These results demonstrate the as yet untapped potential of MALDI-2 to study GSL (dys)regulation in diseased tissues.

### Please explain why your abstract is innovative for mass spectrometry?

This work utilises the enhanced sensitivity offered by MALDI-2 to enable imaging of low abundance glycosphingolipids that further broadens the lipidomics insight provided by MSI approaches.

### Co-authors:

*Shadrack Mutuku, Molecular Horizons and School of Chemistry and Molecular Bioscience, University of Wollongong*  
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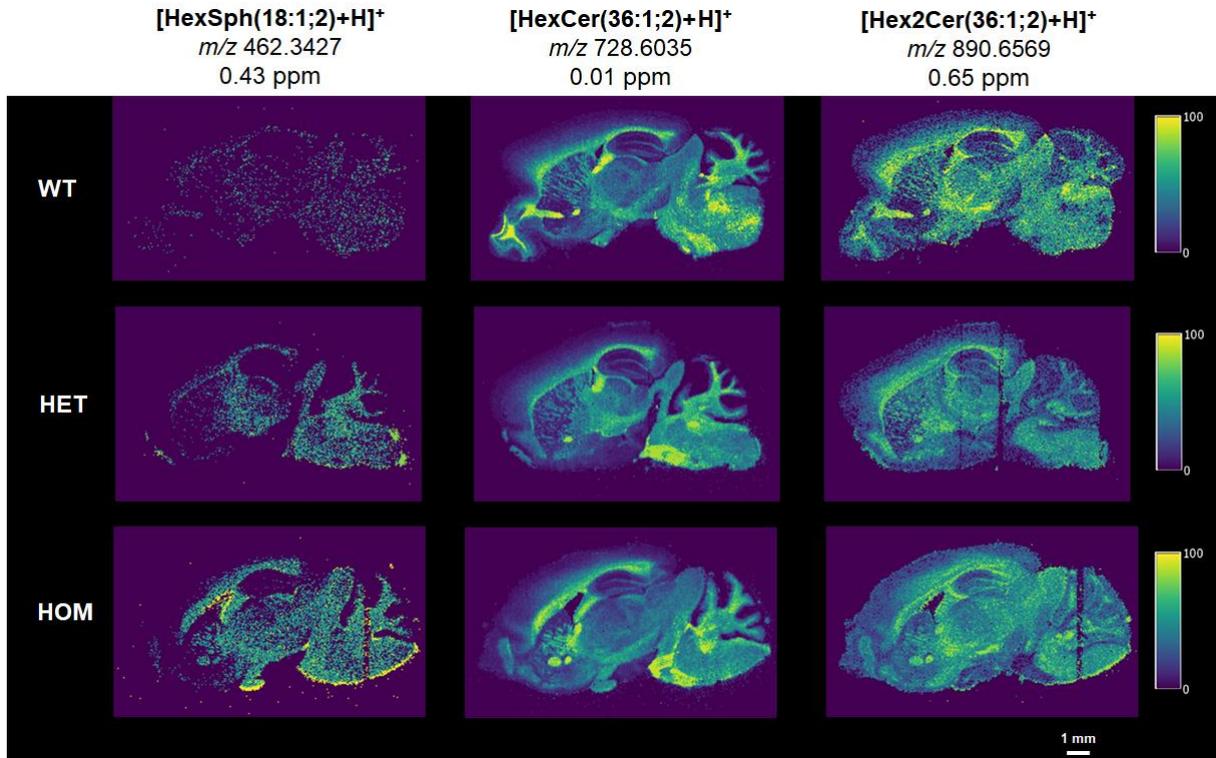
*Nathan Hatcher, Merck & Co., Inc*

*Ron Heeren, Maastricht MultiModal Molecular Imaging (M4I) Institute, Division of Imaging Mass Spectrometry,*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

Maastricht University  
Kim Ekroos, Lipidomics Consulting, Ltd



Accumulation of HexSph species reflecting result of GBA-1 mutation

Poster number: **LS-PB-050**

## **COMBINATION OF MALDI-MSI AND LC-MS TO GAIN A DEEPER UNDERSTANDING OF THE ROLE OF NEW TARGETS IN GLIOBLASTOMA PROGRESSION ON A LIPIDOME AND METABOLOME LEVEL**

Abstract ID: 146

**Presenting author: Dina Rebecca Naomi Vos, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences**

### **Introduction**

Recent advances in glioblastoma research focusses on enzymes that produce amino acid-derived metabolites that signal through ligand-activated transcription factors. Their metabolites suppress T-cell proliferation, promote tumor cell motility, and are associated with reduced survival of glioma patients. The role of these amino acid catabolic enzymes and their functional interaction with transcription factor activity warrants a deeper investigation to gain a deeper understanding of their role and potential involvement in tumor metabolic pathways.

### **Methods**

Four different mouse cohorts with xenografted human U87 glioblastoma cells in CD1 nu/nu mouse brain (enzyme overexpressed, transcription factor knock-down, enzyme overexpressed and receptor knock-down, and control) were analyzed by untargeted MALDI-MS and complementary untargeted LC-MS to investigate the differences in their metabolite profiles. In total 24 mice, 6 per group, were analyzed. MALDI-MSI was performed in dual polarity at 50  $\mu\text{m}$  on a timsToFfleX (Bruker Daltonics). For the LC-MS measurements, the xenograft regions were cut out using laser microdissection (LMD; Leica Microsystems) and then analyzed using a CapLC system on an Impact II (Bruker Daltonics).

### **Preliminary data (results)**

The MSI data were analyzed per modality and ionization by doing pairwise comparisons between the conditions using only the xenograft regions by Supervised Spatial Shrunken Centroids (SSc) clustering and feature extraction. For each comparison, this yielded two lists with a number of m/z values that were statistically significant for one or the other condition. This analysis was repeated with the replicate sections and the m/z values that were identified in both replicates were considered to be biologically significant and subsequently identified. Preliminary results show changes in lipids and metabolites related to the expression of the metabolic enzyme. Among these, differences in ether-linked phospholipids and ceramide levels are prominent. These results are reflected also in the preliminary LC-MS data.

### **Please explain why your abstract is innovative for mass spectrometry?**

Combination of untargeted MSI and LMD-guided LC-MS for lipidomics and metabolomics investigation of a large mouse cohort.

### **Co-authors:**

*Tobias Bausbacher, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences*

*Shad Mohammed, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences*

*Caroline Chipeaux, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences*

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*Luis Felipe Somarribas Patterson, DKTK Brain Cancer Metabolism Group, German Cancer Research Center (DKFZ), Faculty of Bioscience, Heidelberg University*

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Clinic and National Center for Tumor Diseases*

*Carsten Hopf, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, Faculty of Medicine, Heidelberg University*

Poster number: **LS-PB-051**

## **DISCOVERY OF SPATIAL PEPTIDE SIGNATURES TO PREDICT TREATMENT RESPONSE OF PATIENT-DERIVED XENOGRRAFT MODELS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA**

Abstract ID: **193****Presenting author: Benjamin-Florian Hempel, BIH Center for Regenerative Therapies (BCRT), Berlin Institute of Health at Charité – Universitätsmedizin Berlin**

### **Introduction**

Head and neck cancer (HNC) is one of the most prevalent and lethal cancers worldwide with more than 550,000 cases and around 300,000 deaths each year. About 90% of all head and neck cancers are squamous cell carcinomas (HNSCC) and knowledge on pathophysiological alterations that are causal for tumor progression and therapy response often remains largely elusive due to tumor heterogeneity. As a result, therapeutic options are often inadequate and treatment resistance remains a major challenge to date. Therefore, patient-derived xenograft (PDX) HNSCC models provide an excellent opportunity to monitor treatment response under defined conditions and spatial tumor heterogeneity.

### **Methods**

In order to overcome these limitations, we performed matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) on HNSCC-PDX models under different treatment conditions (sensitive: platinum 14, taxane 33, cetuximab 27). Initially, we implemented machine learning (ML) utilizing a random forest (RF) algorithm on the HNSCC-PDX cohort to automate discrimination of non-tumor regions, followed by the prediction of treatment response. Tumor peptide signatures were evaluated based on univariate and multivariate statistical analyses to discover spatial discriminative and risk-associated classifier. To identify associated proteins to previous identified peptide signatures, bottom-up tandem mass spectrometry were applied on adjacent tissue sections.

### **Preliminary data (results)**

Our proof-of-concept study demonstrates the potential of MALDI-MSI in combination with unsupervised analyses to robustly predict spatial discriminative and risk-associated peptide signatures for treatment resistance and overcome limitations such as tumor heterogeneity. ML classification, evaluated based on mean AUC, identified specific molecular profiles that enable to distinguish tumor from non-tumor regions (AUC > 0.9) and several highly HNSCC-associated proteins with different expression in tumor regions related to various systemic chemotherapeutics (AUC > 0.8).

### **Please explain why your abstract is innovative for mass spectrometry?**

These results hold a great promise as alternative risk classification strategy and analytical tool to shed further insights into HNSCC intra-tumor heterogeneity.

### **Co-authors:**

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*Ulrich Keilholz, Charité Comprehensive Cancer Center, Charité – Universitätsmedizin Berlin*  
*Ingeborg Tinhofer-Keilholz, Department of Radiooncology and Radiotherapy, Charité – Universitätsmedizin Berlin*  
*Oliver Klein, BIH Center for Regenerative Therapies (BCRT), Berlin Institute of Health at Charité – Universitätsmedizin Berlin*

Poster number: **LS-PB-052**

## VISUALIZATION OF THE DIFFERENTIAL CARDIOLIPIN PROFILES IN MURINE RETINA CELL LAYERS USING HIGHLY RESOLVED MALDI-MS IMAGING

Abstract ID: **213****Presenting author: Sebastian Bessler, Institute of Hygiene, University of Münster**

### Introduction

Age-related macular degeneration is a common cause for severe visual impairment or even blindness. There is mounting evidence that progression of the disease is closely related to lipid oxidation. Mitochondrial cardiolipins (CL), composed of four fatty acyl chains, many of which with conjugated double bonds, are particular prone to oxidative attack, due to their involvement in the electron transport chain. The precise acyl chain compositions of CLs are cell and tissue specific. Here we employed a trapped ion mobility time-of-flight mass spectrometer (timsTOF fleX MALDI-2, Bruker) to image the CL profiles in individual retina cell layers of murine eyeballs at a pixel size of 10  $\mu\text{m}$ . A detailed insights into the differential CL composition were obtained by using tandem MS in our imaging experiments.

### Methods

Enucleated eyeballs from mice and albino rats were embedded in carboxymethyl cellulose, snap-frozen, and sectioned to 16  $\mu\text{m}$  thickness. Sections were washed with n-heptane and ammonium acetate to remove triacylglycerols and alkali metal salts respectively. Norharmane MALDI matrix was applied using a sublimation/recrystallization protocol. All experiments were conducted in the negative ion mode using standard MALDI-MSI without laser postionization and by applying 100 laser shots per pixel. After the measurement the matrix was removed using isopropanol and the sections H&E-stained. Optical images were recorded with a microscope slide scanner. SCiLS Lab software was used for correlative data visualization.

### Preliminary data (results)

Murine as well as human retina consist of several cell layers, each providing highly specific physiological functions. In size, the layers range from an about 10  $\mu\text{m}$ -wide single cell retinal pigment epithelial (RPE) layer to about 60  $\mu\text{m}$  for the outer nuclear layer. These delicate morphologies pose high demands on an artefact-free sample preparation. Moreover, an unambiguous detection of CLs by MALDI-MSI poses high challenges with regard to the analytical sensitivity. Figure 1 A,B demonstrates, at the examples of CL(78:2) and CL(76:9), that with the here developed protocols high-quality differential ion images are obtained that readily allow to match individual CL species to individual cells. Overall about 30 different species of CL with specific molecular weight were recorded, all detected as  $[M - H]^-$  ions in the mass spectra. The overview spectra for three retinal layers and ocular muscle tissue are provided in Figure 2. Figure 1 C,D furthermore highlights, at the example of overlays, an excellent correlation between the MSI data and scanning microscopy examination of the H&E-stained sections following the MALDI measurement. Not least, this correlative approach enhances the fidelity of layer assignment.

Low-energy CID experiments furthermore revealed that several of the registered groups of CLs actually reflect isomers with varying acyl chain compositions. For example, in the RPE and both plexiform layers CL(72:7) mostly consists of CL(18:2\_18:2/18:2\_18:1), whereas it is mostly composed of CL(16:0\_22:6/16:0\_18:1) in the photoreceptor inner segment.

### Please explain why your abstract is innovative for mass spectrometry?

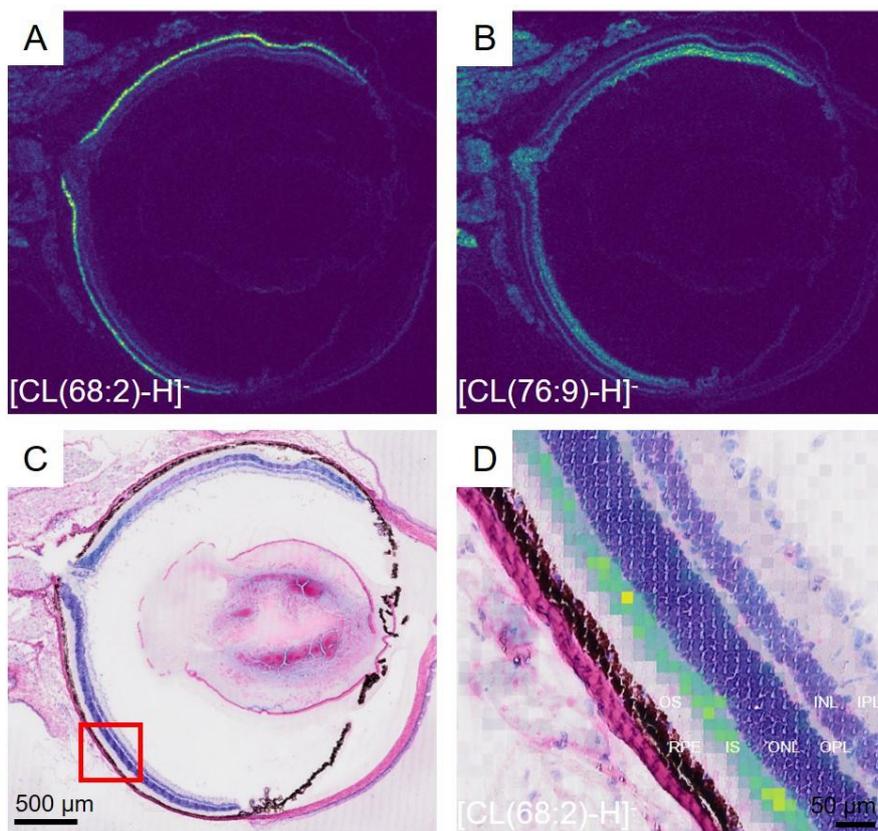
First MALDI-MSI data visualizing the highly differential expression of cardiolipins in murine retina cell layers at close to single-cell resolution.

### Co-authors:

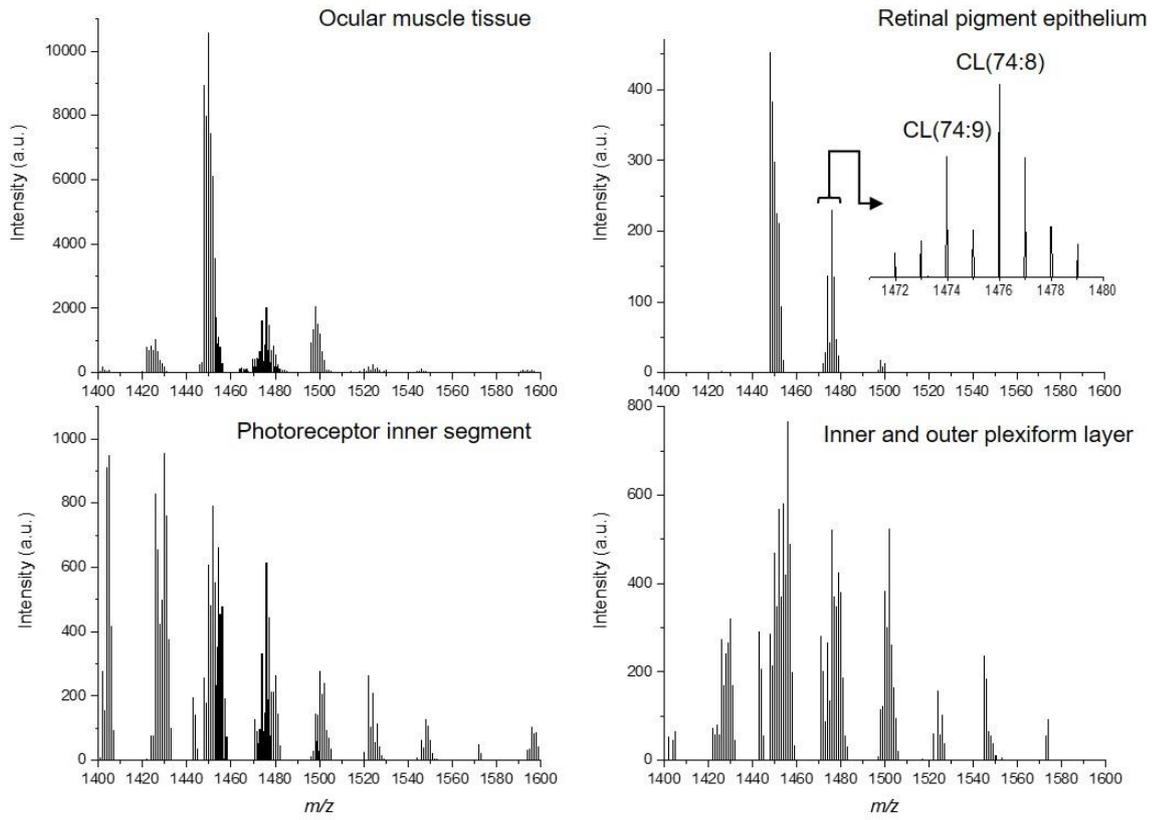
*Jens Soltwisch, Institute of Hygiene, University of Münster*  
*Klaus Dreisewerd, Institute of Hygiene, University of Münster*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Ion images of selected cardiolipin species for a mouse retina.



MALDI-MSI sum spectra recorded from individual retinal cell layers.

Poster number: LS-PB-053

## UNAMBIGUOUS VISUALIZATION OF GLYCOSE IN RENAL TISSUE BY USE OF MALDI-2-MS/MS IMAGING

Abstract ID: 215

Presenting author: Jens Soltwisch, Institute of Hygiene, University of Münster

### Introduction

Mass spectrometry imaging of small metabolites such as glucose is often hampered by the presence of isobaric or isomeric ion species. Consequently, the visualization of a specific  $m/z$ -value may not display the distribution of a single metabolite ion species but much rather an overlay of distributions for a number of molecular ions. To reduce this ambiguity, MS/MS can be employed on the targeted isolated  $m/z$ -region of interest. In this case, spatial distribution of fragment ions linked to a specific isobaric molecular precursor can be utilized to disentangle MS imaging results. Here we present how to employ MALDI-2-MS/MS on a timsTOF fleX instrument to visualize the distribution of glucose in murine renal tissue.

### Methods

Cryo-sections (16  $\mu\text{m}$  thick) from kidneys of mice that received a perfusion with glucose solution and fasting controls were covered with norharmane matrix using ultrasonic spraying. MALDI-2-MS/MS imaging analysis was performed on a timsTOF flex (Bruker Daltonik) at 20  $\mu\text{m}$  pixel size.  $m/z$ -regions of 1 Da width were selected for the deprotonated monoisotopic molecular ion of natural glucose as well as isotopically labeled glucose ions and fragmented at 20 eV collision energy. SCiLS lab software (Bruker) was used for visualization of MSI data.

### Preliminary data (results)

For the analysis of glucose, MALDI-MSI in positive ion mode typically produces sodiated molecular ions. Upon collision-induced dissociation (CID) this ion species does not produce sizeable amounts of diagnostic fragments. Employing MALDI-2 post-ionization, on the other hand, deprotonated glucose ion species are readily detected that yield diagnostic ring cleavage fragments in CID (Fig. 1). Applied to MS imaging analysis, the technique allowed for the unambiguous visualization of glucose in murine renal tissue at a pixel size of 20  $\mu\text{m}$ . While in fasting mice the analyte is mainly found in the renal cortex, in mice perfused with a glucose solution it is also prominently detected in the medulla and renal pelvis. Using isotopically labeled glucose moreover indicates an increased release of stored unlabeled glucose upon perfusion.

Next to glucose, fragments from a number of isomeric or isobaric ions in the same precursor  $m/z$ -window produce specifically different spatial distribution within the kidney. Out of these was identified as deprotonated inositol based on diagnostic fragments (Fig.1), an ion species isomeric to the respective glucose ion. Inositol is prominently detected from the renal pelvis and medulla.

### Please explain why your abstract is innovative for mass spectrometry?

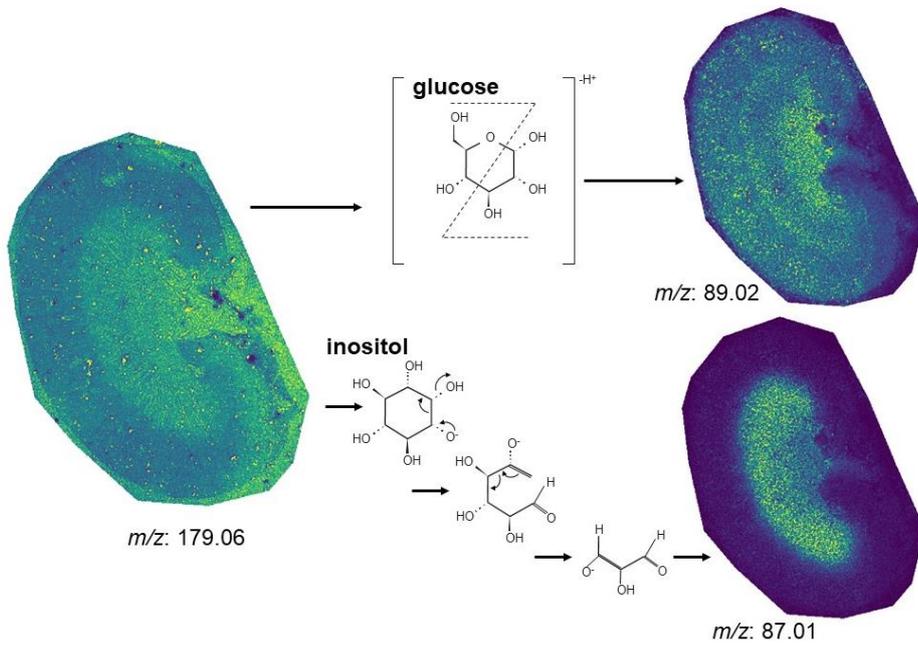
Visualization of isomeric small metabolite ion species using MALDI-2-MS/MS imaging

### Co-authors:

Verena Hoerr, Hybrid MRI physics Group, TRIC, University of Münster

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Visualization of the intensity distribution of precursor, glucose and inositol

Poster number: **LS-PB-054**

## VISUALIZING TYROSINE PATHWAY ALTERATIONS IN A CELL LINE DERIVED XENOGRAFT MODEL

Abstract ID: **226****Presenting author: Tobias Bausbacher, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, 68163 Mannheim, Germany**

### Introduction

Tryptophan catabolizing enzymes (TCE) play a major role in cancer cell migration and metastasis in multiple types of tumors. Especially in glioma patients, high levels of these enzymes are associated with low survival rates. These effects are elicited through the catabolism of tryptophan (Trp) into metabolites that interact with ligand-activated transcription factors highly relevant in cancer progression. Recently a new TCE was identified that plays a key role in glioblastoma and requires a deeper investigation by targeted MALDI MS Imaging (MSI). While the main effects stem from Trp catabolism, other amino acids are also catabolized by this enzyme, such as tyrosine (Tyr). Therefore, monitoring Tyr pathway alterations in glioma is paramount for understanding brain tumor progression.

### Methods

Four different mouse cohorts with xenografted human U87 glioblastoma cells in CD1 nu/nu mouse brain (enzyme overexpressed, transcription factor knock-down, enzyme overexpressed and receptor knock-down, and control) were analyzed. High resolution mass spectrometry imaging (HRMSI) using a 7T FT-ICR (Bruker Daltonics) was used to visualize metabolic changes in the xenograft region. On-tissue chemical derivatization (OTCD) was applied to specifically increase the detectability of tyrosine and its metabolites.

### Preliminary data (results)

We detected and imaged at high spectral resolution key metabolites associated with tyrosine catabolism through FMP-10 derivatization. The enzyme-overexpressing tissue showed Tyr depletion and accumulation of its immediate and downstream metabolites while the control tissue showed the opposite distribution. These findings are consistent in all 6 biological replicates.

### Please explain why your abstract is innovative for mass spectrometry?

The use of HRMSI in combination with an OTCD approach applied to a large mouse cohort.

### Co-authors:

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*Shad Mohammed, Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), Mannheim University of Applied Sciences, 68163 Mannheim, Germany*

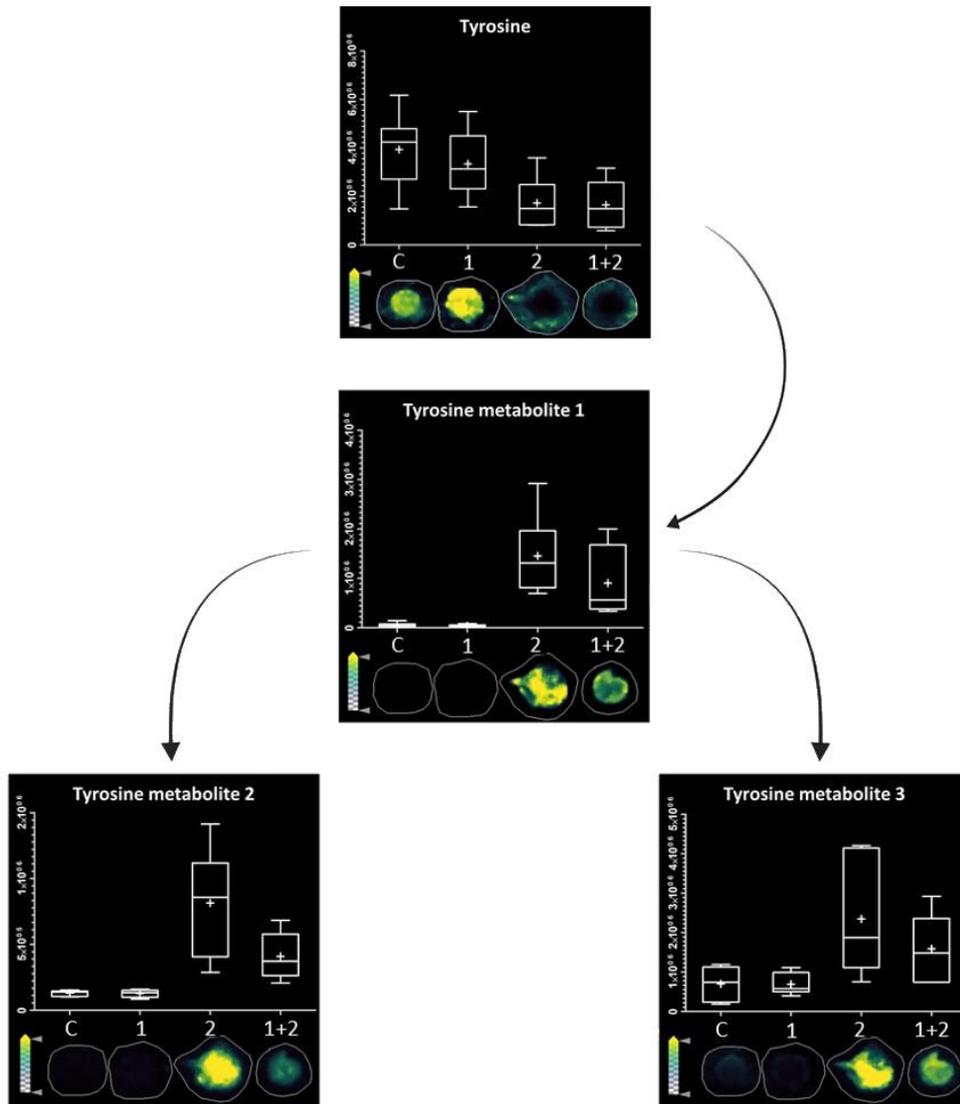
*Luis Felipe Somarribas Patterson, DKTK Brain Cancer Metabolism Group, German Cancer Research Center (DKFZ), Heidelberg, German, Faculty of Bioscience, Heidelberg University, 69120 Heidelberg, Germany*

*Pauline Pfänder, DKTK Brain Cancer Metabolism Group, German Cancer Research Center (DKFZ), Heidelberg, German, Faculty of Bioscience, Heidelberg University, 69120 Heidelberg, Germany*

*Soumya Ranjan Mohapatra, DKTK Brain Cancer Metabolism Group, German Cancer Research Center (DKFZ), Heidelberg, German, Faculty of Bioscience, Heidelberg University, 69120 Heidelberg, Germany*

*Christiane Agnes Opitz, DKTK Brain Cancer Metabolism Group, German Cancer Research Center (DKFZ), Heidelberg, German, Neurology Clinic and National Center for Tumor Diseases, Heidelberg, Germany*

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Distribution of tyrosine and its metabolites in the xenograft region.

Poster number: LS-PB-055

## INVESTIGATING APOE-GENOTYPE AND AGE-DEPENDENT PLAQUE-ASSOCIATED LIPID CHANGES IN AN ALZHEIMER DISEASE MOUSE MODEL USING MATRIX ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY IMAGING

Abstract ID: 238

Presenting author: Christel Kuik, M4i, Maastricht university

### Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder expressed by progressive memory loss and normal brain functioning. One of the neuropathological features of the disease is an abnormal accumulation of amyloid- $\beta$  peptides (A $\beta$ ), resulting in the formation of senile plaques in the brain. The primary risk factors for AD are aging and carrying the  $\epsilon$ 4 allele of the apolipoprotein E gene (APOE4). APOE is involved in cholesterol metabolism, lipid transport and clearance of A $\beta$ . However, the APOE4 clearance method is less effective than the APOE3 variant. Currently, no research has investigated whether individual A $\beta$  plaque-associated lipids changes are associated with AD-related APOE-genotype and age. Hence, this study applied MALDI-MSI for the spatial distribution of lipid profiles by age and APOE-genotype in individual plaques.

### Methods

All mice homozygously expressed five familial AD mutations (5xFAD) crossed with the human APOE3 (E3FAD) or APOE4 (E4FAD) gene. Subsequently, three age groups (2, 5-7, and 9+ months) were selected in each genotype condition ( $n=3$ ). The collected, snap-frozen, mouse brains were cryosectioned at 12  $\mu$ m and sublimed with 2,5-Dihydroxybenzoic acid matrix before being analyzed using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) on a RapifleX Tissue typer at 20  $\mu$ m lateral resolution in positive-ion mode. After MALDI-MSI analysis, the sections were stained by immunohistochemistry to locate the A $\beta$  plaque regions. Data were analyzed using SCiLS lab software.

### Preliminary data (results)

A principal component analysis showed different lipid profiles between A $\beta$  plaque regions and non-plaque areas in E3FAD, and E4FAD genotypes. Accumulation of  $m/z$  values 524.39, 534.30 and 562.36 was found within the A $\beta$  plaque regions, potentially corresponding to lysophosphatidylcholines (LPCs) [LPC 18:0 + H]<sup>+</sup>, [LPC 16:0 + K]<sup>+</sup>, [LPC 18:0 + K]<sup>+</sup> respectively. Furthermore, an increase in lipid distribution of amyloid plaques was seen between the three different age groups (figure 1). Additionally, gender-specific lipid profiles were detected. These results will add insights into the molecular mechanisms that lead to the development of AD in patients with different APOE gene expressions and sex.

### Please explain why your abstract is innovative for mass spectrometry?

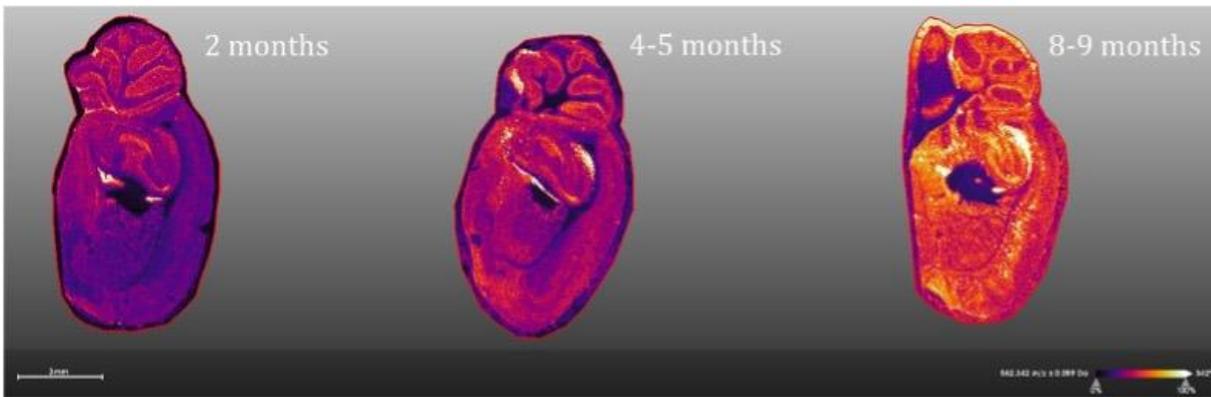
Our protocol developed to study plaque-specific lipid characterization by MALDI-MSI, will add unique novel information on local lipid changes between different Alzheimer genotypes.

### Co-authors:

*Daan van Kruining, Maastricht university Department of Psychiatry and Neuropsychology*  
*Berta Cillero Pastor, M4i, Maastricht university*  
*Maarten Honing, M4i, Maastricht university*  
*Pilar Martinez-Martinez, Maastricht university Department of Psychiatry and Neuropsychology*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



m/z 562.36, showing increased accumulation in the A $\beta$  plaques.

Poster number: LS-PB-056

## MALDI MASS SPECTROMETRY IMAGING WORKFLOWS FOR THE ECOTOXICOLOGICAL MODEL ORGANISMS DAPHNIA MAGNA, DANIO RERIO AND EISENIA FETIDA

Abstract ID: 239

**Presenting author:** Matthias Ochs, Matthias Ochs, Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Germany, Elisabeth Schirmer, Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Germany, Sven Ritschar, Animal Ecology 1, University of Bayreuth, Germany, Christian Laforsch, Animal Ecology 1, University of Bayreuth, Germany, Stefan Schuster, Animal Physiology, University of Bayreuth, Germany, Andreas Römpf, Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Germany

### Introduction

Environmental toxicology aims to understand the sources, fate and effects of chemicals released into the environment. A classical approach to understand toxic mechanisms involves the use of scientifically well-characterized model organisms. In ecotoxicological studies targeting aquatic ecosystems, *Daphnia magna* (waterflea) and *Danio rerio* (zebrafish) are established model organisms while the compostworm *Eisenia fetida* has proven to be a suitable candidate for the investigation of terrestrial ecosystems. MALDI-MSI is a powerful technique for the visualization of molecules within tissue sections. However, for *E. fetida* and *D. rerio* only few and for *D. magna* no suitable MALDI-MSI imaging workflows are existent. Here we introduce three MALDI-MSI workflows for the analysis of *D. magna*, *D. rerio* and *E. fetida* tissue sections with high-spatial resolution (5 to 25  $\mu\text{m}$ ).

### Methods

*D. magna* clones were cultivated on an artificial M4 medium and daily fed with green algae. *D. rerio* fish were held in commercial fish tanks and daily fed with commercial fish food. *E. fetida* worms were held on a substrate recommended by the OECD for ecotoxicological testing. For cryosectioning, worms and fish were quick frozen in LN and subsequently embedded in 3% CMC while daphnids were embedded in 8% gelatine. pNA-matrix was applied using a home-build semi-automatic pneumatic sprayer. Measurements were conducted using a Q-Exactive HF (Thermo Scientific, Bremen, Germany) coupled to an AP-SMALDI-AF5 ion source (TransMIT, Gießen, Germany).

### Preliminary data (results)

In this work, a particular emphasis was put on the development of cryosectioning protocols that preserve the tissue integrity while maximizing the signal intensity during the MALDI-MSI measurement. Crucial factors such as the choice of embedding media, the embedding process, optimal sectioning temperature and thickness were carefully optimized. The sectioning of daphnids still represents a challenge as their encapsulating carapaces which consist of amorphous calcium carbonate tends to fracture during cryosectioning leading to disruption of the body integrity. To overcome this hurdle, daphnids were allowed to swim in a vial filled with gelatin solution. This step is to ensure that the cavity within the carapace and its appendices are filled with embedding medium which strongly contributes to the tissue stability during cryosectioning. Doing so, we were able to obtain coronal sections of 18  $\mu\text{m}$  thickness with preserved tissue integrity. The subsequent MALDI-MSI measurements revealed distinct lipid distributions in embryos/eggs, the carapace lining and parts of the thoracic legs. Sagittal sections (20  $\mu\text{m}$ ) of *D. rerio* fish were sectioned at -15 °C. We were able to obtain distinct lipid-distributions showing anatomical structures of the eye, the brain, gill filaments and the liver. In case of *E. fetida*, the worms were anesthetized using 7% magnesium chloride to enhance muscle relaxation and subsequently embedded in CMC. Sectioning was performed at -20 °C with 20  $\mu\text{m}$  thickness. The resulting lipid ion images showed prominent histological features such as the typhlosolis, the dorsal blood vessel, the colon and the muscle ring.

### Please explain why your abstract is innovative for mass spectrometry?

This work introduces optimized MALDI-MSI workflows for the chemical imaging of important aquatic (*D. magna*, *D. rerio*) and terrestrial (*E. fetida*) model organisms.

Poster number: LS-PB-057

## LASER POST-IONIZATION AS A TOOL IN MASS SPECTROMETRY IMAGING OF PLANT-BACTERIA SYMBIOSIS

Abstract ID: 249

Presenting author: Benjamin Bartels, M4i, University Maastricht

### Introduction

Plants are generally unable to fixate atmospheric dinitrogen and thus are limited by the inorganic nitrogen available in their surroundings to assimilate nitrogen required for growth. Plants of the *Azolla* genus have overcome this challenge through symbiosis with the cyanobacterium *Nostoc azollae*, which are able to assimilate atmospheric dinitrogen directly, making *Azolla* species highly productive. They are known also to produce large amounts of flavonoid compounds, including deoxyanthocyanidins and proanthocyanidins, which have been hypothesized to mediate the molecular communication between the symbiotic partners. Investigation into the metabolite profile and how it is changing with respect to environmental conditions may lead to an improved understanding of the underlying mechanisms and ultimately to better applications of *Azolla* species as biofertilizer and crop.

### Methods

An Ekspla NT230 tunable laser system was connected to a Spectroglyph MALDI/ESI injector ion source to allow MALDI-2 experiments being conducted on an Orbitrap Elite mass spectrometer. Standards of flavonoid compounds, e.g. deoxyanthocyanidins and proanthocyanidins, were used to determine the optimal experimental parameters for laser post-ionization, subsequently used for MSI experiments on *Azolla* sections, as well as confirming the identity of molecular species ionized from the plant tissue via accurate mass and MS/MS. The optimized methodology was applied to investigate the influence of different biotic and abiotic stresses on the flavonoid profile of *Azolla* plants and symbionts.

### Preliminary data (results)

Recent developments in MALDI-MSI, employing a secondary laser for post-ionization (MALDI-2), demonstrated significant increases in ion yield and broadened the scope of molecules detectable [1-3]. Additionally, recent work has shown the MALDI-2 is also capable of ionizing additional analytes via a direct a resonance-enhanced multiphoton ionization (REMPI) process that can selectively enhance the ions yields of aromatic, UV active compounds, such as endogenous antioxidants like vitamin E and ubiquinol [4]. Such REMPI processes result in a complete decoupling of the laser desorption and ionization event and enables sensitive detection at reduced desorption laser energies that results in smaller effective spot sizes and thus facilitates higher resolution imaging. In this work, we investigate the utility of laser post-ionization processes (e.g. REMPI) during MALDI-2 experiments for the detection and imaging of bioflavonoids and other metabolites within plants of the *Azolla* genus. Classic MALDI-MSI experiments served as a testbed for optimizing the sample preparation protocols and as a baseline from which to judge the gained utility of the laser post-ionization. In Figure 1, an optical image (left) of a *Azolla filiculoides* cryosection post-sublimation of 2,5-dihydroxybenzoic acid is shown together with the spatial distribution of  $m/z$  269.045 (right), putatively assigned to the deprotonated ion of the deoxyanthocyanidin Luteolinidin.

[1] Soltwisch et al. 2015, *Science*, 348(6231), 211-215

[2] McMillen et al. 2020, *J Mass Spectrom*, 2020, 55, e4663

[3] Barré et al. 2019. *Anal. Chem.*, 91(16), 10840-10848

[4] Sarretto et al. 2021, *Anal. Sens.*, 1, 1-6

### Please explain why your abstract is innovative for mass spectrometry?

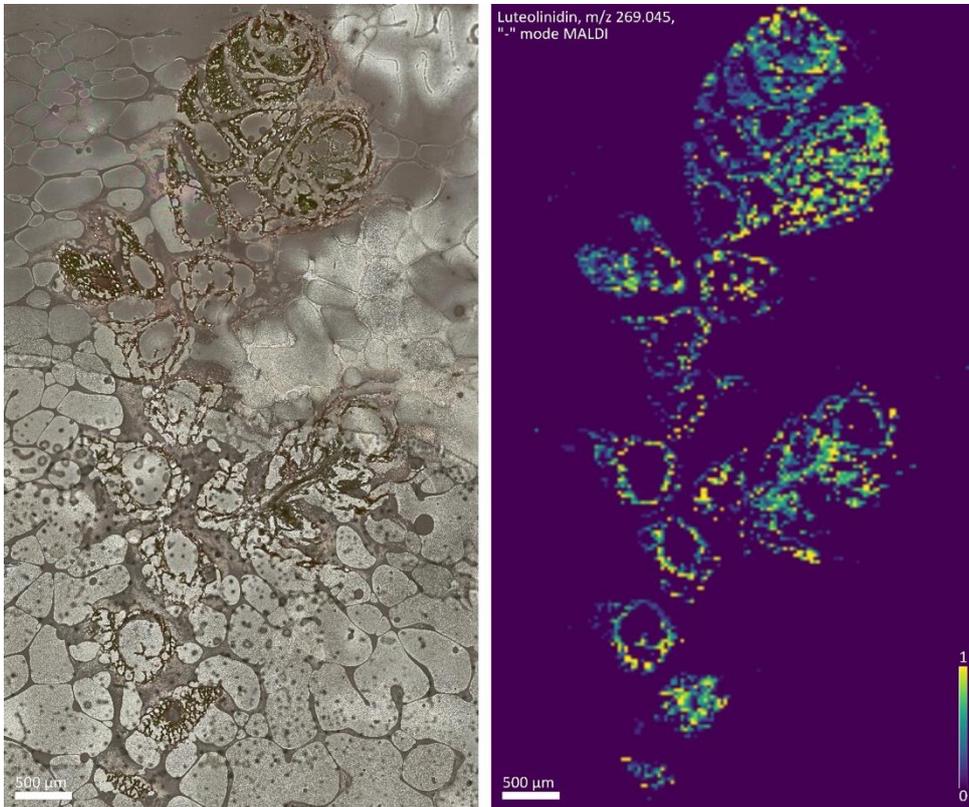
Laser post-ionization (e.g. REMPI) may increase the spacial resolution and lower the limits of detection of UV-active specialized metabolites in MALDI-2 MSI of plants.

Co-authors:

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

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*Henriette Schlupepmann, Molecular Plant Physiology, Utrecht University*  
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Optical image and ion map of an *Azolla filiculoides* cryosection.

Poster number: LS-PB-058

## NEONATAL BRAIN METABOLOME AFTER HYPOXIC-ISCHEMIC INSULT: A MASS SPECTROMETRY IMAGING STUDY IN NEWBORN RATS

Abstract ID: 254

**Presenting author:** Hynek Mácha, Institute of Microbiology of the Czech Academy of Sciences, Videňská 1083, Prague 142 00, Czech Republic, Department of Analytical Chemistry, Faculty of Science, Palacký University, 17. listopadu 12, Olomouc 771 46, Czech Republic

### Introduction

Neonatal brain hypoxic-ischemic (HI) injury represents a major cause of mortality and morbidity in newborns. The pathology is characterized by two phases of brain energy metabolism failure. Primary failure occurs under hypoxia due to a lack of oxygen. The secondary failure occurs under reoxygenation due to hypoxia-induced calcium overload of mitochondria. Importantly, the degree of secondary failure determines the overall severity of the HI insult and thus is responsible for the neonate's outcome.

### Methods

In the present study, using 7-day old rat pups, we focused on metabolite changes in HI-affected brain regions at 12, 24, and 36 hours after experimental HI insult. By means of high-resolution MALDI Fourier transform ion cyclotron resonance mass spectrometry imaging (MSI) using the FMP-10 derivatization method, metabolic and neurotransmitter profiling was spatially visualized and relatively quantified in brain sections at striatal, hippocampal, and substantia nigra levels. Revealed molecular changes were interrelated to findings of Nissl staining histology from corresponding sections.

### Preliminary data (results)

At a 12-hour time point, significant metabolite alterations due to HI insult were found mainly in the cortex, thalamus, and hippocampus; particularly, an increase of polyamines (putrescine, cadaverine, 3-aminopropanol), amino acids (histidine, glycine, valine), and phosphocreatine. In parallel, signs of starting neurodegeneration, including shrunken neurons with condensed chromatin, were detected in the HI-affected regions. From 24 hours after the insult, monoamine neurotransmitter norepinephrine and epinephrine levels significantly increased in the caudate putamen and nucleus accumbens; loss of neurons, apoptotic cell death, and reactive astrogliosis were observed. At a 36-hour time point,  $\alpha$ -tocopherol was increased due to HI insult in globus pallidus, hippocampus, and thalamus; it tended to grow also in other brain regions. Predominantly necrotic cell death was found at this time point. Notably, antioxidant taurine was reduced in HI affected cortex and hippocampus in all three-time points, indicating an ongoing redox dysbalance. On the other hand, increased valine may reflect effective catabolism of glutamate excess responsible for excitotoxicity and increased phosphocreatine an involvement of the antiapoptotic process; overall, both mechanisms indicate activation of brain self-regenerative processes. In conclusion, by means of MALDI MSI, critical region-dependent metabolic events, including changes in the metabolism of macroergic phosphates, catecholamines, amino acids, and polyamines, were readily observed in the HI-insulted neonatal rat brain.

The authors gratefully acknowledge the support from the Czech Science Foundation (22-06771S), the internal grant Agency of Palacký University, Olomouc (IGA\_PrF\_2022\_023), and Slovak Grant Agency (VEGA-2/0166/20).

### Please explain why your abstract is innovative for mass spectrometry?

MALDI mass spectrometry imaging revealed key regionally-dependent metabolic events, including changes in energy and catecholamine metabolism, amino acids and polyamines system, and self-protection mechanism, following hypoxic-ischemic neonatal brain injury.

### Co-authors:

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Poster number: LS-PB-059

## SPATIAL OMICS TO IDENTIFY MOLECULAR REGULATORS OF SULFATIDE ABUNDANCE IN INTRAHEPATIC CHOLANGIOCARCINOMA

Abstract ID: 294

**Presenting author: Kasper K Krestensen, Maastricht Multimodal Molecular Imaging Institute (M4I), Maastricht University**

### Introduction

Cholangiocarcinoma (CCA) is a group of highly heterogeneous tumours arising in the epithelial cells of the biliary tree. CCAs are notoriously aggressive and most patients are asymptomatic during early stages of the disease and therefore present with advanced-stage cancer, once diagnosed, with a 5-year survival rate of <10% (Rizvi et al., *Nature Reviews Clinical Oncology*, 2018). Sulfatides are a group of lipids, within the liver uniquely found in the bile duct. We have recently observed an altered composition of sulfatides in certain CCA patients, characterized by early tumor recurrence (Huizing et al., submitted for publication). The goal of this project is to get a better understanding of the underlying molecular processes leading to altered abundance of sulfatide classes (e.g. hydroxylation- & saturation status) in CCA.

### Methods

Human intrahepatic CCA samples (n=10) were obtained through the biobank programmes of Maastricht University Medical Center and the University Hospital Aachen. Samples were cryosectioned and coated with norharmane-matrix for matrix-assisted laser desorption-ionization mass spectrometry imaging (MALDI-MSI). For lipid imaging, MSI experiments were conducted in negative ion mode on a Solarix-XR FT-ICR mass spectrometer with a pixel size of 25x25  $\mu\text{m}$  and within a mass range of 300-2000 m/z. Following MALDI-MSI, the samples were stained with haematoxylin for tumour region determination. Tumour areas with high sulfatide abundance were excised using a laser microdissection system and submitted to full proteomics analyses.

### Preliminary data (results)

We have recently observed an altered sulfatide metabolism in tumours from patients suffering from CCA or colorectal liver metastases. This project aims to further investigate which proteins/enzymes are causing the altered metabolism through targeted proteomics of high-abundance sulfatide tumour regions from the same samples. Initial results from the high-mass resolution lipid imaging of the CCA samples indicate an overall increased area of abundance of sulfatides compared to control samples (n=3). Furthermore, sulfatide abundance shows a spatial correlation with the tumour area, whereas healthy controls only show abundance in the bile duct, if at all. Given the high sulfatide abundance in the tumour tissue, it is of great interest to investigate the altered metabolism of these lipids in greater detail. Based on image co-registration with a haematoxylin stain of the measured sample, regions of high sulfatide abundance within a tumour region will be cut out using a laser microdissection microscope. The proteomic profile of the regions of interest will then be investigated using LC-MS to find potentially interesting dysregulated markers. Based on the previous experiments, lipid desaturases are of high interest, since there was a clear correlation between sulfatide saturation-levels and tumour recurrence in post-operative patients. Furthermore, other molecules that influence the synthesis of sulfatides are of significant interest as a general increase in sulfatide intensity has been observed in the CCA samples. Thus, with this lipid-imaging guided proteomics workflow we hope to shine a light on the regulatory molecules causing an altered sulfatide metabolism in CCA.

### Please explain why your abstract is innovative for mass spectrometry?

This project combines the spatial information of MSI and quantitative strengths of LC-MS through lipid imaging guided proteomics to provide a novel insight in CCA pathology.

### Co-authors:

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*Ron Heeren, Maastricht Multimodal Molecular Imaging Institute (M4I)*

Poster number: LS-PB-060

## DETERMINATION OF TISSUE ENDOGENOUS PROTEIN-LIGAND BINDING AFFINITIES AND PROTEIN CONCENTRATIONS USING LIQUID EXTRACTION SURFACE ANALYSIS MASS SPECTROMETRY

Abstract ID: 298

**Presenting author:** Bin Yan, National Centre of Excellence in Mass Spectrometry Imaging, National Physical Laboratory, UK

### Introduction

Native mass spectrometry (MS) has been extensively used in measuring protein-ligand binding affinities due to its capability to preserve intact proteins and protein complexes. As the analysis requires information on the protein concentration, most such studies were performed in solution phase. As such, surface and tissue sample studies are still lacking. Results from our solution-phase study indicate that protein-ligand binding affinity is independent of protein concentration at low values. Therefore, we proposed a concentration-dilution based approach to address the challenges in surface studies. This method has proven reliable in measuring binding affinities of ligands to standard proteins deposited on glass slides or spiked on homogenised tissue sections. It was also applied to study binding strength between common drug molecules and tissue endogenous proteins.

### Methods

The study was performed on a Waters Synapt G2Si mass spectrometer with an Advion Triversa Nanomate nano-electrospray (ESI) source. A customized, automated workflow consisting of surface sampling, concentration dilution of extracted proteins, protein-ligand mixing and infusion ESI-MS measurement were developed using Advion ChipSoftX with the new Developers Kit. The well characterized protein-ligand pair of lysozyme and N,N',N''-Triacetylchitotriose (NAG3) was studied both in solution and as surface samples. An additional tissue study examined binding affinities between mouser liver fatty acid-binding protein (L-FABP) and some common drug molecules including paracetamol, fenofibric acid and prednisolone.

### Preliminary data (results)

In the surface study of protein droplets deposited and air-dried on glass slides, the dissociation constant  $K_d$  between extracted lysozyme (theoretical max. concentration 20  $\mu\text{M}$ ) and NAG3 was measured to be  $\sim 95 \mu\text{M}$ . This was much larger than the  $K_d \sim 15 \mu\text{M}$  from the solution phase measurement, indicating that a high concentration of surface extracted protein is interfering with the measurement ( $\gg 0.5 K_d$ , i.e., 7.5  $\mu\text{M}$ ). After 10x and 100x dilution, the  $K_d$  value was measured to be 17.2  $\mu\text{M}$  and 15.5  $\mu\text{M}$ , respectively, which approximately reflected true binding strength of this protein-ligand pair. Besides, based on 10x diluted protein concentration below 0.1 $K_d$ , the surface extracted protein concentration was deduced to be less than 15.5-17.2  $\mu\text{M}$ . For lysozyme spiked on veal brain tissue homogenate sections, very similar affinity was obtained for NAG3 binding ( $K_d$  of 14.1-15.8  $\mu\text{M}$ ).

In the study of ligand binding to tissue proteins, it was found that FABP extracted from mouse liver did not bind to paracetamol, while binding to prednisolone and fenofibric acid was detected at the ratio of 1:1 and 1:2, respectively. The measured  $K_d$  was  $\sim 350 \mu\text{M}$  for prednisolone binding and  $\sim 50 \mu\text{M}$  for both 1st and 2nd fenofibric acid binding. Although as previously reported, the absolute  $K_d$  obtained by native MS could be two orders of magnitude higher (i.e., lower affinity) than that measured by other methods due to different buffer conditions, a satisfactory agreement was observed in terms of relative affinities from different ligands binding.

### Please explain why your abstract is innovative for mass spectrometry?

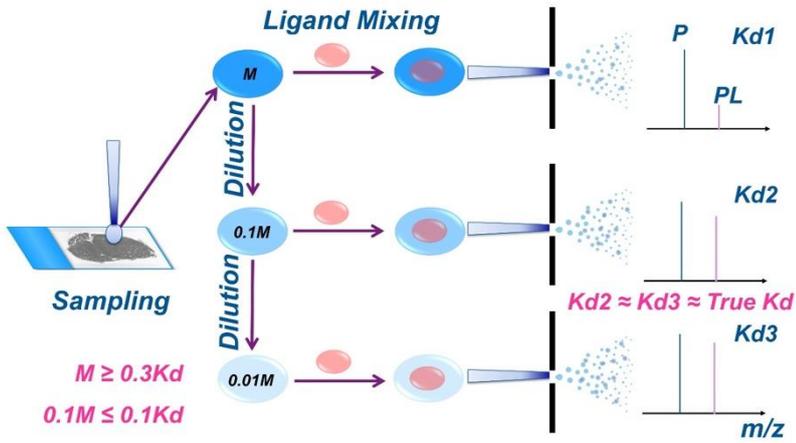
For the first time, native MS was applied to measure tissue endogenous protein-exogenous ligand binding affinities, meanwhile the proposed approach can be used for semi-quantification of sampled protein concentration.

### Co-authors:

Josephine Bunch, National Centre of Excellence in Mass Spectrometry Imaging, National Physical Laboratory, UK, Department of Metabolism, Digestion and Reproduction, Imperial College London, UK, Rosalind Franklin Institute, UK

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Automated workflow for measuring tissue protein-ligand binding affinities

Poster number: **LS-PB-061**

## CHRONOLOGICAL MAPPING OF MEDICATION IN HORSE HAIR BY MALDI-MSI

Abstract ID: **310****Presenting author: Bryn Flinders, Hair Diagnostix, Dutch Screening Group, Gaetano Martinolaan 63A, 6229 GS, Maastricht, The Netherlands.**

### Introduction

Hair is potentially a biological archive documenting environmental exposure and/or incorporation of compounds from blood. Traditionally, hair analysis is performed by chromatographic separation followed by mass spectrometry. Whilst it has become the gold standard, this still requires lengthy sample preparation and can only provide the results reflecting one-month increments. Recently, matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) is being explored as it requires fewer hairs, has simple sample preparation and the ability to provide more accurate and visual information based on the spatial resolution used. The work presented here shows the application of MALDI-MSI to monitor the distribution of medication in horse hair and demonstrate its potential usefulness as a screening tool for medication history or treatment compliance.

### Methods

Horse mane hair samples from a horse undergoing post-surgery treatment and a horse not undergoing any treatment were longitudinally sectioned using an in-house built device. The sectioned hair samples were coated with CHCA (10 mg/mL in 50% ACN with 0.2% TFA) using the Bruker ImagePrep. The samples were then imaged in positive ion mode using a Bruker rapifleX at a spatial resolution of 100 × 36 µm.

### Preliminary data (results)

The MALDI-MS images showed the presence of two overlapping bands at  $m/z$  237.16 and  $m/z$  291.15, which were approximately 5 mm long. Based on the average growth rate of horse mane hair (2 cm/month), this is approximately 7 days of growth. These were identified by exact mass tandem mass spectrometry measurements as procaine (a local anesthetic and marker for intramuscular penicillin G use) and trimethoprim (an antibiotic) respectively. This correlates with the known dosing times of these medications.

### Please explain why your abstract is innovative for mass spectrometry?

MALDI-MSI was used to visualize medication in horse hair samples and provide the potential time frame the medications were administered, which could potentially be applied to a health check.

### Co-authors:

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Poster number: **LS-PB-062**

## MULTIMODAL CHARACTERIZATION OF VASCULAR AMYLOID DEMENTIA IN POSTMORTEM HUMAN BRAIN TISSUE

Abstract ID: 311

Presenting author: Wojciech Michno, University College London

### Introduction

Cerebral amyloid angiopathy (CAA) that leads to dementia, has both hereditary and sporadic forms. Among genetic causes of CAA, mutations that affect either the BRI2 gene (Familial British Dementia [FBD] and Familial Danish Dementia [FDD]), or genes that are directly associated with altered amyloid- $\beta$  ( $A\beta$ ) levels in Alzheimer's disease. In CAA, deposition of shorter, less aggregation prone  $A\beta$ <sub>x-40</sub> rather  $A\beta$ <sub>x-42</sub> has been reported. FBD presents only with ABri peptides, while the FDD displays both ADan and  $A\beta$  peptides. These differences in aggregation have been suggested to be caused by differences in the C-terminus of the mutated BRI2 precursor protein.

### Methods

In this study, we interfaced matrix assisted laser desorption ionization (MALDI) based mass spectrometry imaging (MSI) of  $A\beta$ , ABri and ADan in CAA, FBD, and FDD, across occipital and frontal cortex with fluorescent amyloid staining using electrooptic amyloid probes, luminescent conjugated oligothiophenes (LCOs). Further, we performed laser microdissection pressure catapulting to isolate individual vessels and confirm the peptide sequences of underlying peptides using LC-MSMS. This combination allowed us to delineate both structural differences among CAA lesions, and their underlying peptide composition in respective diseases.

### Preliminary data (results)

Major findings include extensive N-terminal processing in the CAA cases, with only limited pyroglutamate formation at the N-terminus. In case of the BRI2 mutation associated cases, we observed extensive N-terminal processing and pyroglutamate formation that was slightly more prominent in case of FBD. Interestingly, while both the FBD and FDD displayed C-terminal processing, this was much more prominent in the FDD. Indeed, while we did observe  $A\beta$  peptides in FDD, they displayed only very limited spatial correlation with ADan peptides. From structural perspective, the amyloid in CAA cases appeared more densely packed than in BRI2 mutations. Finally, there were no difference between brain regions.

### Please explain why your abstract is innovative for mass spectrometry?

We developed a multimodal MALDI IMS analysis approach for peptide composition and structural characterization of amyloid lesions in post mortem human brain tissue.

### Co-authors:

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Poster number: LS-PB-063

## A DISTINCT PEPTIDE SIGNATURE FOR REMODELING AFTER MYOCARDIAL INFARCTION IN A MOUSE HEART

Abstract ID: 334

**Presenting author: Charles Henri X.L. Van Assche, The Maastricht MultiModal Molecular Imaging Institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, The Netherlands**

### Introduction

Myocardial infarction (MI) remains the most common type of cardiovascular disease and the main cause of death worldwide. MI leads to myocardial ischemia, subsequent molecular changes, inflammatory pathway activation, and remodeling. In this work we aim to find specific peptide signatures, linked to cardiac remodeling in the mouse heart after MI using matrix assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI).

### Methods

Mice susceptible for cardiac ischemic injury (miR-216a knock-out in a BL6CBAF1 background) were subjected to myocardial infarction by permanent ligation of the left coronary artery or sham surgery. 4 weeks after surgery, the animals were sacrificed, and the hearts were excised and formalin fixed paraffin embedded. Transverse sections underwent on-tissue digestion before peptide analysis using MALDI-MSI on a Rapiflex TissueTyper (Bruker, Bremen, Germany). Segmentation was performed in SCiLS to obtain region of interest information. From the selected ROIs, areas were dissected using the Leica LMD 7000 (Leica Microsystems, Wetzlar, Germany) and processed for proteomics to perform protein identification.

### Preliminary data (results)

Probabilistic latent semantic analysis (PLSA) revealed that peptides belonging to haemoglobin in blood ( $m/z$  1529.73) and actin in ventricles ( $m/z$  976.45 and 1198.70) were the biggest contributors in components 1 and 2, respectively. Component 5 highlighted peptide masses co-localizing with the affected area in the heart as assigned by Sirius Red stain. Further spectral clustering analysis suggested a decrease of  $m/z$  1906.84 and 2093.11 as compared to the unaffected area.

The segmentation results showed 17 clusters based on the peptide signature, we highlighted 3 clusters of interest; Cluster 5 (control region which is related to the healthy part of tissue), Cluster 10 (penumbra of the ischemic region), and Cluster 12 (ischemic region).

### Please explain why your abstract is innovative for mass spectrometry?

The use of protein MALDI-MSI in the spatialOMx workflow on mouse cardiac ischemic tissue provides supplementary informations on the pathways involved in such disease.

### Co-authors:

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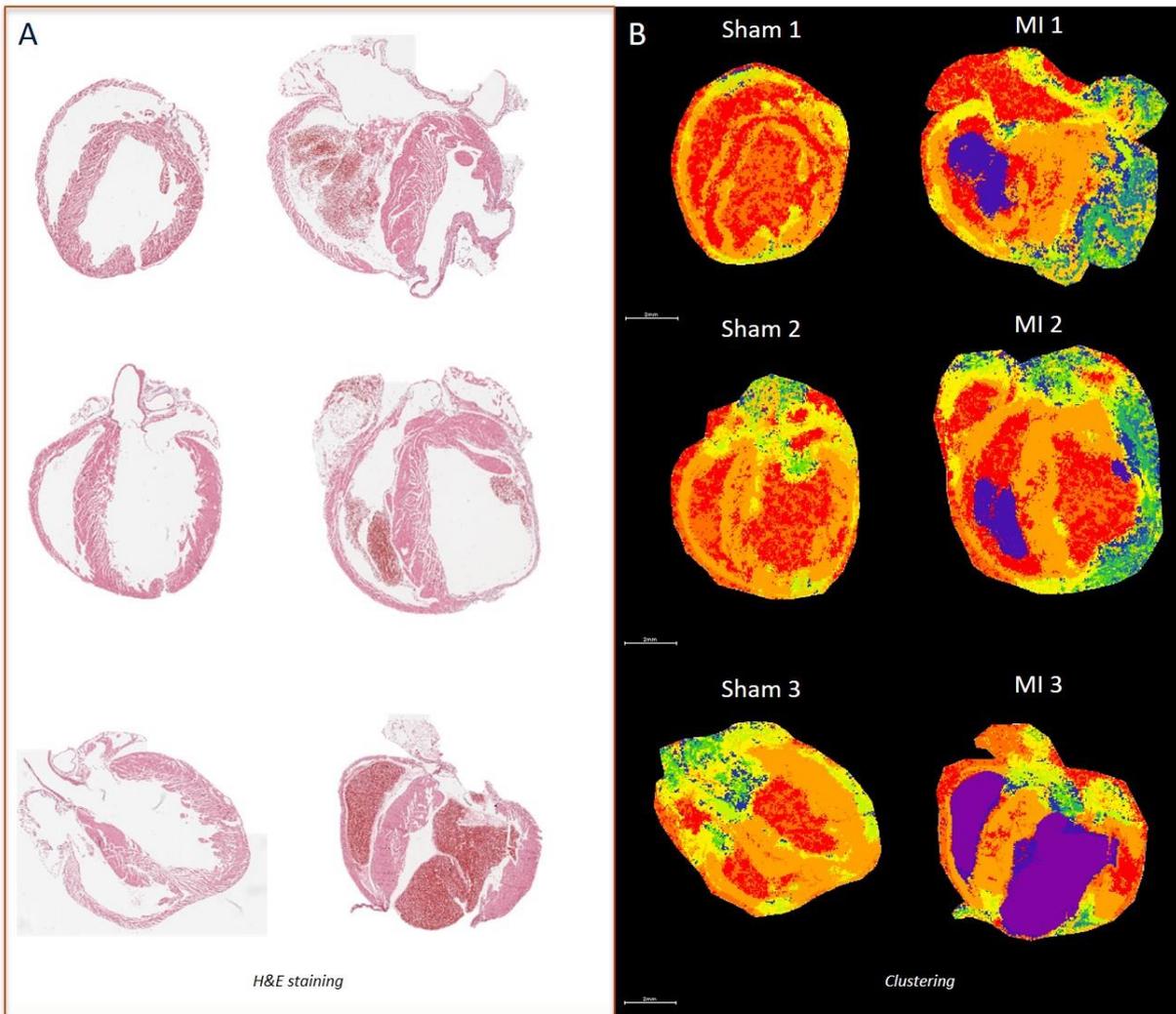
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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Segmentation analysis for Sham hearts and Ischemic hearts

Poster number: **LS-PB-064**

## COMPLEMENTARY BIOIMAGING FOR THE DETERMINATION OF METALLOPHARMACEUTICALS AND LIPIDS IN LIVER TISSUE

Abstract ID: **360****Presenting author: Katharina Kronenberg, Institute of Inorganic and Analytical Chemistry, University of Münster**

### Introduction

Liver cancer is the sixth most occurring cancer type worldwide. Symptoms and signs of liver cancer usually appear late in the course of the disease, which is often medicated by the antitumor agent cisplatin. Due to the late diagnosis, survival rates are poor and therefore, often palliative therapies are chosen. Hence, an early and reliable diagnosis of liver carcinomas and tumor stages plays an important role in suggesting an appropriate therapy and increasing survival rates. In this context, magnetic resonance imaging in combination with liver-specific contrast agents significantly improves the detection and characterization of liver lesions. This study focuses on the complementary determination of lipid species and the metal-based pharmaceuticals cisplatin and gadoxetic acid in liver lesions.

### Methods

Rats with liver tumors were treated with the gadolinium-based contrast agent gadoxetic acid as well as cisplatin and sacrificed 15 minutes after injection. The resection of tumor tissue with surrounding liver tissue allows the direct comparison of gadolinium, platinum and other endogenous elements as well as lipids in both tissue types. For elemental bioimaging, a 213 nm laser ablation system (LA) hyphenated to inductively coupled plasma-mass spectrometry (ICP-MS) revealed quantitative and spatially resolved information in the tissue thin sections. A complementary molecular imaging approach was used for the investigation of lipids via matrix-assisted laser desorption/ionization (MALDI) mass spectrometry.

### Preliminary data (results)

Elemental and lipid distributions were correlated with different pathological tissue types, which were examined by hematoxylin and eosin staining. Furthermore, gadolinium and platinum were quantified in regions of interest, providing information on how the uptake of gadoxetic acid and cisplatin varies in different tumor regions and stages. Liver lesions and tumors show decreased gadolinium and iron concentrations, indicating the expected low uptake of gadoxetic acid and the absence of iron-storing hepatocytes. In contrast, the copper content reveals accumulations while cisplatin is distributed heterogeneously in the various tissue types. Furthermore, the complementary MALDI-MS analysis supports the pathological findings on a molecular basis.

### Please explain why your abstract is innovative for mass spectrometry?

The combination of molecular and elemental mass spectrometry imaging methods allows an in-depth analysis of pathological tissue types.

### Co-authors:

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Poster number: LS-PB-065

## DISCRIMINATION OF MAMMARY TUMORS IN MICE WITH SPIDERMASS TOPOGRAPHICAL MASS SPECTROMETRY IMAGING

Abstract ID: 366

Presenting author: Nina Ogrinc, Laboratory PRISM, Inserm U1192, University of Lille

### Introduction

Surgery is a key approach for treatment and diagnosis of solid tumours. However, *in vivo* margin delineation during surgery remains a grand challenge, particularly in breast cancer. Despite several image guidance techniques and intraoperative assessment, clear surgical margins and debulking efficiency remain scarce. To reduce the risk of resections there is, therefore, an urgent need to develop rapid and sensitive intraoperative imaging tools. The SpiderMass, based on water-assisted laser desorption-ionization (WALDI), is an intraoperative MS technique which retrieves molecular information directly *in-vivo* and in real-time. Through robotic assistance, the SpiderMass, has recently paved its way to topographical molecular imaging of biological samples. It can, therefore, provide imaging of the defined areas within the body and help with the precise discrimination of tumoural and peritumoral regions.

### Methods

Tg C3(1)Tag mice with mammary tumors were imaged by robot-assisted SpiderMass. The SpiderMass is composed of a remote laser microprobe tuned to 2.94  $\mu\text{m}$ , a transfer line connected to a dedicated interface and a QTOF mass spectrometer. The system is coupled to a stiff 6D-axis precision MECA robotic arm (MECADEMIC) with repeatability of 5  $\mu\text{m}$ . The "home-made" 3D printed is equipped with a distance sensor. The distance sensor and the microprobe enable a simultaneous collection of topographical and molecular data with 1.6 pixels/s. The collected topographical and molecular data was subjected to AMX and Matlab developed software.

### Preliminary data (results)

Six Tg C3(1)Tag mice were sacrificed and exposed to reveal the locations of mammary tumors. The mice were placed under the distance sensor and the SpiderMass microprobe to simultaneously collect the topographical x, y, z coordinates and the corresponding molecular data at each pixel. Several tumors and the surrounding peritumoral and healthy tissue were imaged at 400-500  $\mu\text{m}$  spatial resolution within the exposed bodies of the mice. The tumors were then dissected and frozen for further *ex-vivo* analysis. The molecular data was coregistered to the topographical images and revealed specific molecular profiles allowing a clear discrimination between the tumoral, peritumoral tissues and the healthy mammary gland. The specific molecular profiles were then subjected to PCA-LDA analysis to create tumour and non-tumour tissue classification models. The developed classification models were then validated on three separate Tg C3(1)Tag mice. Each pixel of the topographical tumour image was classified using real-time recognition software. The *ex-vivo* collected tumours were then further cross-validated with Matrix-Assisted Laser Desorption Ionization mass spectrometry imaging displaying over 90% similarity between the molecular data obtained.

### Please explain why your abstract is innovative for mass spectrometry?

SpiderMass topographical molecular imaging of cancers and machine learning/deep learning solutions are a stepping stone for the next generation of *in vivo* molecular image guidance in a surgical room.

### Co-authors:

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Chann Lagadec, UMR9020 – UMR-S 1277 - Canther, University of Lille  
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Michel Salzet, Laboratory PRISM, Inserm U1192, University of Lille  
Isabelle Fournier, Laboratory PRISM, Inserm U1192, University of Lille

Poster number: LS-PB-066

## INVESTIGATION OF THE IDH1 MUTATION IN A CHONDROSARCOMA 3D IN VITRO MODEL USING MALDI-MSI

Abstract ID: 399

**Presenting author: Rick Ursem, Pathology, Leiden University Medical Center, Center for Proteomics and Metabolomics, Leiden University Medical Center**

### Introduction

Chondrosarcoma (CS) is the second most common primary malignant bone tumor. To date, the only treatment option is surgical removal due to CS resistance to both radiation and chemotherapy. However, around 10% of CS cases are unresectable due to their location or metastases. Thus, there is a need for novel therapeutic options, and therefore a better understanding of the underlying biology. Here, we focus on the effect of the isocitrate dehydrogenase 1 mutation (IDH1<sup>MUT</sup>), found in ~55% of CS cases, on the proteome and the extracellular matrix. Using isogenic wild type and mutant cell lines, chondrosarcoma 3D *in vitro* models, exhibiting a more representative extracellular matrix composition, were created. These were analyzed by bottom-up proteomics and Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI)

### Methods

An IDH1 wild-type (IDH1<sup>WT</sup>) CS cell line was transduced with a lentiviral vector encoding the IDH1<sup>R132C</sup> mutant gene. Both the IDH1<sup>WT</sup> and IDH1<sup>R132C</sup> cell lines were cultured as 3D chondrogenic pellets to better mimic the *in vivo* situation, with 3D cell-cell interactions, nutrient gradients, and abundant hyaline extracellular matrix (ECM) production. Chondrogenic pellets were fixed with formalin and embedded in paraffin. Sections were mounted onto a slide, underwent antigen retrieval, trypsin digestion then sprayed with  $\alpha$ -CHCA matrix for subsequent measurement. Mass spectrometry imaging was performed on a 12T MALDI-FTICR-MS, followed by data analysis in R.

### Preliminary data (results)

The chondrogenic pellets showed abundant ECM production, as well as a necrotic core which indicated a physicochemical gradient. These features were visible to a similar degree in IDH1<sup>WT</sup> and IDH1<sup>R132C</sup>. MSI analysis of chondrogenic pellets was able to identify distinct layers in these spheroids, which would normally be lost in typical bottom-up proteomics approaches. These layers likely correspond to the actively proliferating cells in the outer layers, and the non-proliferating cells in the inner layer. Next, using an interpretable machine learning approach it was possible to identify features distinguishing the IDH1<sup>WT</sup> and IDH1<sup>R132C</sup> CS pellets. The IDH1 gene is involved in metabolic processes, which provide energy but also the building blocks for cells. Here we observe that the IDH1 mutation results in proteomic alterations. On one hand, this can be due to the wide-spread downstream effects of the mutation. The IDH1 mutation is known to cause epigenetic alterations which alter downstream transcription and translation. On the other hand the IDH1 gene is additionally involved in the hydroxylation of ECM proteins, and the lack thereof may reorganize the ECM to make certain epitopes more or less accessible to trypsinization. Future work will focus on investigation of proteomic alterations which could be targetable for novel therapies.

### Please explain why your abstract is innovative for mass spectrometry?

This work shows the first characterization of a 3D chondrosarcoma model with MSI and the first proteomic imaging study of samples rich in type II collagen.

### Co-authors:

Julia Sidorov, Pathology, Leiden University Medical Center, Center for Proteomics and Metabolomics, Leiden University Medical Center

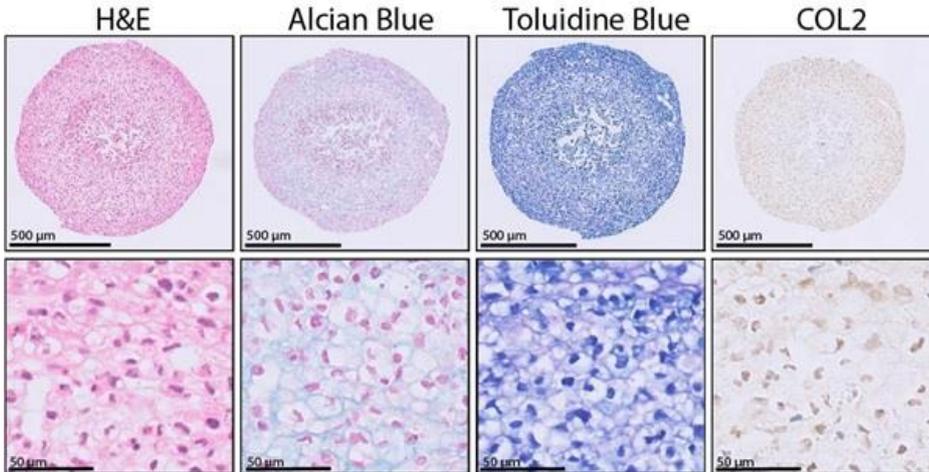
Ieva Palubeckaitė, Pathology, Leiden University Medical Center, Center for Proteomics and Metabolomics, Leiden University Medical Center

Inge Briaire-de Bruijn, Pathology, Leiden University Medical Center

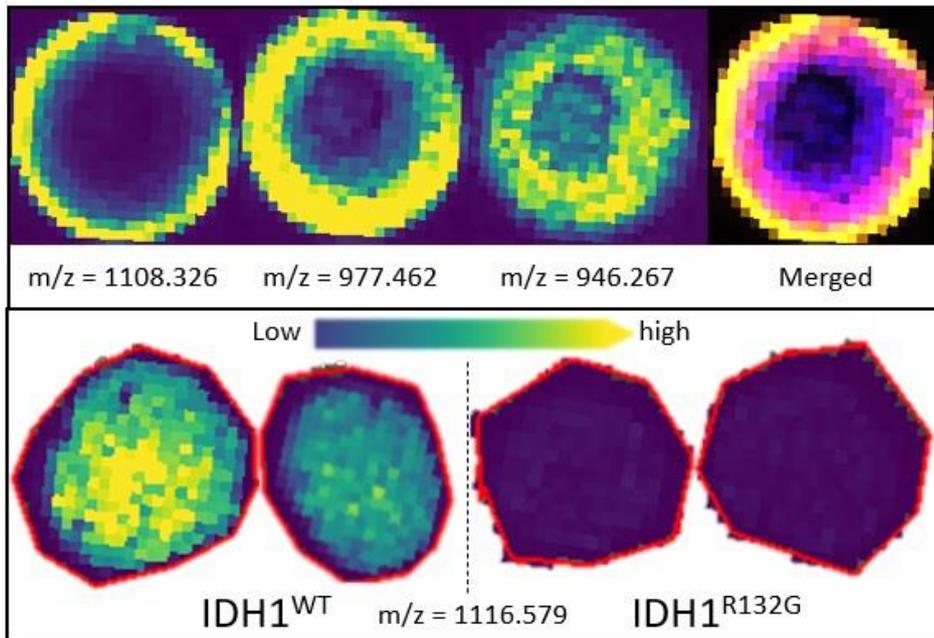
Liam McDonnell, Laboratory of Proteomics and Metabolomics, Fondazione Pisana per la Scienza Onlus

Bram Heijs, Center for Proteomics and Metabolomics, Leiden University Medical Center

Judith Bovee, Pathology, Leiden University Medical Center



**Figure 1.** Histology of IDH1<sup>R132C</sup> cell line chondrogenic pellets



**Figure 2.** MSI of peptides released from chondrogenic pellets

Poster number: LS-PB-067

## CHANGES IN LIPID METABOLISM AS EARLY INDICATOR FOR DIABETES INDUCED RENAL PATHOLOGY

Abstract ID: 404

**Presenting author: Rosalie Rietjens, Department of Internal Medicine (Nephrology) & Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands**

### Introduction

Diabetic nephropathy (DN), chronic loss of kidney function due to diabetes mellitus, is a common complication in diabetes currently lacking effective long-term treatment. Microalbuminuria is now the earliest marker for DN, thereby disregarding renal changes that occur before this first indication of decreased kidney function. Mass spectrometry imaging (MSI) allows to study lipid metabolism in the context of tissue histology without the need for labeling. Existing literature applying this technique focuses on later stages of DN, thereby overlooking its potential to serve at an earlier diagnostic stage. The aim of this project is to identify renal cell-specific lipid changes using MSI, which could serve as damage markers for early diabetes induced renal pathology.

### Methods

To study diabetic renal changes, apolipoprotein E-knockout mice were treated with streptozotocin (STZ) and put on an enriched cholesterol diet to induce diabetes. After 12 weeks, both control (n=4) and diabetic (n=4) mice were sacrificed and kidneys were harvested for immunohistochemical and matrix assisted laser desorption/ionization (MALDI) MSI. Post-MSI immunofluorescent staining in combination with tissue morphology was used to identify different renal cell types. Analysis of MALDI-MSI data allowed us to spatially segment the results per cell type for further statistical analysis of the metabolic MSI data (Figure 1A).

### Preliminary data (results)

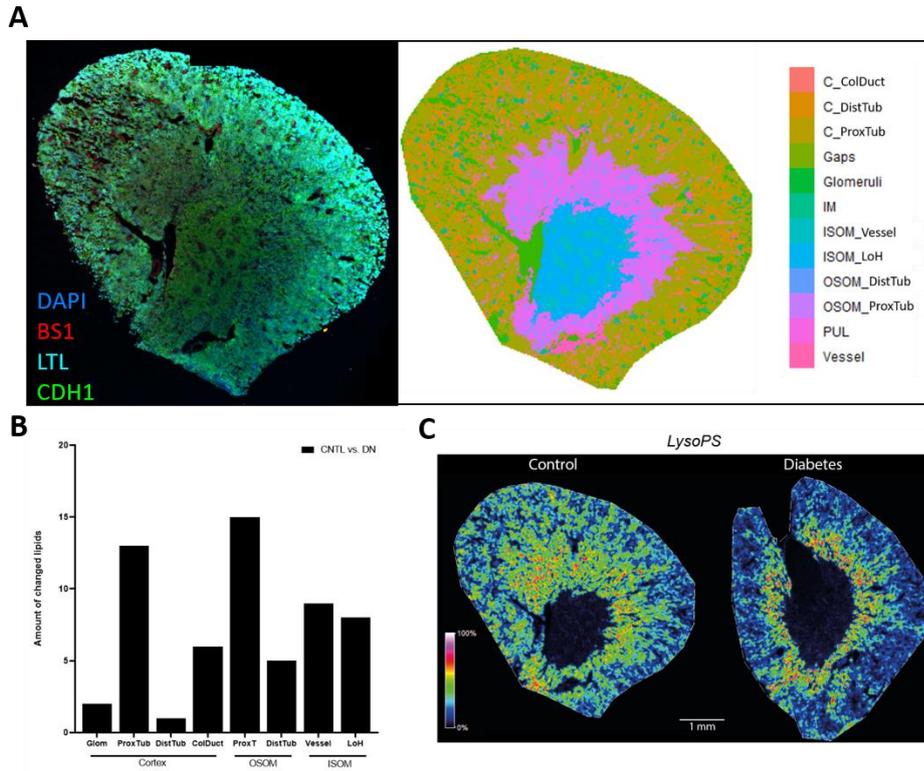
Two weeks after STZ induction, blood glucose levels of the diabetic mice were significantly elevated compared to control indicating successful induction of diabetes. Dimensionality reduction of the processed MSI data allowed us to identify 11 renal cell types with a unique molecular signature, which could be annotated using spatial segmentation analysis (Figure 1A). Using this immunofluorescence-based cell type annotation, we zoomed in on various morphological regions of the kidney to specifically test for changes in lipid profiles. Here, the proximal tubular cells in the cortex and outer stripe of the outer medulla had the highest number of significantly changed lipids (Figure 1B). Focusing the analysis on these two groups of cells revealed specific molecular signatures to be discriminative between diabetes and control (Figure 1C). These molecular signatures might serve as an early indicator of diabetes induced renal changes, thereby opening up a potential window for treatment before the kidney is damaged beyond repair.

### Please explain why your abstract is innovative for mass spectrometry?

MSI can provide new lipid biomarkers for diabetes induced renal changes. Linking these to human plasma and blood data might present new diagnostic strategies for earlier detection of DN.

### Co-authors:

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*Wendy Sol, Department of Internal Medicine (Nephrology) & Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands*  
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*Ton Rabelink, Department of Internal Medicine (Nephrology) & Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands*



**Figure 1. Identification of a cell type specific lipid profile indicating early kidney changes induced by diabetes.** Spatial segmentation analysis corresponds to renal cell type specific staining (A). Amount of lipid changed in different morphological regions of the kidney (B). Example lipid distribution in a control and diabetic mouse kidney (C).

Identification of cell type-specific lipid profile indicating diabetes-induced kidney changes.

Poster number: **LS-PB-068**

## **MALDI-MSI OF A THREE-DIMENSIONAL CELL CULTURE MICROARRAY '3MA'**

Abstract ID: **409**

**Presenting author: Ieva Palubeckaitė, Department of Pathology, Leiden University Medical Center**

### **Introduction**

We demonstrate a novel sample format called '3MA' which allows the transfer of 3D cell culture in vitro samples into microarrays for mass spectrometry imaging experiments, allowing higher repeatability and more efficient analysis. Compatibility and repeatability of the 3MA format was demonstrated using a selection of dissimilar cell types cultured as spheroids. Subsequently, doxorubicin treatment-induced proteomic changes were investigated within chondrosarcoma 3MAs. Chondrosarcomas are highly chemotherapy and radiotherapy resistant cartilaginous malignant bone tumors. Surgical resection is currently the only curative treatment, which is particularly detrimental in unresectable cases. Therefore, an enhanced analytical method would aid understanding of the treatment resistance mechanisms in the case of chondrosarcoma. 3MAs would additionally aid analysis of other large 3D culture model cohorts, where efficiency is essential.

### **Methods**

Three chondrosarcoma (CH2879, JJ012, SW1353), two osteosarcoma (SJS1, MHM, SAOS2), as well as colorectal carcinoma (HCT116), pancreatic carcinoma (PANC1) and breast carcinoma (MCF7) cell lines were cultured as multicellular tumor spheroids (MCTS) for 7 days. MCTS were collected, formalin-fixed, paraffin-embedded and used to create a 30-core '3MA mix' block, including 6 reference tissue cores. These were tested for use in pathology and MALDI-MSI workflows.

Chondrosarcoma MCTS were subsequently treated using varying concentrations of doxorubicin. These were used to create 40-core '3MA chondrosarcoma treatment' blocks (n=2), including 6 reference tissue cores, to investigate treatment-dependent variations in the proteome.

### **Preliminary data (results)**

MCTS of five different cancer types, originating in bone, cartilage, colon, pancreas and breast, were successfully cultured as MCTS and transferred to a 3MA format. The '3MA mix' block was used to demonstrate quality, reproducibility and applicability to many cell types, as well as compatibility of the format with typical lipidomic, glycomic and proteomic MALDI-MSI workflows. Section to section reproducibility was demonstrated using histological staining of sections throughout the block (Figure 1). The '3MA mix' block produced approximately 40 total sections, each containing >54% of the MCTS cores. To achieve coverage of all cores within the block, two sections from differing depths could be utilized. The 3MA format was demonstrated to be compatible and reproducible with MSI, using both TOF and FT-ICR-based MS instruments.

Untargeted proteomic investigation of doxorubicin-treated chondrosarcoma MCTS demonstrated treatment group specific species. These results require further investigation to examine chondrosarcoma chemotherapeutic resistance pathways.

### **Please explain why your abstract is innovative for mass spectrometry?**

This is the first demonstration of large-scale 3D cell culture microarrays, fully compatible with pathology workflows, as well as MSI. Use is demonstrated with difficult to handle 3D cultures.

### **Co-authors:**

*Inge Briare-de Bruijn, Department of Pathology, Leiden University Medical Center*

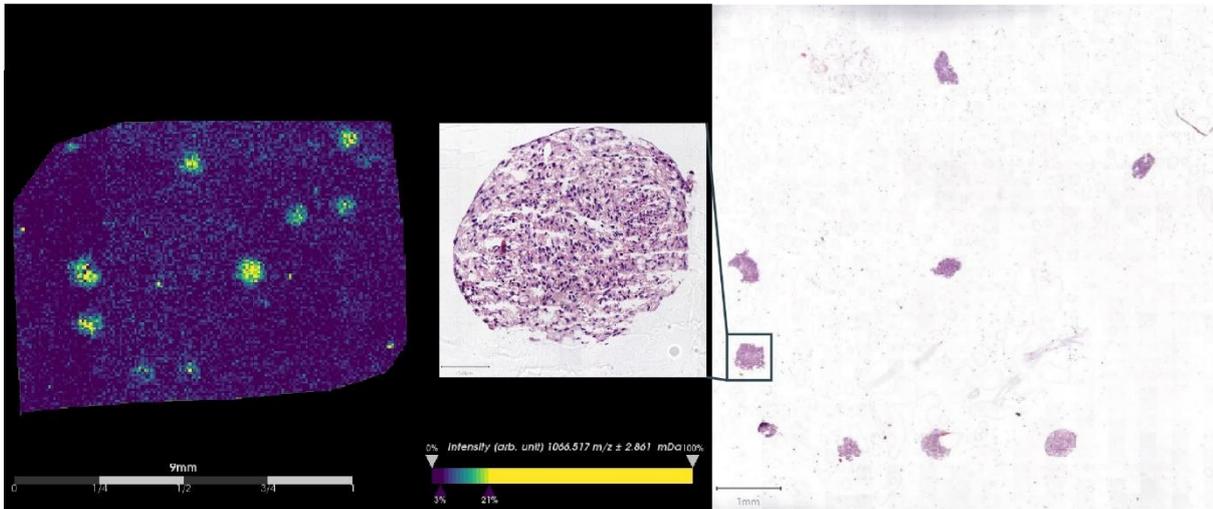
*Hans Dalebout, Department for Proteomics and Metabolomics, Leiden University Medical Center*

*Bram Heijs, Department for Proteomics and Metabolomics, Leiden University Medical Center*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Liam McDonnell, Fondazione Pisana per la Scienza ONLUS*  
*Judith Bovee, Department of Pathology, Leiden University Medical Center*



3MA block peptide image (MALDI-MSI) and MCTS histology (H&E).

Poster number: LS-PB-069

## CORRELATED LIPIDOMICS MASS SPECTROMETRY IMAGING AND SPATIALLY RESOLVED PROTEIN/TRANSCRIPTOME MAPPING TO UNDERSTAND THE PROGRESSION OF INFECTIOUS DISEASES

Abstract ID: 422

**Presenting author: Jianhua Cao, Maastricht MultiModal Molecular Imaging Institute (M4i), Division of Imaging Mass Spectrometry, Maastricht University, the Netherlands**

### Introduction

Infectious diseases are usually characterized by local inflammation of the infected tissues. Research shows that our ability to control infection is determined among others by the metabolic response of our immune cells which can result in the production of different types of lipids. What is not known is how immune cells regulate lipid metabolism in complex tissue microenvironments. In this study, we aim to correlate lipidomics, mass spectrometry imaging (MSI) and spatially resolved protein/transcriptome mapping to holistically understand the progression of leishmaniasis.

### Methods

Matrix-assisted laser desorption/ionization MSI was used for non-targeted lipidomics of *L. donovani*-infected mice liver sections at two different time points (day 28 and day 36, n=5 per time point) of infection, in comparison with healthy controls. MSI guided regions of interest were excised by laser microdissection and analysed with liquid chromatography-MS/MS based lipidomic. In addition, the spatially resolved protein and transcriptome profiles from consecutive sections were obtained by a Nanostring GeoMx Digital Spatial Profiler and a Genomics Visium platform, respectively. Later, spatial lipidomics data is coupled to the proteomic and transcriptomic data using bioinformatic and machine learning approaches.

### Preliminary data (results)

Preliminary MSI data indicates distinct lipid composition differences in inflammatory hepatic granulomas and surrounding parenchyma. Moreover, in granulomas, heterogeneous spatial distributions of lipids and proteins were observed with combined MSI and Nanostring GeoMx Digital Spatial Profiler (DSP), respectively. A more in-depth lipids characterization of MSI-highlighted regions of interest (ROIs) is unveiled with conventional LC-MS/MS based lipidomics. Following the same approach, the heterogeneity at the protein or transcriptome level is further investigated with targeted DSP. Dynamic changes of different molecules over time upon infection will be further evaluated. Finally, complementing spatially resolved lipidomics, proteomics and transcriptomics datasets will allow the identification of specific enzymatic pathways related to disease phenotype and evolve into a tool for further discovery research and clinical application.

### Please explain why your abstract is innovative for mass spectrometry?

A spatial multi-omics approach using different imaging and 'omics' techniques would allow comprehensive delineation of local metabolic signatures at focal immune microenvironments and identify novel drug targets for therapeutic intervention.

### Co-authors:

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*Nidhi Sharma Dey, York Biomedical Research Institute, Hull York Medical School, University of York*

*Grant Calder, Department of Biology, University of York*

*Peter O'Toole, Department of Biology, University of York*

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Poster number: LS-PB-070

## LIPID ANALYSIS OF FRACTURE HEMATOMA WITH MALDI-MSI

Abstract ID: 449

**Presenting author: Sylvia Nauta, Division of Imaging Mass Spectrometry, Maastricht MultiModal Molecular Imaging (M4i) Institute, Maastricht University, Maastricht, the Netherlands, Department of Orthopedic Surgery and Traumasurgery, Maastricht University Medical Center, Maastricht, the Netherlands**

### Introduction

Bone fracture healing is a complex process involving numerous cell types and molecular pathways. A substantial number of fractures shows delayed healing or non-union development, despite improvements in bone fracture treatment. The fracture hematoma (fxh) is formed immediately after fracture, and plays an important role in successful bone fracture healing. However, the exact molecular and cellular mechanisms of the role of the fxh in bone healing are mostly unknown. Analysis of lipids in fxh can contribute to improved understanding, as lipids are key molecular components of the cell and involved in cellular signaling, inflammation, and metabolism. The aim of this study is the analysis of lipid signals in fxh with matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), including protocol development.

### Methods

Six porcine and six human fxh samples were surgically removed at different time points after fracture (porcine: 72 hours; human samples: range 1-19 days) and snap-frozen. Sample preparation consisted of sectioning, washing, and matrix application (norharmane, TM Sprayer HTX). MALDI-MSI was performed in positive and negative ion mode on a RapifleX MALDI TissueTyper mass spectrometer (Bruker). Different washing methods were compared for the removal of heme in porcine and human fxh. The intra- and inter-variability of fxh were studied. Time-dependent lipid patterns were identified in human fxh for different time points after fracture using principal component and linear discriminant analyses.

### Preliminary data (results)

The washing of fxh is essential, as ion suppression occurs due to the easy ionization of heme. Ammonium formate and acetone washes for different durations were compared for both porcine and human samples in positive and negative ion mode. The optimal fxh washing method was an ammonium formate wash for 15 seconds twice based on the increased intensity of selected  $m/z$  values, decreased heme intensity, and minimum delocalization (figure 1). The effect on the molecular profile of the sampling location within a fxh was studied for porcine samples, as fxh are heterogeneous tissues. This comparison showed that the inter-variability between samples is higher than the intra-variability within a sample. The lipid profiles of human fxh at different time points after fracture (2, 9, and 19 days) were compared in positive and negative ion mode. Time-dependent lipid patterns could be found for both ion modes, for example, day 2 and day 19 could be distinguished from each other (figure 2). In negative ion mode, certain phosphatidylinositols (PIs) and cardiolipins (CLs) are more characteristic of day 19, while a phosphatidylethanolamine (PE) is more characteristic of day 2. Phosphatidic acids (PAs) and phosphatidylserines (PSs) are present on both day 2 and 19, but the fatty acid chain composition differs. In positive ion mode, certain lysophosphatidylcholines (LPCs) have are more characteristic for day 2. The main class of identified lipids is phosphatidylcholines (PCs), which are present in both day 2 and day 19 with differences in the fatty acid chain composition.

### Please explain why your abstract is innovative for mass spectrometry?

Fracture hematoma were analyzed for the first time with MALDI-MSI with an optimized protocol for the removal of heme. In addition, time-dependent lipid patterns in fracture hematoma were shown.

### Co-authors:

*Rald Groven, Division of Traumasurgery, Department of Surgery, Maastricht University Medical Center, Maastricht, the Netherlands, Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands*

*Jane Gruisen, Division of Imaging Mass Spectrometry, Maastricht MultiModal Molecular Imaging (M4i) Institute, Maastricht University, Maastricht, the Netherlands, Division of Traumasurgery, Department of Surgery, Maastricht University Medical Center, Maastricht, the Netherlands*

*Britt Claes, Division of Imaging Mass Spectrometry, Maastricht MultiModal Molecular Imaging (M4i) Institute, Maastricht*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

University, Maastricht, the Netherlands

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Martijn van Griensven, Department of Cell Biology-Inspired Tissue Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

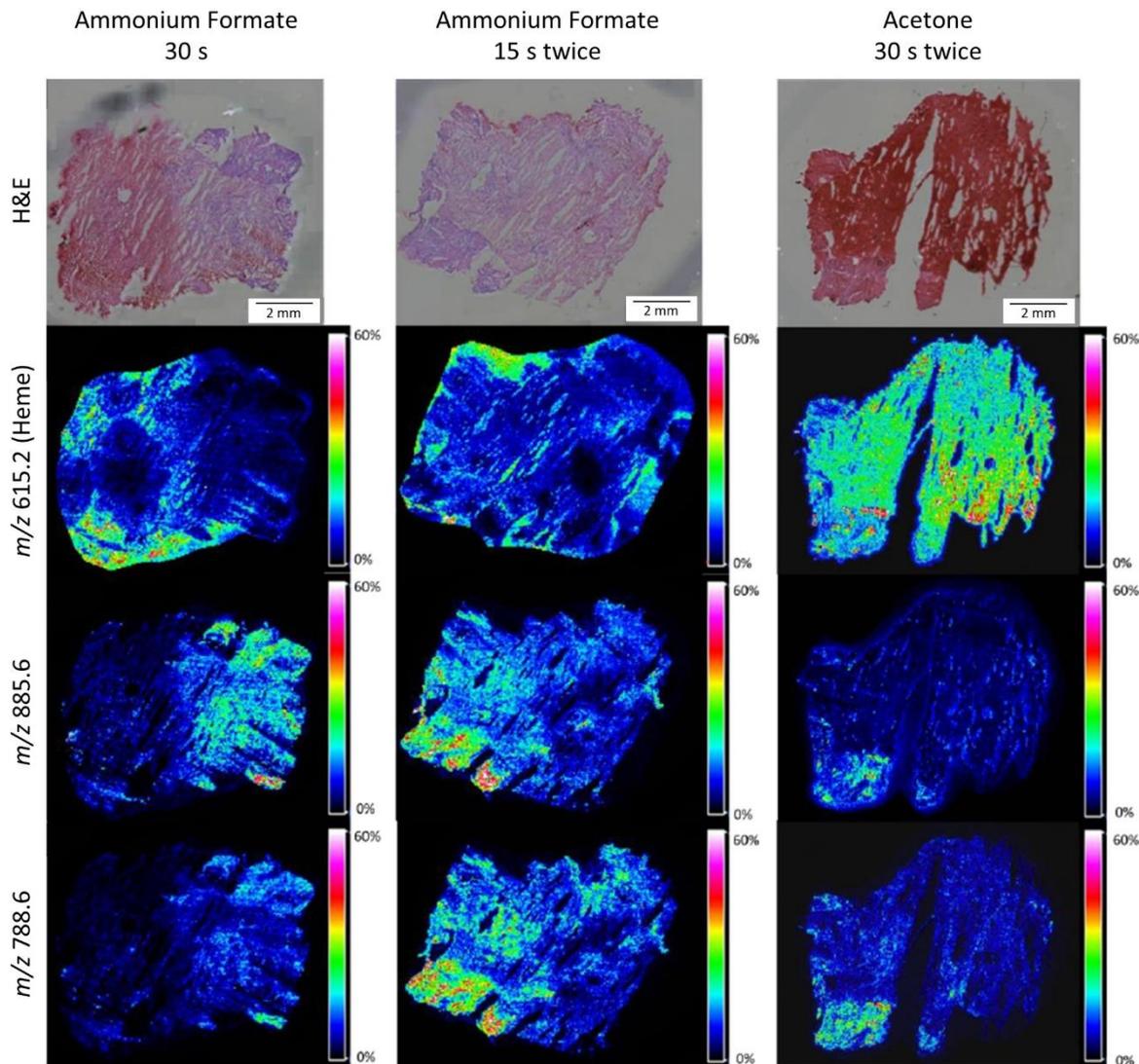
Martijn Poeze, Division of Traumasurgery, Department of Surgery, Maastricht University Medical Center, Maastricht, the Netherlands, NUTRIM, School for Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands

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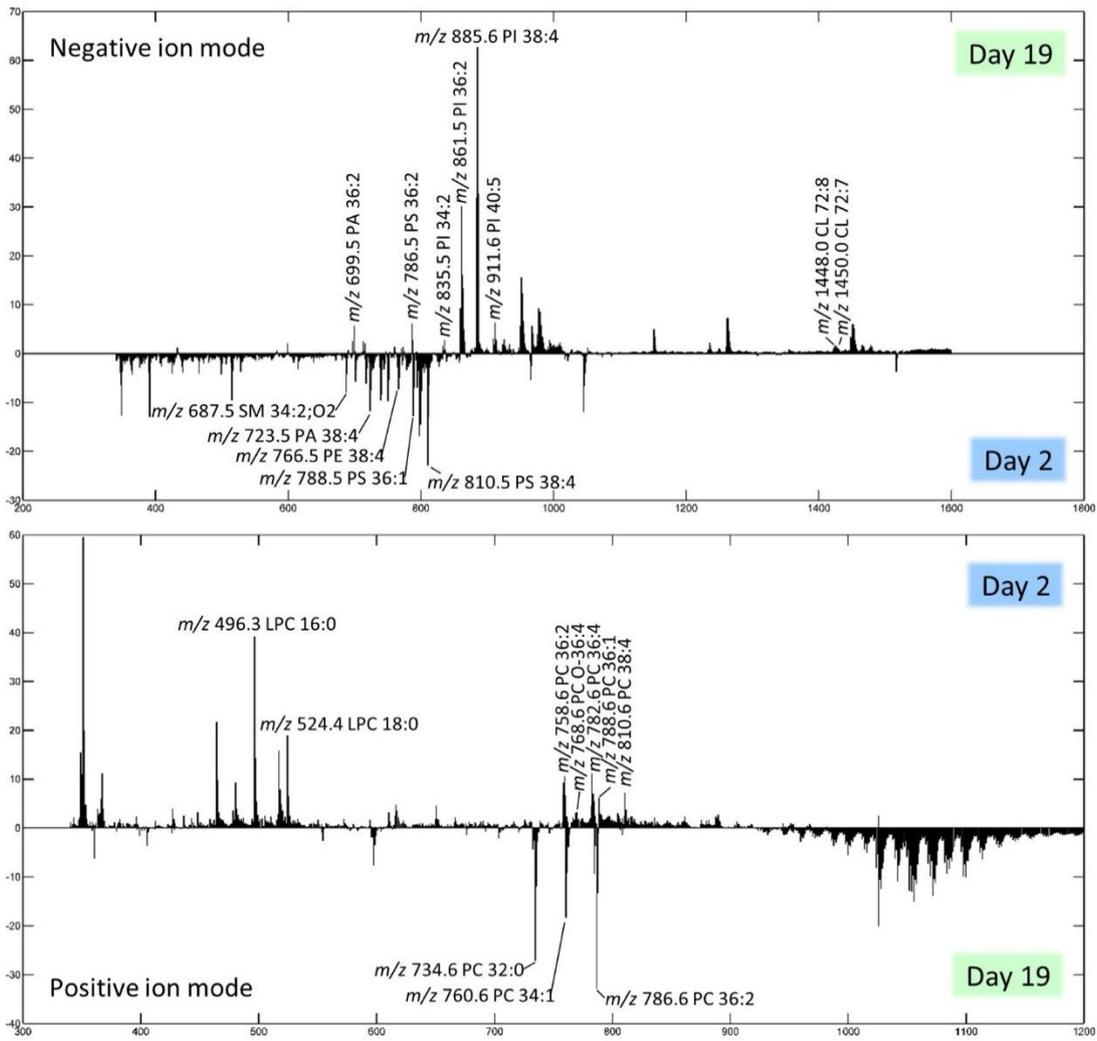
Taco Blokhuis, Division of Traumasurgery, Department of Surgery, Maastricht University Medical Center, Maastricht, the Netherlands, NUTRIM, School for Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands



Comparison of washing methods for heme removal from fracture hematoma

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Identified lipids separating day 2 and day 19 fracture hematoma

Poster number: **LS-PB-071**

## **A DEEP LEARNING APPROACH TO INCORPORATE MICROSCOPY AND MALDI IMAGING ENHANCES CLASSIFICATION OF SKIN LESIONS**

Abstract ID: **503**

**Presenting author: Wanqiu Zhang, KU Leuven, ESAT-STADIUS, Leuven, Belgium, Aspect Analytics NV, C-mine 12, 3600 Genk, Belgium**

### **Introduction**

Imaging mass spectrometry (IMS) has previously been applied to the analysis of melanocytic neoplasia of the skin and has shown promising results diagnosing melanoma. Multimodal IMS data analysis approaches have attracted growing attention in recent years, with different strategies being developed and applied to different tasks and diseases.

Since its advent, deep learning has advanced many applications, including the computer vision domain. It is now possible to automatically capture meaningful morphological features from histopathological images, which are relied on for melanocytic lesions evaluation by most dermatopathologists.

In this work, we present a multimodal, deep learning-based melanoma diagnosis strategy, using MALDI IMS data together with digital histopathological images of tissue sections. Using this combined information, our multimodal strategy enables improved classification of melanoma and nevus.

### **Methods**

Dermatopathologists annotated H&E-stained images of FFPE melanocytic lesions using an online portal (Aspect Analytics Annotation Studio). These annotations were used to guide IMS data acquisition, via an ultrafleXtreme MALDI-TOF MS (Bruker Daltonics), generating one mass spectrum per 50  $\mu\text{m}$  spot after coregistration.

Serial sections were deparaffinized, antigen retrieved, and digested with trypsin. H&E slides were scanned at 20X magnification using a Leica SCN-400 digital slide scanner at a resolution of 0.5x0.5 $\mu\text{m}$ .

A pre-trained deep learning model is used to extract morphological features from H&E images, which are then fused with coregistered IMS data for downstream classification.

### **Preliminary data (results)**

We tested our multimodal strategy on 1050 co-registered microscopy and IMS images & spectra, respectively, from 50 samples labeled as either nevus or melanoma by dermatopathologists. IMS data preprocessing included mass recalibration, TIC normalization, spectral realignment and peak detection.

Firstly, we applied a pre-trained deep neural network model to extract morphology and color features from the microscopy images. Secondly, we combined the resulting high-level microscopy features with coregistered IMS data for downstream classification tasks. We then built a linear SVM model on the fused data to classify nevus versus melanoma. Finally, to visualize the high dimensional fused data, we perform UMAP to embed the data based on the cosine distance and construct 2D and 3D scatter plots.

For comparison, we built classifiers directly on the unimodal microscopy and unimodal IMS data as baseline models. To evaluate the models, the mean ROC AUC values from nested cross-validation were calculated for the multimodal IMS & microscopy, unimodal IMS, and unimodal microscopy. This resulted in ROC AUCs of 0.97, 0.95, and 0.93 with standard deviations of 0.03, 0.04, 0.06, respectively, showing a 2%-4% improvement in classification for the multimodal approach, in terms of both performance and stability. UMAP results from multimodal strategy also showed improved visual separation between two classes over unimodal pipelines.

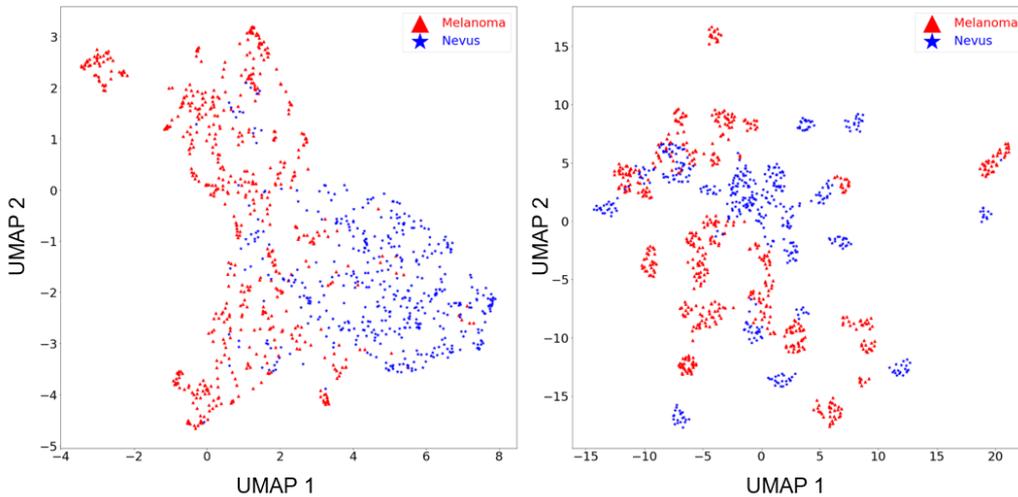
In conclusion, we propose a deep learning-based multimodal strategy for IMS data analysis, enabling direct incorporation of morphological features (as microscopy data is collected in the majority of IMS experiments) and improvements in downstream classification tasks.

**Please explain why your abstract is innovative for mass spectrometry?**

Multimodal analysis of IMS and histology data using deep learning improves melanoma classification results and provides a wide range of applications, such as clinical practice benefits.

**Co-authors:**

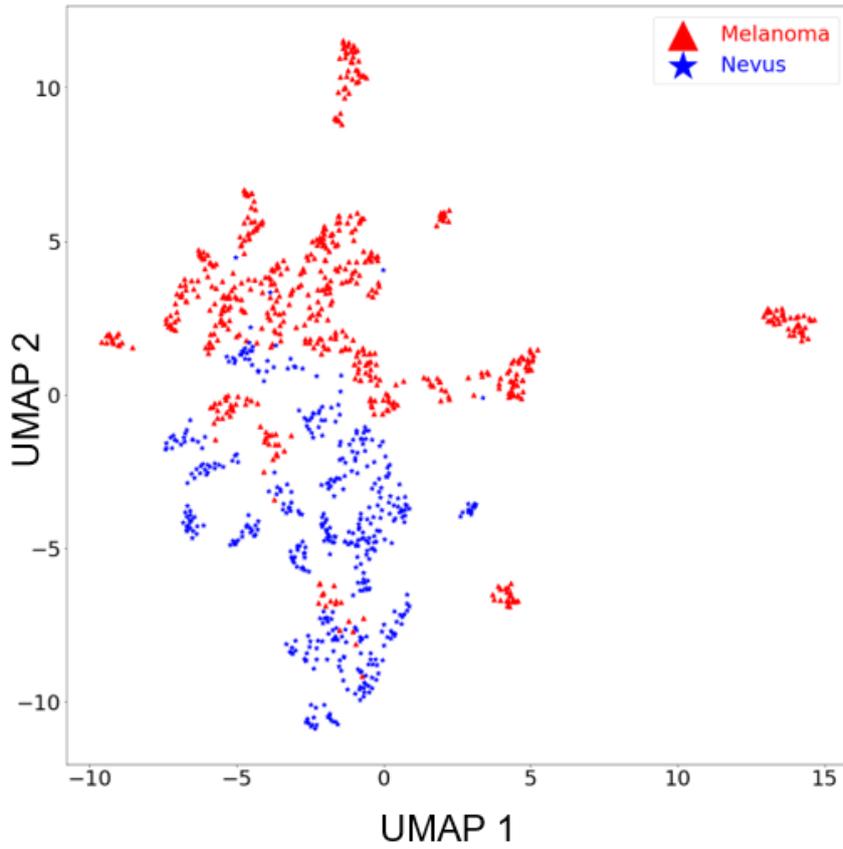
*Nathan Heath Patterson, Frontier Diagnostics, LLC, Nashville, Tennessee, USA, Mass Spectrometry Research Center, Department of Biochemistry, Vanderbilt University, Nashville, Tennessee, USA*  
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*Alice Ly, Aspect Analytics NV, C-mine 12, 3600 Genk, Belgium*  
*Richard M. Caprioli, Frontier Diagnostics, LLC, Nashville, Tennessee, USA, Mass Spectrometry Research Center, Department of Biochemistry, Vanderbilt University, Nashville, Tennessee, USA*  
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UMAP embedding of unimodal microscopy (left) and unimodal IMS (right)

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



UMAP embedding of multimodal IMS and microscopy

Poster number: **LS-PB-072**

## **LEVERAGING IMAGING MS OF MULTIPLEXED N-GLYCAN MARKERS IN BIOFLUIDS TO CLASSIFY CIRRHOTIC PATIENT SAMPLES**

Abstract ID: **572**

**Presenting author: Alice Ly, Aspect Analytics NV**

### **Introduction**

Changes in glycosylation patterns of immunoglobulin G (IgG) have been reported in patient serum and plasma for different diseases including cancer, rheumatoid arthritis, and liver disease. While alterations in N-glycan signatures have been proposed for diagnostic use e.g., identifying disease stage, determining protein-specific N-glycosylation changes from patient samples using traditional methods are laborious.

We recently demonstrated the ability to accurately detect a N-glycan known to be characteristically increased in cirrhotic serum by using a multiplexed array and antibody capture to directly profile N-glycans of serum IgG, PNGaseF cleavage and MALDI mass spectrometry. In this follow-up study, we present a proof-of-concept data analysis workflow for the N-glycans detected by our protein immunocapture method.

### **Methods**

Capture antibodies (IgG, IgG1-4) were spotted onto nitrocellulose-coated slides, dried overnight, then rinsed to remove unbound protein. After adding patient serum, slides were incubated at room temperature for 2 hours in a humidity chamber and washed to remove residual salt. PNGaseF was applied using a spraying robot, incubated overnight at 37°C in a humidity chamber, then CHCA matrix applied using the same sprayer.

Data were acquired using a solariX 7T FT-ICR in positive ion mode over  $m/z$  600–4000, exported using FlexImaging v5.0 to the sqlite format, and then imported into our custom Python data processing pipeline.

### **Preliminary data (results)**

This study compared serum from 10 stage 4 cirrhotic patients versus 10 healthy controls. We created multiplexed arrays capturing 5 different glycoproteins: IgG and IgG1-4. The array included two technical replicates for healthy samples, and four technical replicates for cirrhosis samples for each of the glycoproteins.

A custom pipeline for extraction and classification of the resulting N-glycan data was developed in Python. Spectra were collected across measurements, TIC normalized, then realigned based on known reference peaks and mean spectrum across all spectra. The combined data was then peak picked, resulting in around 7000 peaks. Spots were automatically detected using computer vision techniques. For each spot, spectra were extracted and grouped per patient and glycoprotein combination.

To investigate underlying trends and potential batch effects, unsupervised explorative analysis was performed on the combined data using NMF and PCA. Technical replicates were screened for outliers. Spots outside of acceptable bounds were removed. Next, different classification models were developed to distinguish the cirrhotic samples from control samples. Classification models were made on both the full peak-picked data and on 15 previously identified N-glycan peaks.

A linear SVM model was made separately for each of the glycoproteins and on the combined data for all glycoproteins, resulting in 10 different models. Nested and clustered cross validation was used to measure the different models' performance and technical replicates were used to estimate model robustness. Despite limited sample size, several of the resulting models showed good classification results, confidently distinguishing between control and disease samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

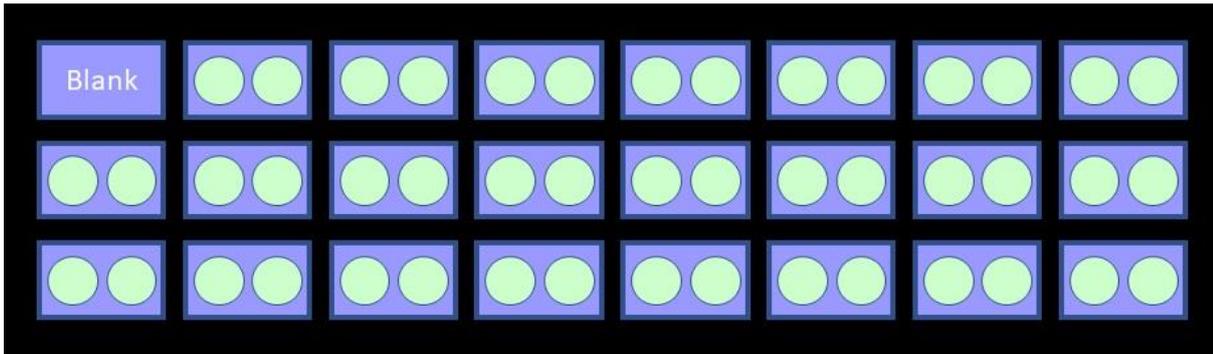
We present a proof-of-concept analysis pipeline for classification of cirrhotic patients based on mass spectrometry of immunocaptured serum N-glycans. This can also be used for QC purposes.

**POSTER SESSION B**

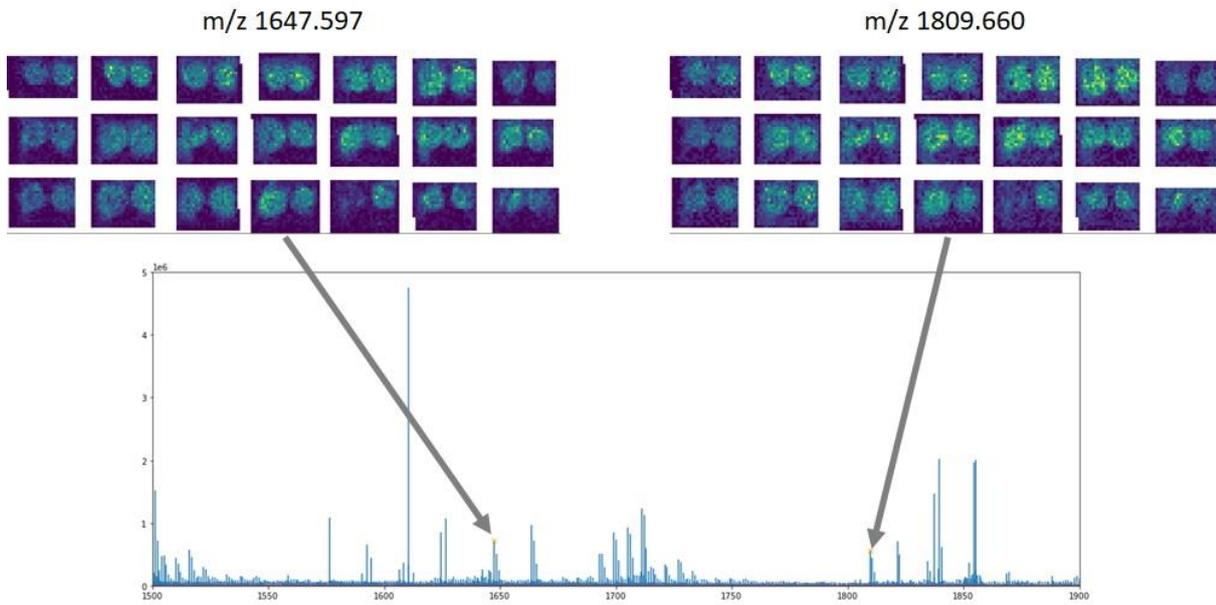
Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

**Co-authors:**

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*Richard R Drake, Medical University of South Carolina, Glycopath Inc.*  
*Peggi M Angel, Medical University of South Carolina, Glycopath Inc.*  
*Anand S Mehta, Medical University of South Carolina, Glycopath Inc.*  
*Marc Claesen, Aspect Analytics NV*



Array configuration showing 21 wells with 2 capture spots/well



Example data showing abundance of two glycans across the array

Poster number: LS-PB-073

## FUNCTIONAL DIAGNOSTICS FOR CONGENITAL DISORDERS OF GLYCOSYLATION FROM PLASMA GLYCOPEPTIDE PASEF-DDA DATA

Abstract ID: 589

**Presenting author: Hans Wessels, Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands**

### Introduction

Molecular diagnostics is on the verge of implementing high-throughput functional Omics data in routine clinical practice for high-precision personalized healthcare. Glycoproteomics in blood plasma offers unique possibilities for clinical diagnostics by providing site-specific glycosylation data for up to hundreds of proteins in a single measurement. Since both biomarker discovery and diagnostics can be performed using the same holistic data, the use of PASEF-DDA<sup>1</sup> effectively eliminates the tedious process of developing and applying different methods for untargeted biomarker discovery and target biomarker measurement. In this work we will share results for diagnosis of Congenital Disorders of Glycosylation (CDG) obtained via targeted data extraction on holistic PASEF-DDA data.

### Methods

Patient and control plasma samples were subjected to tryptic digestion and enriched for glycopeptides. Glycopeptide mixtures were separated by nanoflow C18RP liquid chromatography using 15min linear gradients of 3-45% acetonitrile in 0.1% formic acid, 0.02% TFA at 45°C. Eluting peptides were analyzed by Parallel Accumulation SErial Fragmentation (PASEF®) on a timsTOF Pro 2 instrument. Acquired data were first analyzed using MS Fragger to identify glycopeptides from which glycopeptides of TRFE, IGHG1 and IGHG2 were selected for targeted data extraction. Signal intensities were extracted using Skyline and subsequently processed into glycoform and glycan trait fractions (%) for downstream analyses.

### Preliminary data (results)

We started to apply our optimized PASEF-DDA data generation workflow to a cohort of n=100 healthy individuals and n=150 CDG patient samples with primary defects in the N-glycosylation pathway. First, we established a target list of TRFE, IGHG1 and IGHG2 glycopeptide precursors by analyzing a subset of patients and controls that, based on diagnostic experience and previous glycoproteomics results, should include the vast majority of glycoforms required to diagnose CDG. This initial target list of 217 glycopeptide precursors was then used to extract signal intensities and subsequent glycoform and glycan trait fractions for a first subset of 10 control and 7 patient samples. In all cases the observed shift in glycoform distributions could be explained directly by the genetic defect underlying glycobiochemistry changes: e.g. loss of fucosylated glycans in SLC35C1 GDP-fucose transporter deficiency, increase in galactose lacking glycans in B4GALT1 galactosyl transferase deficiency, or increase in hybrid and high-mannose glycans in MAN1B1 mannosidase deficiency. On top of these results, we will also evaluate the diagnostic performance of this method compared to the current CDG diagnostics standard that is based on immunocaptured transferrin glycoform profiling by intact protein mass spectrometry. Finally, based on obtained results, this glycopeptide technology will be implemented in routine patient care for CDG patients at the Radboud university medical center.

### Please explain why your abstract is innovative for mass spectrometry?

Disease diagnosis by holistic plasma glycopeptide profiling

### Co-authors:

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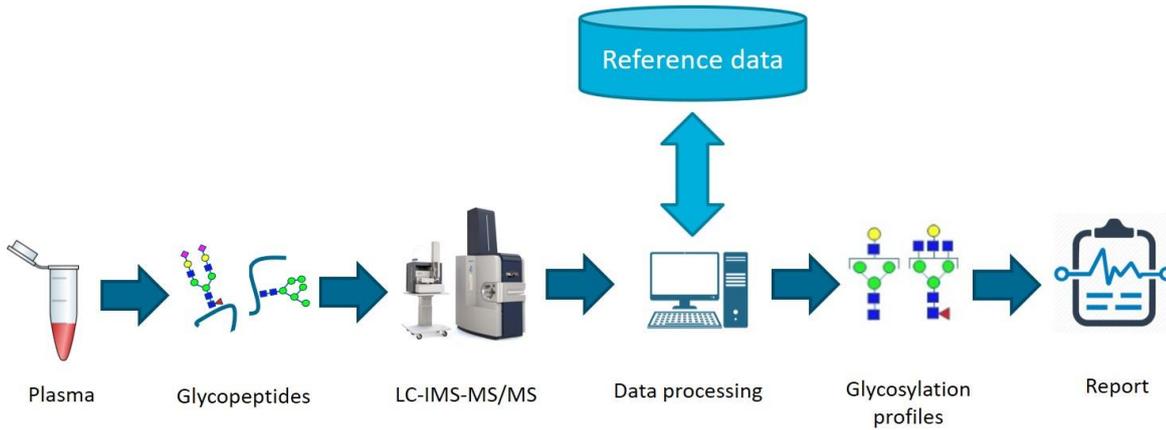
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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

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Workflow for glycopeptide-based diagnostics of congenital disorders of glycosylation

Poster number: **LS-PB-074**

## **MASS DEFECT FILTERING FOR IMPROVED VISUALIZATION OF PEPTIDE FEATURES IMAGED BY AP-MALDI MASS SPECTROMETRY HISTOCHEMISTRY (MSHC)**

Abstract ID: **592**

**Presenting author: Nivedita Bhattacharya, ProteoFormiX Janssen Pharmaceutica Campus Turnhoutseweg 30, 2340 Beerse, Belgium, MassTech LAS Applications Demo Lab, 100 NCL Innovation Park, Dr. Homi Bhabha Road, Pune- 411008, India**

### **Introduction**

MSHC (mass spectrometry histochemistry) is a recent development in mass spectrometry imaging (MSI) which focuses on the analysis of histological sections of biological samples. One of the prime applications of MSHC is the study of biomolecules in human clinical material from well documented biobanked tissues. Especially the countless formaldehyde-fixed paraffin-embedded (FFPE) tissue blocks which until not long ago were almost exclusively used for microscopy investigations by histo(patho)logists, have now become accessible for MS(I) based studies. A physiologically (and very likely also pathologically) relevant class of molecules which have now become accessible for MSHC are the endogenous/secretory peptides, which are produced by many different tissues, albeit in very low quantities. We here report on a method to filter peptide information out of multi GB MSHC datasets.

### **Methods**

FFPE samples (surgically resected hypophysary adenomas) had been stored in the Leuven University Neuropathology Biobank for several years. They had been fixed in formaldehyde and embedded in paraffin following hospital standard operating procedures. Tissues sectioned at 5  $\mu\text{m}$  thickness (semi-automatic microtome) were mounted on regular microscope glass slides and coated with 3 layers of 2,5-DHB matrix (HTX-TM sprayer). MSHC was further performed on a HRMS instrument (LTQ Orbitrap Velos, ThermoFisher) using an atmospheric pressure MALDI source (AP-MALDI UHR (ng); MassTech). Data were analyzed with Mozaic (Spectroswiss) software. Additionally, mass defect plots were generated with the open-source platform mMass.

### **Preliminary data (results)**

MSHC datasets of FFPE tissues, especially when collected at high special resolution (MALDI analysis at pixel dimensions between 5 and 20  $\mu\text{m}$  wide), can easily increase beyond 10 GB of raw data, often a relatively large proportion of which are so-called background signals, not seldom high abundant molecules of little biological relevance and/or high intensity MALDI matrix cluster derived signals.

- 1) With the employed AP-MALDI HRMS combination, MSHC allows for the high resolution (down to 10  $\mu\text{m}$  pixel diameter) imaging of neuropeptides (relatively abundant) in particular areas of human clinical FFPE pituitary tissues being multiple years old.
- 2) MSHC images of vasopressin and oxytocin visually correlate well with immunohistochemical data using anti-vasopressin and anti-oxytocin antibodies performed on adjacent FFPE tissue sections.
- 3) Complex MSHC datasets are generated and mass defect filtering (intensity-based; with an optimized S/N ratio of 3) can be successfully applied to reduce data complexity.
- 4) In addition to vasopressin and oxytocin ions (protonated as well as sodiated and potassiated), other peptide-like ion features could be identified at 1037.4655, 1238.4736 by this HRMS-based data filtering strategy.
- 5) The detected peptide-like ion features co-localized with vasopressin.
- 6) The built-in mass defect feature of Mozaic, that was developed for petroleomics applications, could be successfully employed for feature identifications in our complex MSHC datasets.
- 7) Confirmation of the newly identified deduced peptide structures can be achieved by tandem MS.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

**Please explain why your abstract is innovative for mass spectrometry?**

Mass defect filtering to extract biomolecular class specific information out of large (HR)MSHC datasets.

**Co-authors:**

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Poster number: LS-PB-075

## CHARACTERIZATION OF CUTANEOUS SQUAMOUS CELL CARCINOMAS BY MEANS OF MULTIVARIATE STATISTICS AND MALDI IMAGING

Abstract ID: 645

**Presenting author:** Lauritz Brorsen, Department of Dermatology, Copenhagen University Hospital - Bispebjerg and Frederiksberg, Department of Pharmacy, University of Copenhagen, Department of Digestion, Metabolism and Reproduction, Imperial College London

### Introduction

Cutaneous squamous cell carcinoma (cSCC) is the second most prevalent type of skin cancer in humans and in recent years the incidence rate has been increasing. Despite the increase in incidences, technological advances that can assist surgeons during tumor excision are lacking. Current methods for cSCC excisions are time-consuming, costly, and prone to interpersonal bias. This can be of severe consequences for patients suffering from cSCC. The risk is associated with either excision of too much tissue or leaving behind undetected malignant tissue.

We aim to demonstrate the potential for mass spectrometry imaging as a valuable tool in the elucidation of the lipidomic differences between healthy skin and cSCC.

### Methods

Cryosections (10  $\mu\text{m}$ ) of cSCC and healthy skin were obtained from mice. One section from each tumor underwent HE-staining and subsequent detailed pathological annotation. A section adjacent to the annotated section was analyzed with matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) imaging. The MALDI-MS setup was comprised of an AP-SMALDI10 ion source coupled to a Q-Exactive Orbitrap mass analyzer. 1,5-diaminonaphtalene was used as the matrix and applied by spraying. Univariate statistical methods were used to identify ions that correlate with the annotated region in the HE-stained section.

### Preliminary data (results)

As seen in figure 1, the area of cSCC can be tentatively delineated to match the annotated region using the distribution of single lipid markers. The use of multivariate statistics to include more data for a more robust model will be presented. The results shown in figure 1 demonstrate are based on a univariate model and show that a multivariate approach would likely yield a better classification of cSCC. Results from this model will be used to build a database providing a strong statistical foundation for the classification of cSCC and other types of skin cancer. This database will be applied with the aim of providing a diagnostic and surgical tool based on rapid evaporative ionization mass spectrometry (REIMS).

### Please explain why your abstract is innovative for mass spectrometry?

MALDI used for high resolution mass spectrometry imaging of cSCC. Multivariate statistics used for sub-classification of cSCC.

### Co-authors:

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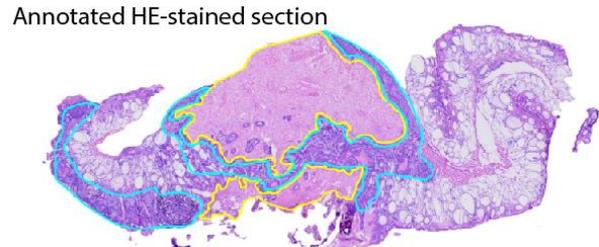
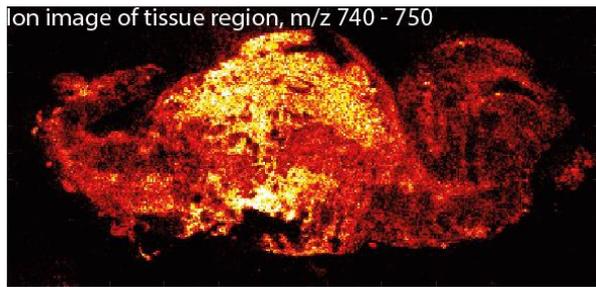
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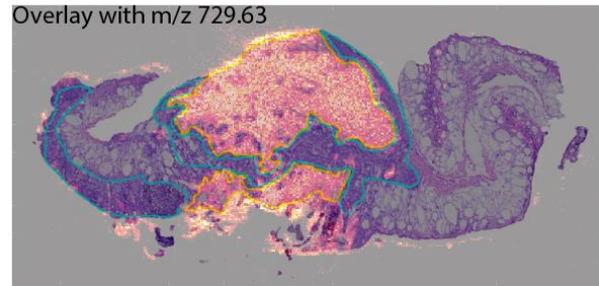
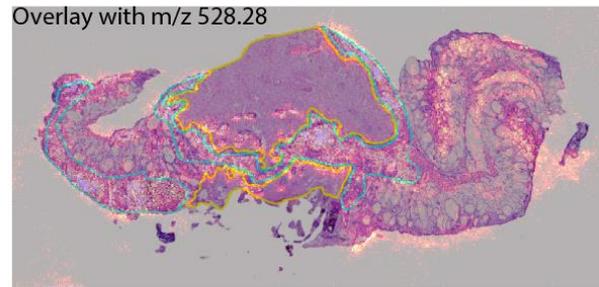
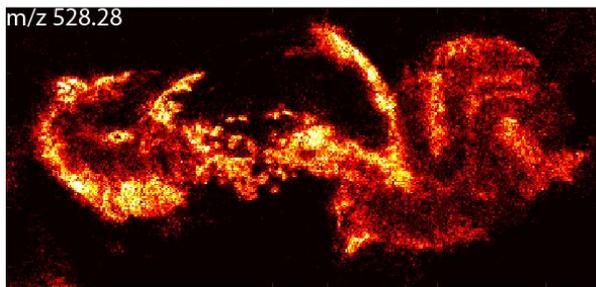
*Catharina M. Lerche, Department of Dermatology, Copenhagen University Hospital - Bispebjerg and Frederiksberg*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Blue: cSCC regions  
Yellow: Orthokeratotic hyperkeratosis



Left column: Ion-images, Right column: HE-stained section and ion-image overlay

Poster number: LS-PB-076

## INCREASED IMAGE CLARITY AND SPECIFICITY USING DUAL MSI SOURCES AND MULTI-REFLECTING TIME OF FLIGHT ANALYSING LIPID METABOLITES IN MOUSE TESTIS

Abstract ID: 680

Presenting author: Emmanuelle Claude, Waters Corporation

### Introduction

Tight regulation in lipid homeostasis is critical for male fertility and in the normal process of germ cell development, extensive lipid remodelling occurs in different cell types which is poorly understood. Mass spectrometry imaging provides spatial localization of different molecular species in the adult mouse testis but there are challenges with conventional imaging given the highly complex tissue structure and marked heterogeneity in cell types.

With the improvements developed for desorption electrospray ionisation (DESI) and matrix-assisted laser desorption ionization (MALDI) sources such as higher spatial resolutions as well as advances in time-of-flight technology, we have investigated lipid metabolites of the different compartments within a mouse testis using high resolution mass spectrometry imaging (MSI) for increased specificity and high mass accuracy.

### Methods

MSI experiments were carried out on a Q-oaTOF and multi-reflecting Q-ToF (MRT) mass spectrometers equipped with a DESI source or a MALDI source. DESI spray conditions were set at 2  $\mu\text{l}/\text{min}$ , 95:5 MeOH: water with 100pg/ $\mu\text{L}$  Leu-enkephalin. The  $\text{N}_2$  nebulising gas pressure was set at 10 psi and a stage speed was 100  $\mu\text{m}/\text{sec}$ .

When the intermediate vacuum MALDI source was in operation, a solid-state diode-pumped Nd:YAG laser with a repetition rate of 1KHz. MALDI matrix was sprayed using the HTX M5 (HTX Technologies) automated sprayer. Subsequent analyses were performed using positive and negative ion modes.

### Preliminary data (results)

In positive ion mode, mainly glycerophospholipids and triglycerides were detected with a mix of potential cation types such as  $\text{H}^+$ ,  $\text{Na}^+$  and  $\text{K}^+$ , increasing the complexity in lipid identification.

PCA was performed using eight ROIs drawn on the three main compartments of the testis using MetaboAnalyst. From the score plot, the groups were clearly separated. The MRT instrument yields ppb mass accuracy, allowing possible identification of PC (36:1) and PC (38:4) which was strongly localised in Leydig cells. PC (34:1) and PC (36:4) ( $\text{Na}^+$  and  $\text{K}^+$ ) ions were mainly localised in the Sertoli cells/early germ cell type and PC (38:5) and PC 38:6) were localised in the more mature germ cells.

Comparing the datasets from Q-oaTOF (FWHM ~ 25,000) and MRT (FWHM ~ 200,000), there are numerous examples where the increased mass resolution obtained with the MRT allows several peaks to be resolved whereas only one was detected with the Q-oaTOF.

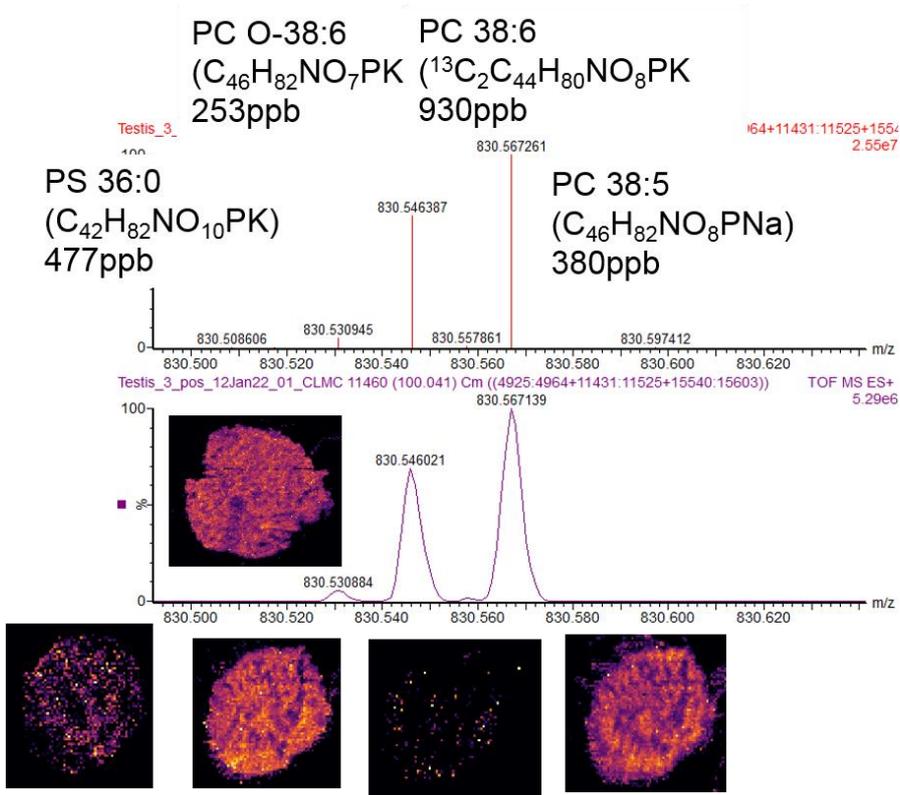
For example, for  $m/z$  830.55, four peaks were detected with the MRT. By order of intensity, tentative identification was PC 38:5 ( $\text{C}_{46}\text{H}_{82}\text{NO}_8\text{PNa}^+$ , 380ppb), PC O-38:6 ( $\text{C}_{46}\text{H}_{82}\text{NO}_7\text{PK}^+$ , 253ppb), PS 36:0 ( $\text{C}_{42}\text{H}_{82}\text{NO}_{10}\text{PK}^+$ , 477 ppb) and PC 38:6 ( $^{13}\text{C}_2\text{C}_{44}\text{H}_{80}\text{NO}_8\text{PK}^+$ , 930ppb), with the first two lipids being relatively intense. However, only one peak was detected using the Q-oaTOF and the mass accuracy for PC 38:5 was over 10ppm likely due to the interference of the PC O-38:6.

### Please explain why your abstract is innovative for mass spectrometry?

Increased confidence in lipid identification with high resolution MSI by dual sources, showing distinct molecular differences in tissue substructure.

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



MRT DESI imaging of m/z 830.5 lipids

Poster number: LS-PB-077

## MAPPING GLUCOSE UPTAKE AND METABOLISM IN THE NORMAL BOVINE LENS

Abstract ID: 744

**Presenting author:** Ali Zahraei, Department of Physiology in the School of Medical Sciences, University of Auckland

### Introduction

As a primary nutrient, glucose is required to drive the functional processes that actively maintain lens transparency. In lieu of a blood supply, glucose is taken up from its surrounding humours, and it can be utilised primarily in three metabolic pathways; glycolysis, the pentose phosphate pathway, and the polyol pathway. However, there is a lack of understanding of how glucose uptake and metabolism occur in the lens in physiological and pathological conditions.

This study aims to optimise the methodology for the spatially precise mapping of glucose uptake and metabolism in cultured normal bovine lenses and correlate the pattern of glucose uptake to glucose transporter distributions and abundance.

### Methods

Bovine lenses were incubated in artificial aqueous humour containing normoglycaemic stable isotopically-labelled (SIL) glucose from 5 min to 20 hrs. Following these incubations, the lenses were frozen for subsequent MALDI IMS, or micro-dissected and analysed by GC-MS to validate the identification of spatially-mapped metabolites. MALDI IMS of lens sections was acquired using a spatial sampling resolution of 150  $\mu\text{m}$  on SolariX XR 7T FT-ICR. Data were acquired in negative mode and range  $m/z$  100 – 1000. Additionally, glucose transporters (GLUTs) were mapped throughout the lens by spatial micro-punching of lens tissue regions and subsequent gel-based proteomic analysis.

### Preliminary data (results)

SIL glucose uptake at 5 min was localised predominantly in the lens equatorial region. Glucose is gradually distributed throughout the epithelium and the cortical lens fibres and eventually to the deeper lens nucleus at subsequent timepoints. Several SIL glucose metabolites were also mapped and indicated the presence of metabolism via glycolysis and the pentose phosphate pathway. Furthermore, the conversion of glucose to sorbitol was detected, initially concentrated at the anterior lens surface, likely in the lens epithelium. From its primary uptake site of the equatorial region of the lens, glucose was rapidly metabolised in epithelial and fibre cells into its metabolites, which were most abundant in the metabolically more active cortical fibre cells, in comparison to central fibres. Spatial proteomic analysis of the lens epithelium detected the presence and differential expression of GLUT1 and GLUT3. GLUT3 was expressed in higher abundance than GLUT1 throughout the epithelium, while GLUT1 was more abundant in lens fibre cells. The establishment of this methodology will allow us to map pathological changes in a hyperglycaemia model to mimic diabetic cataract. This approach extends our understanding of normal lens function and may inform metabolic changes related to lens pathology.

### Please explain why your abstract is innovative for mass spectrometry?

This work establishes a consolidated research pipeline to study metabolomics and proteomics in the lens to enable future spatially-resolved screening of metabolic changes in a diabetic lens cataract model.

### Co-authors:

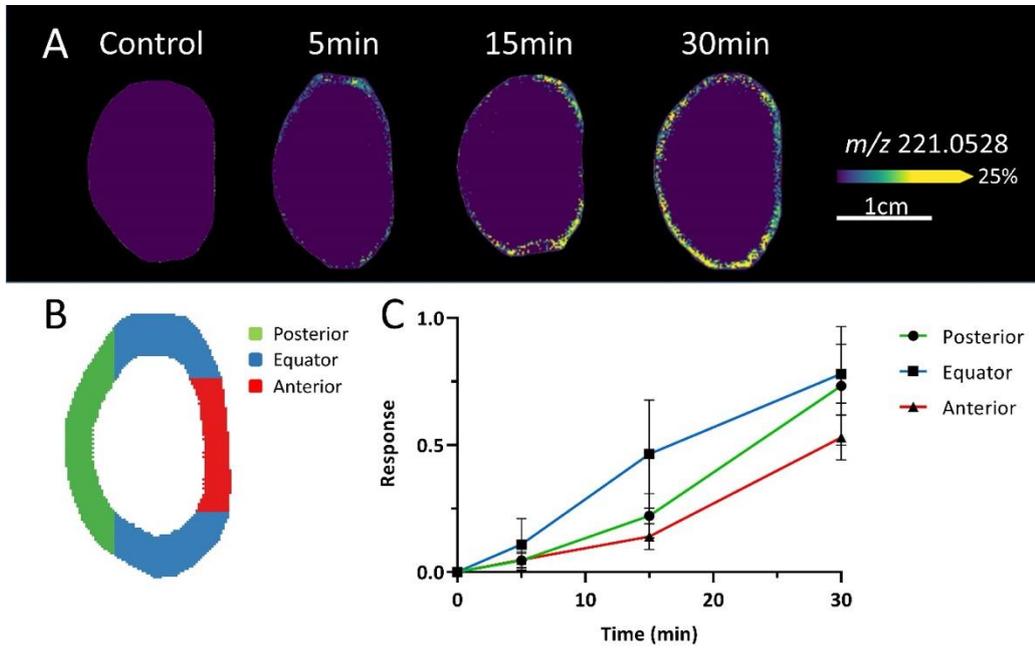
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*George Guo, Department of Physiology in the School of Medical Sciences, University of Auckland, Mass Spectrometry Hub, University of Auckland*

*Nicholas Demarais, Mass Spectrometry Hub, University of Auckland, School of Biological Sciences, University of Auckland*

*Paul Donaldson, Department of Physiology in the School of Medical Sciences, University of Auckland*

Gus Grey, Department of Physiology in the School of Medical Sciences, University of Auckland, Mass Spectrometry Hub, University of Auckland



Comparison of initial glucose uptake at the lens surface.

Poster number: LS-PB-078

## A SENSITIVE METHOD FOR NEUTRAL GLYCOSPHINGOLIPIDS DISTRIBUTION IN GLUCOSYLCEREBROSIDASE-1 KNOCKED OUT MOUSE MODEL USING MASS SPECTROMETRY IMAGING

Abstract ID: 749

**Presenting author: Farid Jahouh, Discovery and Exploratory Bioanalysis – DMPK, Janssen Research and Development a division of Janssen Pharmaceutica NV**

### Introduction

Mutations in Glucosylcerebrosidase1 (GBA1) result in reduced hydrolysis, and subsequent accumulation of its substrate, glucosylceramide in Gaucher's Disease. GBA1 mutations are also the most common genetic risk factor for Parkinson's disease.

Several methods have been applied to study glycosphingolipids concentration in PD and GD mouse models, among those, mass spectrometry imaging was also applied to determine lipids distribution in brain tissue. However, the signal intensity of neutral lipids and glycosphingolipids in MSI is rather poor since they exhibit a low ionization efficiency, they experience a severe matrix effect of phospholipids and these species are generally detected as sodium and potassium adducts.

In this work we will present a sensitive method for neutral glycosphingolipids distribution in mouse brain tissue of WT and homozygous GBA1 KO mice.

### Methods

The method consists of the application of phospholipase C on 10 mm tissue sections on glass slide to digest and remove the phospholipids headgroup for 1h at 37°C. Then, the tissue sections are washed with 1M NaCl for 30 seconds, allowing to increase neutral lipids-sodium adducts formation during analysis. 2,5-dihydroxybenzoic acid was then applied to the tissue section, and mass spectrometry imaging data acquired on a MALDI-timsTOF system in positive ionization mode at a 20x20 mm spatial resolution.

The SCILS software was used for the glycosphingolipids distribution comparison in WT and GBA1 KO mouse brain tissue sections.

### Preliminary data (results)

During the method development, the effect of phospholipids digestion using phospholipase C followed by NaCl tissue treatment were compared in cerebellum of WT mouse brain tissue sections.

As shown in Figure 1 (a), the extracted ion images of different sodiated hexosylceramides (HexCer) in mouse brain cerebellum are mainly distributed in the *Arbor Vitae*. The tissue treatment with NaCl also clearly enhances the HexCer detection sensitivity by factors varying from 192% (HexCer d18:1/18:0) to 314% (HexCer d18:1/20:0).

The phospholipids digestion step using PLC (Figure 1 (b)) followed by the tissue treatment with NaCl also allowed to gain more sensitivity in the detection of the HexCer: 181% for HexCer d18:1/24:0 to 696% HexCer d18:1/18:0. However, some HexCer delocalisation was observed, probably due to the phospholipids digestion condition where condensation and diffusion on tissue was observed.

Figure 2 shows the mass spectral overlay at the *Arbor Vitae* of non-digested and phospholipase C digested phospholipids mouse brain cerebellum tissue. As expected, in the PLC treated tissue, the peak intensity at the phospholipids mass range is decreased compared to the non-treated. In addition, the peak intensity at a higher mass range, corresponding to neutral lipids is much more increased, proving that the reduced matrix effect of the phospholipids enhances the signal intensity of the neutral lipids.

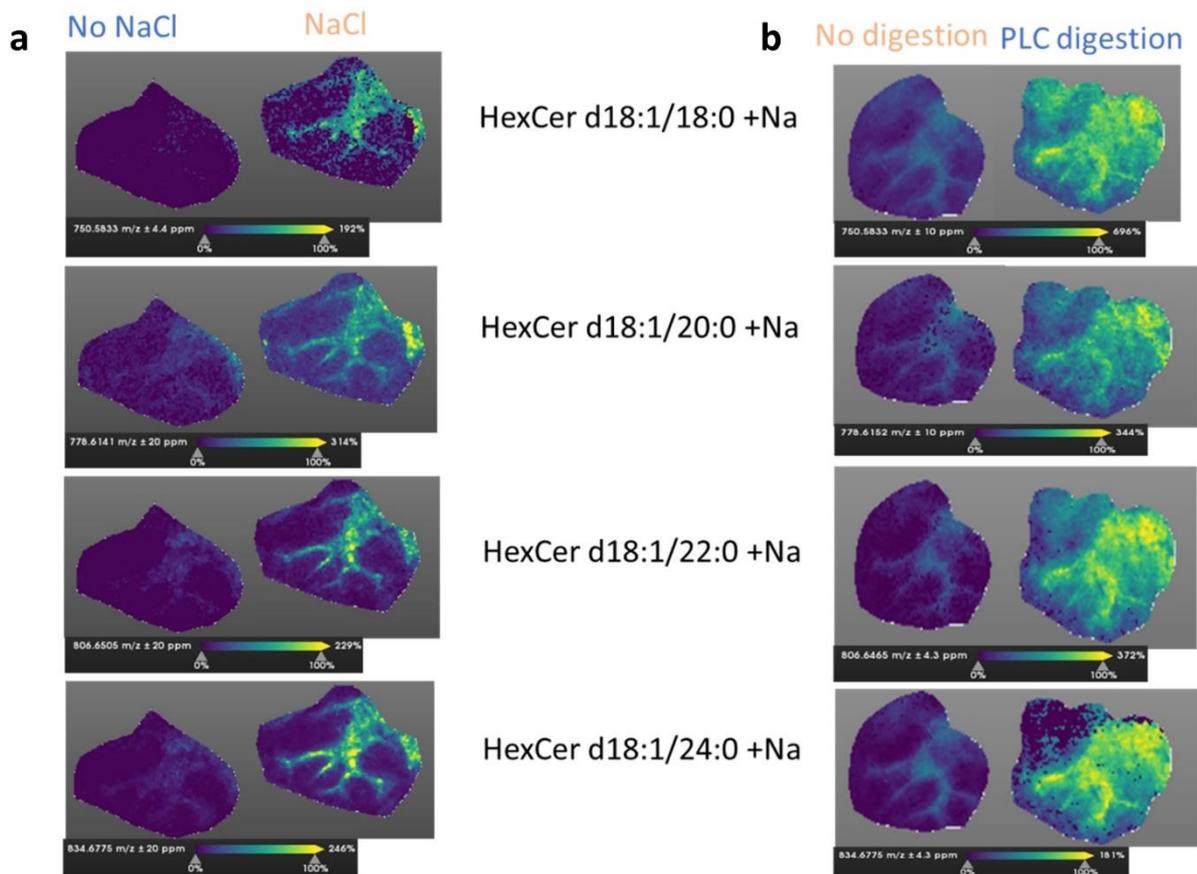
This workflow will be applied to reveal the HexCer and neutral sphingolipid levels differences in homozygous GBA1 KO compared to WT mouse brain tissue.

**Please explain why your abstract is innovative for mass spectrometry?**

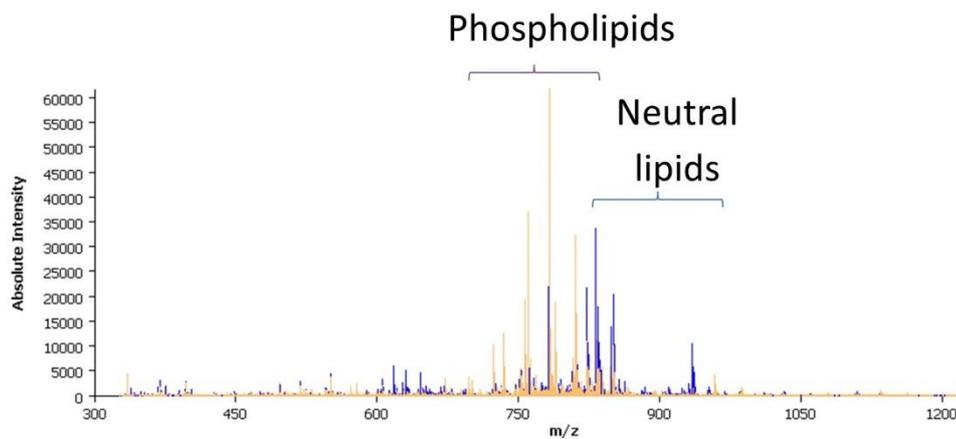
With this method we enhance the detection sensitivity of neutral lipids. The workflow will be applied in GBA1 KO mouse brain tissue to study the distribution of neutral glycosphingolipids.

**Co-authors:**

*Brian Hrupka, Research & Development, Neurosciences, Janssen Pharmaceutica NV*  
*Viktor Neimann, Discovery and Exploratory Bioanalysis – DMPK, Janssen Research and Development a division of Janssen Pharmaceutica NV*  
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Hexosylceramides distribution: NaCl and PLC digestion+NaCl treatment



Overlay lipid mass spectra after non-digested and phospholipaseC digested phospholipids

Poster number: LS-PB-079

## DEVELOPMENT OF A TISSUE STAINING METHOD COMPATIBLE WITH AND IMPROVING LASER CAPTURE MICRODISSECTION, EXTRACTION AND LC-MS/MS ANALYSIS OF DRUGS

Abstract ID: 750

**Presenting author: Farid Jahouh, Discovery and Exploratory Bioanalysis – DMPK, Janssen Research and Development a division of Janssen Pharmaceutica NV**

### Introduction

Laser capture microdissection (LCM) followed by LC-MS/MS analysis of drug extracts is seen as a complementary technique to mass spectrometry imaging because of its higher sensitivity and specificity.

However, the classical staining methods to visualize the tissue region to be microdissected include tissue fixation and treatment steps using cross bridging reagents such as formaldehyde and organic solvents that can bind to drugs or wash them out of the tissue, respectively. Moreover, the selection of the tissue staining dye is critical, since some of them are either non soluble in aqueous solution or do target basic functions, that can bind to certain drugs.

We propose a staining workflow that prevents the loss of drugs and that allows further LCM and LC-MS/MS analysis of drugs.

### Methods

18 mm rat brain and kidney tissue sections were fresh frozen mounted on LCM PEN membrane frame slides and incubated in a petri dish containing trifluoroacetic acid in order to ionize the drugs. The tissue section is then fixed with n-hexane and stained with methylene blue prepared in 10 mM ammonium acetate for 30 seconds. After staining, LCM is performed in different tissue regions and drugs extracted and quantified using reverse phase LC-MS/MS. In order not to contaminate the electrospray source, a divert valve is used to direct the eluting methylene blue to the waste.

### Preliminary data (results)

The first tests were carried out on fresh frozen kidney sections. **Figure 1** shows a 18 mm rat sagittal kidney tissue section stained with methylene blue using our mass spectrometry compatible workflow. The staining allowed us to observe the cortex, medulla and pelvis regions with different color gradients, under the LCM microscope. LCM was then performed on the different tissue regions and around 0.1 mm<sup>2</sup> of tissue was then extracted using the following buffer system: methanol/water/trifluoroacetic acid (50/50/0.2; v/v/v) y sonication in a water bath.

The extract was then dried down and reconstituted in sample injection buffer for LC-MS/MS analysis.

### Please explain why your abstract is innovative for mass spectrometry?

The method describes a tissue staining method that can be used for on-tissue LCM and drug extraction for LC-MS/MS quantification.

### Co-authors:

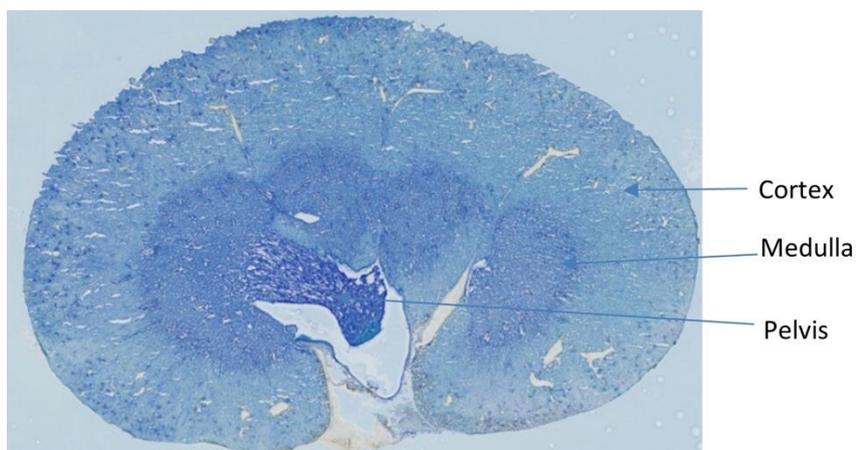
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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



MB stained kidney tissue section using a MS compatible workflow

Poster number: **LS-PB-080**

## **SPATIAL METABOLOMICS FOR EVALUATING RESPONSE TO NEOADJUVANT THERAPY IN NON-SMALL CELL LUNG CANCER PATIENTS**

Abstract ID: **759****Presenting author: Jian Shen, Helmholtz Zentrum München**

### **Introduction**

The response to neoadjuvant chemotherapy (NAC) differs substantially among individual patients. Major pathological response (MPR) is a histomorphological read-out used to assess treatment response and prognosis in patients with non-small cell lung cancer (NSCLC) after NAC. Although spatial metabolomics is a promising tool for evaluating metabolic phenotypes, it has not yet been utilized to assess therapy responses in patients with NSCLC. We evaluated the potential application of spatial metabolomics in cancer tissues for assessing the response to NAC, using a metabolic classifier that utilizes imaging mass spectrometry combined with machine learning.

### **Methods**

Resected NSCLC tissue specimens obtained after NAC (N=88) were subjected to high mass resolution mass spectrometry, and these data were used to develop an approach for assessing the response to NAC in patients with NSCLC. The specificities of the generated tumor cell and stroma classifiers were validated by the application of this approach to a cohort of biologically matched chemotherapy-naïve patients with NSCLC (N=85).

### **Preliminary data (results)**

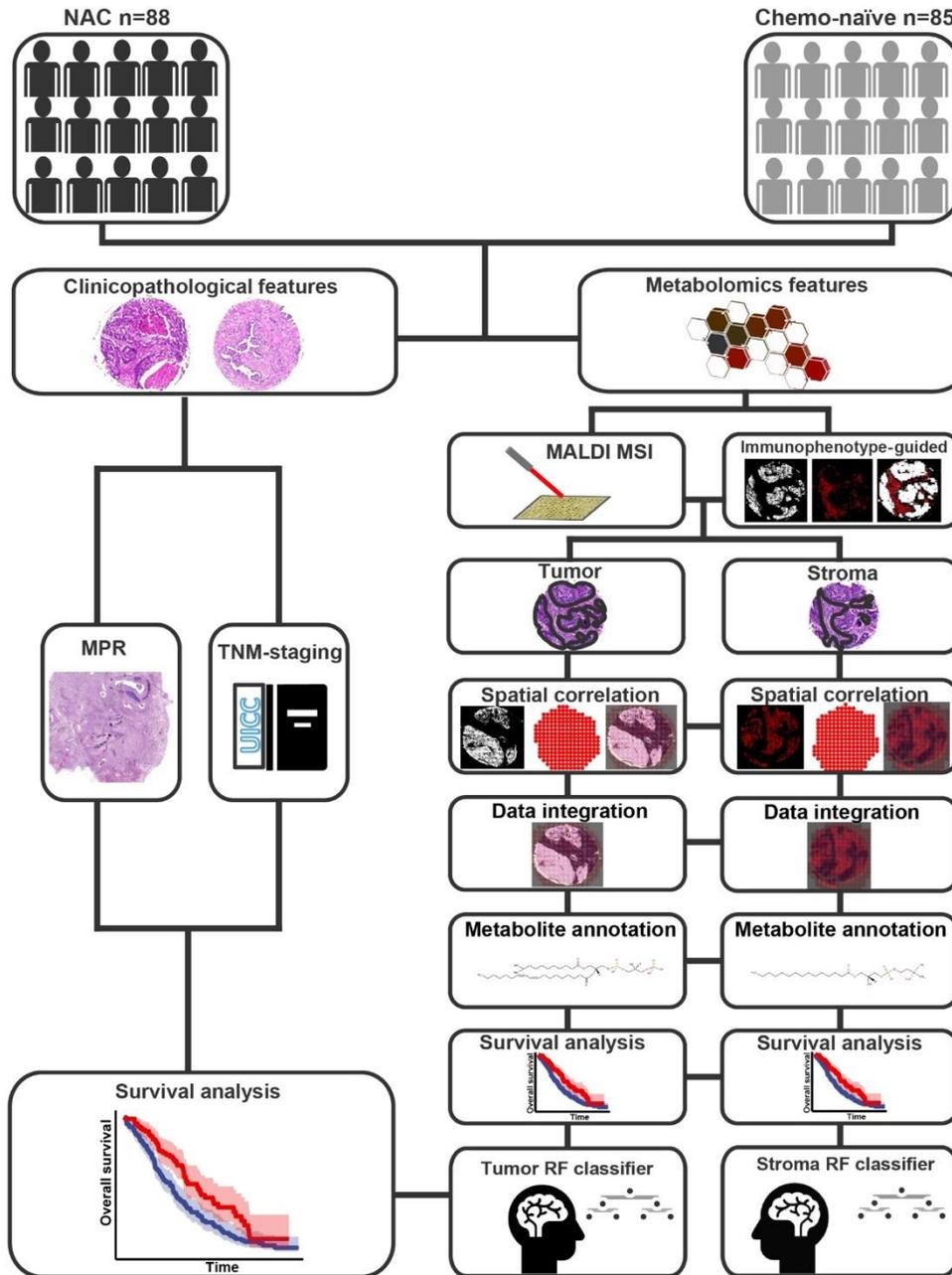
The developed tumor cell metabolic classifier stratified patients into different prognostic groups with 81.6% accuracy, whereas the stroma metabolic classifier displayed 78.4% accuracy. By contrast, the accuracies achieved using either MPR or TNM stage for stratification were 62.5% and 54.1%, respectively. The combination of metabolic and MPR classifiers showed slightly lower accuracy than either individual metabolic classifier. In multivariate analysis, the metabolic classifiers were the only independent prognostic factors identified (tumor:  $P = 0.001$ , hazard ratio [HR] = 3.823, 95% confidence interval [CI] = 1.716–8.514; stroma:  $P = 0.049$ , HR = 2.180, 95% CI = 1.004–4.737), whereas MPR ( $P = 0.804$ ; HR = 0.913; 95% CI = 0.445–1.874) and TNM stage ( $P = 0.078$ ; HR = 1.223; 95% CI = 0.977–1.550) were not significant. The metabolic classifiers allow for further stratification of patients within the MPR categories.

### **Please explain why your abstract is innovative for mass spectrometry?**

Our findings indicate that the metabolic constitutions of both tumor cells and the stroma are valuable additions to the classical histomorphology-based assessment of tumor response.

### **Co-authors:**

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Study design for the development of a metabolic classifier.

The performance of metabolic classifiers and pathological parameters.

Poster number: **LS-PB-081**

## REVEALING THE MOLECULAR UNIVERSE OF ENVIRONMENTAL MICROBIOMES USING SPATIALLY RESOLVED MASS SPECTROMETRY AND MULTIMODAL IMAGING

Abstract ID: **865****Presenting author: Christopher Anderton, Pacific Northwest National Laboratory**

### Introduction

Terrestrial systems and environmental microbiomes represent a complex mixture of interacting species with diverse physiologies and phylogenetic origins, and their functional outcomes are critical to biogeochemical cycles. Little is known about the molecular niches within these systems and exchanges that occur within multi-kingdom systems, where, for example, measuring the molecular transactions among interacting species is a major technical challenge. Our team has developed novel instrumentation and methods to explore the spatial metallome, metabolome, lipidome, and N-glycome of environmental samples ranging from plant tissue to microbial communities to their inter-kingdom interactions, helping us reveal molecular processes responsible for regulated global biogeochemical cycles.

### Methods

A number of spatially-resolved MS and MS imaging (MSI) approaches were used to explore a variety of terrestrial samples and environmental interkingdom interactions. In some cases, these sources were coupled to high mass-resolution mass spectrometers (e.g., 21T FTICR-MS) for high-confidence molecular formula annotations, whereas in other cases we utilized an ultrahigh-resolution pre-mass analysis ion mobility mass spectrometer for confident identification and localization of isomeric compounds. In many examples, we used optical microscopy methods for correlative analysis with our spatial mass spectrometry and MSI approaches, in an effort to link structural information with molecular localization and identification of our detected molecules.

### Preliminary data (results)

Using matrix-assisted laser desorption/ionization (MALDI)-MSI, we determined the molecular location of key metabolites and lipids within multiple plant-based interkingdom interactions and microbial microbiomes. Our results demonstrated how metabolic asymmetry exists within specialized soybean root organs (i.e., 'nodules') as a function of the plant's symbiosis with soil bacteria capable of fixing nitrogen. Recently, we developed a protocol to explore the spatial N-glycome of soybean root nodules using enzyme-assisted MALDI-MSI, where we observed changes in the N-glycome as a function of altered biological nitrogen fixation ability. Coupling MALDI with liquid extraction surface analysis (LESA), we were able to measure the changing disaccharide profiles within a Sphagnum (peat moss) microbiome. Using SIMS, we were able to determine how key micronutrients are acquired from environmental sinks and biotically re-distributed across soil and microbial microenvironments, which was supplemented with X-ray microspectroscopy to measure elemental speciation. In general, we have found that the use of ambient ionization, liquid extraction-based MSI methods (i.e., LESA, nanospray desorption electrospray ionization; nano-DESI) have afforded us the ability to measure agar-based microbial interactions under native conditions. Development of laser-ablation electrospray ionization (LAESI) has allowed us to molecularly profile native plant tissue and has thus shown promise in high-throughput spatial metabolomics of living plants down to the single-cell level.

### Please explain why your abstract is innovative for mass spectrometry?

Spatially resolved MS and MSI approaches, used in combination with other imaging modalities, provided us the ability to visualize the molecular processes within environmental samples.

### Co-authors:

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Poster number: **LS-PB-082**

## **MALDI IMAGING LIPID ANALYSIS INTEGRATED WITH HISTOLOGICAL DATA FOR A COMPLETE SPATIAL-CONTEXTUAL EVALUATION**

Abstract ID: **884****Presenting author: Janina Oetjen, Bruker Daltonics GmbH & Co. KG**

### **Introduction**

In MALDI imaging data analysis, histological evaluation is a key aspect that serves to provide biological context to the imaging results. Therefore, it is critical that software solutions exist to support the integration of histological data with MALDI imaging data. QuPath is an open-source pathology platform for bioimage analysis. While incredibly powerful for pathological analyses, the software does not work with MALDI imaging data. Recently, a QuPath plug-in was released for the MALDI imaging data analysis software SCiLS Lab, making it possible to import histological annotations into SCiLS Lab for downstream analysis. In this study, we demonstrated successful application of this workflow to a rat kidney that revealed specific lipid accumulations in the renal corpuscle.

### **Methods**

MALDI imaging data was acquired on a fresh-frozen rat kidney using a timsTOF fleX instrument at 10  $\mu\text{m}$  pixel size. The same section was stained with H&E post matrix removal and scanned for histological annotation in QuPath 0.3.2. The annotations were exported to SCiLS Lab 2022b via the "Export annotations to SCiLS Lab" extension in QuPath, then co-registered with the optical image of the H&E stain and the previously imported MALDI imaging data in SCiLS Lab. Finally, the co-localization tool of SCiLS Lab was applied to determine lipids accumulating in glomeruli, with lipid species annotated with MetaboScape 2022.

### **Preliminary data (results)**

The QuPath to SCiLS plug-in was used to integrate histological region annotations with statistical analysis of MALDI imaging data. As a proof of principle, we exported renal corpuscle region annotations to SCiLS Lab to detect lipids accumulating with higher abundance in these anatomical structures of the renal nephron.

MALDI imaging of the kidney section resulted in a list of 75 lipid features based on annotations with MetaboScape. These included phosphatidylcholines (PCs), phosphatidylethanolamines (PEs) and sphingomyelins (SMs). With this list, the co-localization tool of SCiLS Lab identified eleven annotated lipids to be highly abundant or uniquely localized in the renal corpuscle such as SMs, PCs and lyso-PEs. Of these, we confirmed the annotations of sphingomyelin (SM) 36:1;O2 and SM 40:1;O2 by on-tissue MALDI TIMS MS/MS analysis.

Based on alignment with the H&E annotations imported from QuPath, we found SM 36:1;O2 to be mainly present in the renal corpuscles' interior, i.e. Mesangium, Glomerulus capillaries, and visceral layer of the Bowman's capsule (Podocytes), while SM(d40:1) was localized in the parietal layer of the Bowman's capsule. The integration of QuPath to SCiLS Lab facilitated the interpretation of ion distributions by MALDI Imaging and helped to bring this information into a spatial-contextual environment.

### **Please explain why your abstract is innovative for mass spectrometry?**

The QuPath plug-in to SCiLS Lab is the first fully integrated tool for combining histology with MALDI imaging statistical analysis.

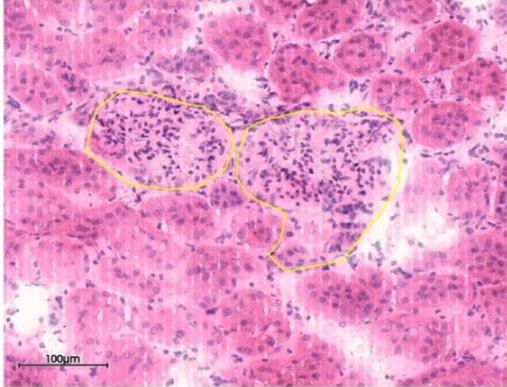
### **Co-authors:**

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*Soeren-Oliver Deininger, Bruker Daltonics GmbH & Co. KG*  
*Jonas Singe, Bruker Daltonics GmbH & Co. KG*

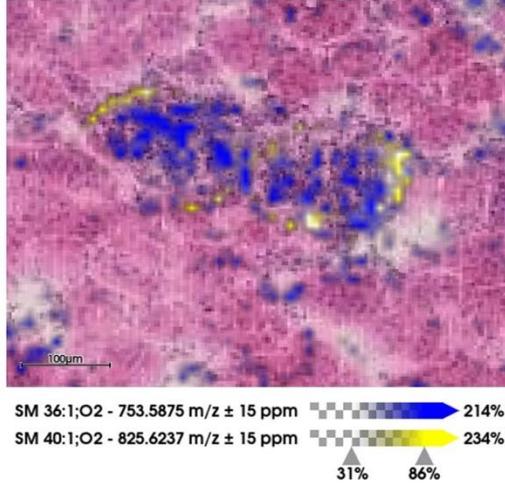
**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

QuPath region annotation



MALDI Imaging data integration with histology



Integration of QuPath region annotations with SCiLS Lab.

Poster number: LS-PB-083

## MAPPING STEROLS IN A MARINE FLATWORM-ALGAE-SYSTEM USING MALDI-2 TIMS MS-IMAGING

Abstract ID: 899

Presenting author: Andras Kiss, Bruker Daltonics GmbH &amp; Co. KG

### Introduction

From humans to sponges, symbiosis with microbes is recognized to be a widespread phenomenon with significant impacts on development, cell and ecosystem function, and evolution. For example, symbiosis between marine animal hosts and algae allows animals to flourish in harsh environments due to symbiont transfer of photosynthetically fixed nutrients. In many of these systems, transfer of a variety of sterols between guest and host plays an important role. In contrast to conventional MALDI-MSI, the addition of laser post-ionization (MALDI-2) allows for a sensitive detection of sterols. This gain in sensitivity enables an improvement in spatial resolution. Here we present MALDI-2-MSI data of sterols from the symbiosis of the *Waminoa* flatworm with two dinoflagellates at a pixel size of 5  $\mu\text{m}$ .

### Methods

Symbiotic systems of *Waminoa* sp. flatworms hosting intracellular symbiotic *Amphidinium* and *Symbiodiniaceae* dinoflagellates were cultured in artificial seawater aquaria, embedded in a mixture of 5% 2-hydroxyethylcellulose / 10% gelatin, and 14  $\mu\text{m}$  thick cryo sections were prepared thaw mounted and stored at  $-80^\circ\text{C}$ . Right before analysis, samples were thawed and dried under a stream of nitrogen and sublimated with 2,5-dihydroxyacetophenone (DHAP) to a layer thickness of about 5-10  $\mu\text{m}$  using a custom-built apparatus. MALDI-2-MSI images were acquired on a timsTOF flex MALDI-2 instrument.

### Preliminary data (results)

The combination of MALDI-2 and TIMS MS Imaging enables the sensitive analysis of 32 individual sterol species directly from tissue without prior chemical derivatization. The signal intensity boost enabled by laser post-ionization allows to perform MS imaging experiments with a pixel size as low as 5  $\mu\text{m}$ , approaching a cellular resolution for algal systems. While MALDI-2 critically increases signal intensity, TIMS crucially aids with the tentative assignment of sterols. Besides the targeted analysis of sterols, collected MALDI-2-MSI data contain spatial information about a great amount of other molecular ion species. Next to different phospholipid species colocalized with regions of the *Waminoa* host, signals were detected that exclusively colocalized with the symbiotic dinoflagellates, including intact chlorophyll and some of its fragments as well as yet unidentified species.

The observed distribution of symbiont-produced sterols within host flatworm tissues reveals that sterol transfer is a conserved element of *Symbiodiniaceae* symbioses in host backgrounds. Surprisingly, the different spatial distribution of stigmasterol compared to cholesterol in an area corresponding to the syncytial gut region could indicate an unprecedented differential use of the various symbiont-produced sterols. The accumulation of these sterols could be based on several reasons, yet the mechanisms and function remain unknown.

### Please explain why your abstract is innovative for mass spectrometry?

Laser post-ionization (MALDI-2) enables the MS-imaging analysis of sterols directly from tissue at a pixel size down to 5  $\mu\text{m}$ .

### Co-authors:

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Elizabeth Hambleton, Centre for Organismal Studies (COS), Heidelberg University

Klaus Dreisewerd, Institute for Hygiene, University of Münster, Interdisciplinary Center for Clinical Research (IZKF), University of Münster

Jens Soltwisch, Institute for Hygiene, University of Münster, Interdisciplinary Center for Clinical Research (IZKF), University of Münster

Poster number: **LS-PB-084**

## **HOST – MICROBE INTERACTION: SPATIALOMX AS KEY TECHNOLOGY TO GAIN INSIGHT INTO BACTERIA LOCALIZATION AND FUNCTION IN MUSSEL GILLS**

Abstract ID: **900**

**Presenting author: Michele Genangeli, Bruker Daltonics GmbH & Co. KG**

### **Introduction**

Host–microorganism associations range from marine symbioses to the human gut. In these environments where symbiotic bacteria provide nutrition for the host, extreme bacterial genomic strain-variation can lead to immense heterogeneity of bacterial phenotypes. Current methods do not allow for the differentiation of phenotypically different strains. On the other hand, SpatialOMx methods, such as spatial metabolomic and spatial proteomics, provide a unique opportunity to reveal the varied genotypes and the heterogeneous distribution of these different strains. In this study, we successfully applied the SpatialOMx workflow to spatially differentiate the phenotypes of the major symbiotic bacterial strains found on mussels.

### **Methods**

Frozen mussel sections of *Bathymodiolus azoricus* were sliced with 10  $\mu\text{m}$  thickness and mounted on IntelliSlides (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). Slides were coated with MALDI DHAP matrix using a TM-sprayer (HTX Technologies, Chapel Hill, USA) and measured on a timsTOFflex MALDI-2 (Bruker Daltonics) in positive polarity for lipids and metabolites. Previous LC-MS data from the same instrument were used as Target list in MetaboScape® to annotate the MALDI imaging data. Interesting metabolites were selected to define specific areas and removed through microdissection with the Leica LMD 7000 for downstream 4D-Proteomics™.

### **Preliminary data (results)**

Mass spectrometry imaging results revealed two different, major, spatially segregated bacterial phenotypes (chemotypes) on the lipid level: the so-called SOX (sulfur oxidizing symbionts) bacteria and MOX (methane oxidizing symbionts) bacteria. To link the molecular machinery behind the heterogeneous metabolite production to the intracellular microbes, we further analyzed both chemotypes with spatially targeted proteomics. Applying our novel pipeline of spatial metabolomics-guided laser capture microdissection, we extracted samples from the immediate host-microbe interface representing each of the chemotypes and successfully detected key proteins from the same bacterial genome as well as key host proteins. In total we found about 400 bacteria specific protein groups in both analyses and about 1200 mussel specific protein groups. Interestingly a methane oxidation related cofactor metabolite was spatially dissolved with MSI ( $m/z$  869)- and the Methane Mono Oxygenase (MMO) which is in the same pathway, was identified with 4D-Proteomics™.

### **Please explain why your abstract is innovative for mass spectrometry?**

SpatialOMx revealed metabolic pathways that drive hidden phenotypic heterogeneity among genetically nearly identical intracellular symbionts of a marine invertebrate.

### **Co-authors:**

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*Janine Beckmann, Max Planck Institute for Marine Microbiology*  
*Benedikt Geier, Max Planck Institute for Marine Microbiology*  
*Michael Easterling, Bruker Daltonics Inc.*  
*Manuel Liebeke, Max Planck Institute for Marine Microbiology*

Poster number: LS-PB-085

## MALDI-MSI: A POWERFUL APPROACH TO UNDERSTAND PRIMARY PANCREATIC DUCTAL ADENOCARCINOMA AND METASTASES

Abstract ID: 931

**Presenting author: Juliana Gonçalves, Institute of Pathology, School of Medicine, Technical University of Munich**

### Introduction

The survival rate of patients affected with Pancreatic Ductal Adenocarcinoma (PDAC) is lower than 10%. That is due to the in-existent symptoms during the initial stage of tumor development. At the time of diagnosis, the patient often already presents distant metastasis. The development of distant metastasis is not yet fully understood and treatment of the primary lesion is not always successful in treating the metastasis - dissociate treatment response. In this study, we demonstrate that proteomic analysis of primary PDAC and distant metastasis provide significant information to further understand the disease development.

### Methods

One mixed tissue microarray (TMAs) containing samples from primary PDAC and distant metastasis, from 13 patients was employed as sample set. From 5 of these patients, it was possible to include samples from both primary and corresponding metastasis. Tryptic digested samples were analyzed utilizing a Bruker RapifleX MALDI-TOF mass spectrometer. Subsequently, the matrix was removed, sections were stained by H&E, and scanned for histopathological annotation. Data analysis was performed by using the SCiLS Lab (Bruker) and statistical analysis was performed on R. Further sample pairs of primary PDAC and distant metastasis were used as an external validation dataset.

### Preliminary data (results)

The built classification models presented very high accuracy. Random Forest (RF) and Support Vector Machine (SVM) both presented accuracies over 93% and sensitivities over 95%, while Linear Discriminant Analysis (LDA) yielded 90% accuracy and 98% sensitivity. When employing the built classification methods on an external dataset with samples of primary PDAC and corresponding distant metastasis of five different patients, we confirmed that the developed method is applicable to further sample sets, with very satisfactory outcomes. As per feature analysis, we have also observed that collagens play a pivotal role in the development of distant metastasis. AUC-ROC analysis revealed that MACROH2A1, HPF1, COL4A3, TBB2C, actin, and H2B are overexpressed in the metastatic tissue, possibly due to their pivotal function in cellular proliferation.

### Please explain why your abstract is innovative for mass spectrometry?

In this study, we have shown that proteomics, and mass spectrometry imaging, in particular, can be employed to further understand the meanders of PDAC distant metastasis development.

### Co-authors:

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Poster number: **LS-PB-086**

## **THE IMPACT OF ABDOMINAL ADIPOSE TISSUE COMPOSITION ON RADIODENSITY IN COMPUTED TOMOGRAPHY**

Abstract ID: **958**

**Presenting author: Amani Zoabi, Pharmacy, Medicine**

### **Introduction**

Adipose tissue radiodensity values in computed tomography (CT) images taken prior to the surgery may predict the difficulty of the surgery. Despite the potential clinical importance of radiodensity measurement, little is known about how the chemical composition of fat tissue influences this parameter. This study combined Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) and Electrospray Ionization (ESI) Mass Spectrometry with unsupervised machine learning and deep learning to unveil the impact of the chemical composition of the abdominal adipose tissue on its CT radiodensity.

### **Methods**

Patients underwent kidney, bladder, or prostate surgery. Before the surgery, the CT-radiodensity of selected fat tissue in proximity to the operated organ was measured. Fifty-three fat samples were obtained from these zones and analyzed by DESI-MSI and ESI and compared to the HU density and to demographic parameters. In order to identify the molecular ions, which have an impact on tissue radiodensity from massspectral data, a non-negative matrix factorization (NMF) based machine learning algorithm was developed. A correlation-based method was employed afterward as an alternative to NMF, and finally, a simple convolutional neural network was built to verify the results.

### **Preliminary data (results)**

No associations between fat radiodensity and patient gender, age, weight, height, or source of fat were found. Body mass index (BMI) however, showed a weak, but significant negative correlation with radiodensity.

Nevertheless, a substantial difference in chemical composition between adipose tissues of high and low radiodensity was observed. High fat radiodensity exhibits a larger relative abundance of high molecular weight species, such as phospholipids of various types and waxy lipids, while low radiodensity shows mostly short fatty acids.

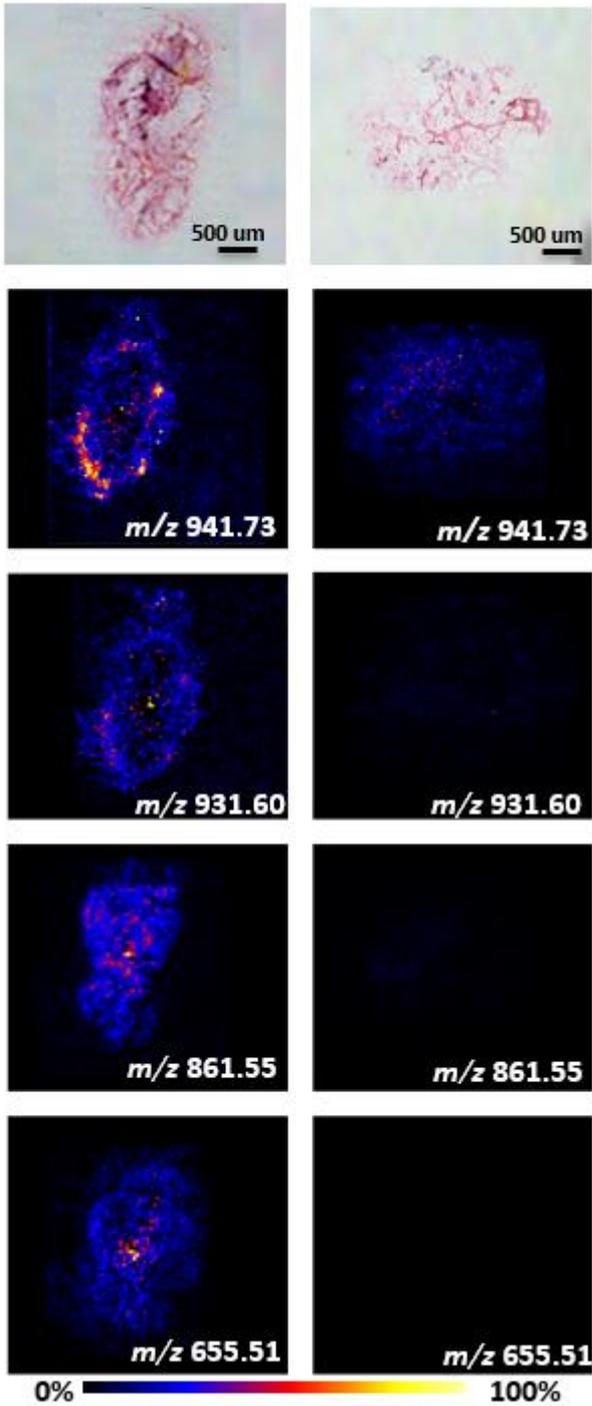
### **Please explain why your abstract is innovative for mass spectrometry?**

We used the unique properties of DESI-MSI of measuring the spatial ditribution of lipids in tissues of similar thickness &ESI to establish a correlation with this ditribution and their radiodensity

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

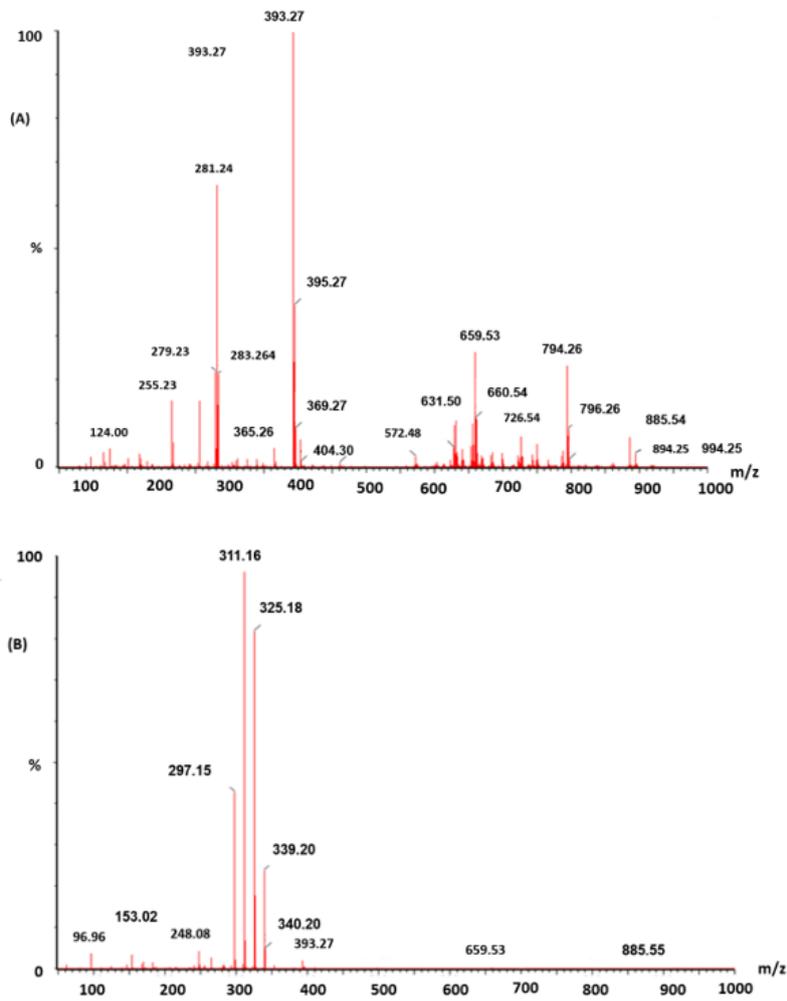
**Tissue with high HU (-69)    Tissue with low HU (-120)**



DESI-MSI ion images in tissues with high and low HU

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Negative ion mode DESI-MS data-of tissues with high/low radiodensities.

Poster number: **LS-PB-087**

## A MALDI IMAGING ROUTINE FOR CAFFEINE AND THEOPHYLLINE

Abstract ID: **963**

**Presenting author: Marius Herbst, Kekulé-Institute of Organic Chemistry and Biochemistry, University of Bonn**

### Introduction

Central nervous system (CNS) diseases are difficult to treat. The blood-brain barrier (BBB) regulates the entry of compounds in our bloodstream to the CNS to prevent damage to the brain. For the development of potential therapeutic drugs, it is essential to study if the compounds will pass the BBB. Additionally, the accumulation of a compound in specific regions of the brain could serve as aid in understanding of CNS diseases and treatment strategy optimizations.

The aim of this study was the establishment of a matrix-assisted laser desorption/ionization (MALDI) imaging method to enable the detection of biologically active compounds of low molecular weight (LMW) in various tissues including kidney, liver and brain. Caffeine and theophylline were selected as model drugs to establish the method conditions.

### Methods

MALDI Imaging is a technique that allows sensitive detection of molecules in tissue sections. Tissues from pig and mouse kidneys were sectioned with a SLEE MEV floorstanding ECO cryostat. The thin sections were immediately stored under vacuum until measurement. For method optimization, droplets of caffeine and theophylline solutions were placed on top. The matrix layer was applied with a HTX Imaging TM-Sprayer in combination with an Azura P 4.1 S pump from Knauer. Measurements were performed with an ultrafleXtreme MALDI-TOF/TOF (Bruker Daltonik) with the flexControl and flexImaging software. Analysis was done with SCiLS Lab.

### Preliminary data (results)

Caffeine, a widely used CNS stimulant, and its structurally related theophylline, a bronchodilator for the treatment of asthma, were chosen as model drugs for a start. MALDI Imaging of these compounds in thin tissue sections has not been reported before. The sample preparation proved to be the most critical task to achieve reproducible measurements of high quality. We optimized the protocol with respect to matrix type, solvent composition and matrix concentration, and thickness of matrix layer. A fine and even layer was achieved through low flowrates and a small number of passes with the automatic sprayer. As therapeutics that are found *in situ* will most likely have a rather low abundance, it was important to find the detection limit. We achieved to safely detect both caffeine and theophylline in low millimolar concentrations. The two compounds showed interesting differences in their ionization behaviour despite their structural similarities. HCCA proved to be a reasonable choice of matrix, while measurements with DCTB were problematic because of vacuum instability and fragmentation.

### Please explain why your abstract is innovative for mass spectrometry?

A MALDI imaging routine to detect the LMW compounds caffeine and theophylline in thin tissue sections at reasonably low concentrations was successfully established.

### Co-authors:

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*Marianne Engeser, Kekulé-Institute of Organic Chemistry and Biochemistry, University of Bonn*  
*Ali El-Tayeb, Department of Pharmaceutical & Medicinal Chemistry, University of Bonn*  
*Christa Müller, Department of Pharmaceutical & Medicinal Chemistry, University of Bonn*

Poster number: LS-PB-088

## LIPIDOMIC PROFILING FOR UNRAVELLING PATHOLOGICAL MECHANISMS IN ACUTE INTESTINAL ISCHEMIA-REPERFUSION INJURY IN HUMAN IN VIVO MODEL.

Abstract ID: 1000

**Presenting author: Annet Duivenvoorden, Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands**

### Introduction

Acute intestinal ischemia-reperfusion (IR) injury is a severe life-threatening condition with a high mortality rate. Prolonged periods of ischemia lead to tissue damage and intestinal barrier loss facilitating bacterial translocation. Reestablishment of blood flow (reperfusion) is necessary in order to save intestinal tissue, but unintentionally exacerbate previously obtained tissue damage. Therefore, new insights are required into the complex series of molecular and cellular mechanisms that contribute to intestinal damage. Previous data indicates that there is a potential involvement of lipids in mediating IR-induced damage, suggesting lipids may be targets for future treatment. To this end, we will investigate this condition by using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) and study changes in lipid distribution in intestinal IR injury.

### Methods

Fresh frozen intestinal tissues were obtained from a human *in vivo* experimental intestinal IR model. In short, in patients undergoing pancreaticoduodenectomy (n=6), an isolated part of jejunum was subjected to 45 min of ischemia (45I), followed by 30 (30R) and 120 min (120R) of reperfusion. Tissues were cryo-sectioned (12  $\mu$ m, -18°C) and thaw-mounted onto indium tin oxide (ITO) slides. Samples were coated with 40 mg norharmane matrix using sublimation (140°C for 180s, HTX technologies) and measured with a Bruker RapifleX MALDI Tissue typer instrument with a spatial raster at 30  $\mu$ m and 5  $\mu$ m.

### Preliminary data (results)

Earlier studies have shown multiple factorial changes in intestinal tissue on RNA and protein level under the influence of IR. In this study, we aimed to identify changes in lipid profiles of distinct histological structures in the small intestine. We first evaluated lipid distribution within histological layers (mucosa, submucosa and muscle) in both negative and positive ion mode. Preliminary measurements of untreated (control) intestinal tissue showed a broad range of lipid species among the intestinal layers, for example, phosphatidylcholines (PC 34:1), lysophosphatidylcholine (LPC 16:0), phosphatidylinositol (PI 34:1 and PI 38:4), glycerophosphoethanolamine (PE 40:4). Interestingly, spectra acquired at 30  $\mu$ m provided useful insight on the spatial localization of these lipids, however, were unable to reveal structural differences in certain intestinal-specific histological features (such as, intestinal crypts, lymphatic vessels, etc.). Most importantly, data acquired at 5  $\mu$ m could distinguish between most of these spatial features. In this study, we will use high resolution MALDI-MSI to investigate the IR-induced changes in specific lipid expression among the histological layers, and more specifically in the intestinal crypts. Further investigation of the changes in lipid metabolism under different conditions of IR injury are being conducted.

### Please explain why your abstract is innovative for mass spectrometry?

This study combines the use of a clinically relevant human model of IR injury with high-resolution MALDI-MSI to explore new grounds on the involvement of lipids as targets for treatment.

### Co-authors:

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
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Poster number: **LS-PB-089**

## **TOPICAL DELIVERY OF ANTIFUNGAL DRUGS WITH NANO-VESICULAR FORMULATIONS CHARACTERIZED BY DESI-MSI**

Abstract ID: **1002**

**Presenting author: Ravit Yakobi, The Hebrew University**

### **Introduction**

The design of antifungal drug delivery systems (DDSs) for topical applications attracts considerable attention in recent years. A variety of potent antifungal drugs are being developed by the pharma industry; however, their effective topical delivery is still challenging.

First, drug penetration through the skin is often negligible. Second, various fungal species reside in different skin strata and therefore control of the precise depth of drug penetration into the skin is highly desirable. Third, long-term topical exposure to drugs can cause irritation.

To address these challenges, we design nanometric DDSs that can effectively penetrate drugs into various skin layers and release their cargo in the infected area. To evaluate the effectiveness of our DDSs we develop a quantitative molecular visualization method based on DESI-MSI.

### **Methods**

Franz diffusion cells are a widely used methodology to evaluate *in vitro* drug penetration and permeation into skin layers. This method controls temperature and humidity to mimic physiological conditions. Often fluorescently labeled drugs or surrogate molecules are used for DDS performance evaluation in the Franz cells.

We developed a quantitative method based on desorption electrospray ionization mass spectrometry imaging (DESI-MSI) to measure the dynamic distribution of drugs in various skin layers after administrating with our developed DDSs. Importantly, we compared this direct evaluation method that does not require any fluorescent labeling to the results obtained by the Franz cells methodology.

### **Preliminary data (results)**

Four different nano-vesicular drug delivery systems were designed and prepared to deliver the hydrophobic antifungal drug - Terbinafine Hydrochloride (TBF): Ethosome, transfersome, liposome and microemulsion. The nano size of these systems was determined via dynamic light scattering measurements.

In the first step, each system was applied on porcine ear skin portions under controlled conditions in Franz diffusion cells. As a control and comparison experiment, the fluorescence molecule fluorescein isothiocyanate (FITC) was added to the skin in each formulation as well. The skin portions were then measured by confocal laser scanning microscopy (CLSM) at z-stack mode to quantify the fluorescence signal from the different skin depths. The ethosomal drug delivery system has shown the highest penetration of FITC to skin by CLSM.

In the second step, each skin portion was sectioned into 10  $\mu\text{m}$  sections using a cryostat. Each section was scanned in DESI-MSI in a positive ionization mode and the distribution of terbinafine in the skin was visualized. The microemulsion formulation showed to significantly enhance the penetration of the drug to the skin, especially through hair follicles. The quantitative DESI-MSI method indicated a higher accumulation of terbinafine released from the microemulsion in the *stratum corneum* (SC) layer of porcine skin, compared to its concentration in deeper layers.

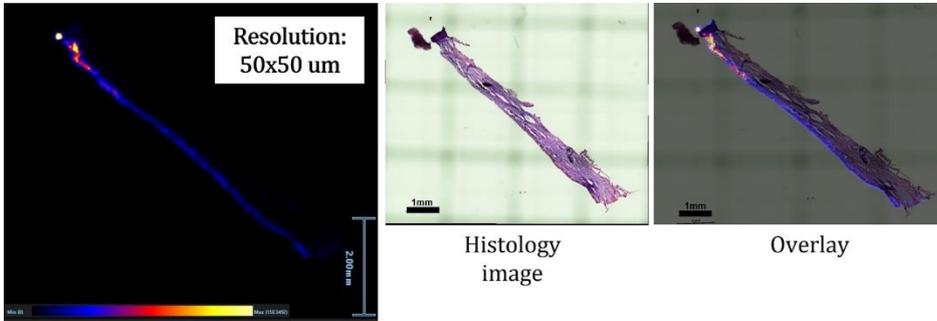
### **Please explain why your abstract is innovative for mass spectrometry?**

The quantitative DESI-MSI method was developed to evaluate drug penetration to skin layers and compared to Franz cell methodology.

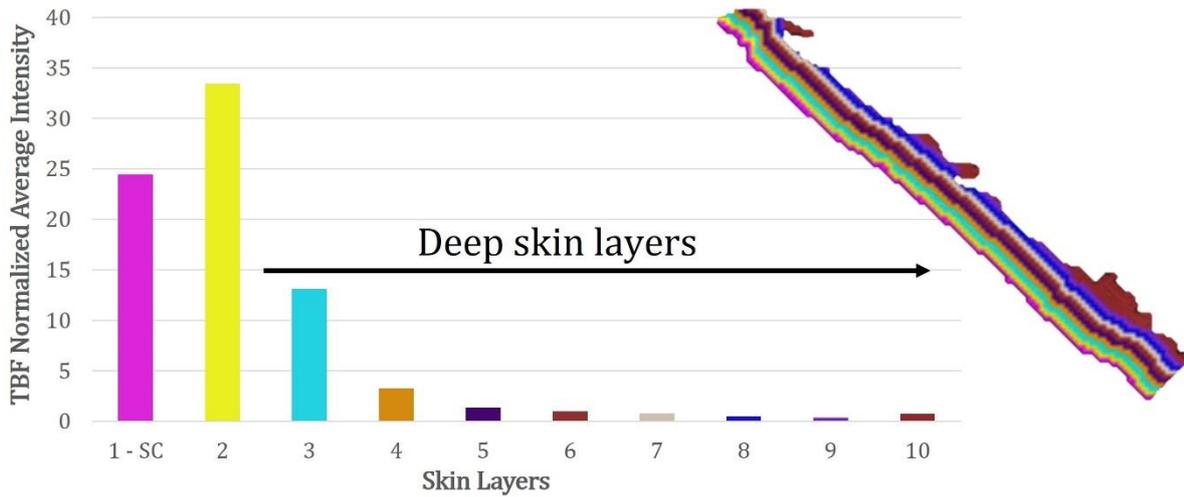
DESI-MSI was established as a tool for topical DDS evaluation.

**Co-authors:**

Hiba Natsheh, *The Hebrew University*  
 Elka Touitou, *The Hebrew University*  
 Katy Margulis, *The Hebrew University*



m/z 292.2 (M+H) Terbinafine distribution from microemulsion.



Quantitative result of Terbinafine penetration to skin layers from microemulsion.

Poster number: LS-PB-090

## MULTI-OMICS ANALYSIS OF HEPATOCELLULAR CARCINOMA USING MALDI IMAGING MASS SPECTROMETRY

Abstract ID: 1009

Presenting author: Katherine Stumpo, Bruker Scientific LLC

### Introduction

The triple helical region (THR) of fibrillar collagens is sensitive to subtle alterations, such as the post-translational (PTM) conversion of proline to hydroxyproline (HYP), impacting biological status. In nearly all cancer types, HYP PTMs are known to change cell processes of signaling, recruitment, proliferation and migration status. A full systems biology analysis of cancer requires multiple levels of interrogation, including glycans, targeted proteins, and the extracellular stroma. In this study, we have a multiomics approach to visualizing changes in tissue, with multiple classes of molecules investigated, with MALDI-2 enhancement being critical for the detection of nonpolar peptides from collagen THR.

### Methods

Hepatocellular carcinoma FFPE tissue was sectioned at 5- $\mu$ m and mounted on to glass slides. Next, slides were heated, dewaxed, and epitope retrieval done using standard procedures. Enzymes were sprayed with a TM-Sprayer (HTX-Imaging, Chapel Hill, NC), samples were incubated in humidity chambers for digestion at 37.5 °C for 5 hours, and were then sprayed with CHCA matrix. Data analysis was performed using SCiLS Lab and TIMS DataViewer (Bruker Scientific). Samples were analyzed on a Bruker Daltonics timsTOF fleX with MALDI-2 in positive ion mode with the primary laser power normalized between normal MALDI and MALDI-2 experiments.

### Preliminary data (results)

In initial studies, previously published methods using collagenase type III were used to target the THR from hepatocellular carcinoma FFPE sections. Tissues were analyzed with conventional MALDI approaches followed by MALDI-2 imaging. MALDI-2 methods typically boost the laser power of the primary MALDI laser which can obscure enhancement effects due to the plume interacting with the MALDI-2 laser. In these experiments, the MALDI-1 laser was optimized and controlled for power so that direct observations could be made on enhancement due to the use of MALDI-2. A significant peak intensity enhancement was observed for collagen peptides mapped across tissue. The majority of peptides reported a two-fold increase in signal intensity such as the doubly hydroxylated proline COL1A2 peptide GPPGESGREGAP ( $m/z$  1142.507). However, certain peptides were detected above the noise threshold that had previously not been detected using conventional MALDI. This included COL1A1 peptide GGPGSRGFP ( $m/z$  863.401), with only 1 HYP, detected only in specific stroma regions and also in a thin 200- $\mu$ m ring around the tumor. The unmodified version of this peptide ( $m/z$  847.400) was not detected in the MALDI-1 analyzed tissue and showed low detection in the MALDI-2 analysis. Continued work combines glycan analysis as well as targeted protein imaging utilizing the MALDI HiPLEX-IHC workflow.

### Please explain why your abstract is innovative for mass spectrometry?

Enhancement of non-polar peptides utilizing MALDI-2 Imaging Mass Spectrometry. Targeted protein imaging using the MALDI HiPLEX-IHC workflow is demonstrated. Multiomics analysis of multiple biological classes is highlighted.

### Co-authors:

*Peggi Angel, Medical University of South Carolina*  
*Richard Drake, Medical University of South Carolina*

Poster number: **LS-PB-091**

## **Metabolic and Genetic Changes In Human Colorectal Cancer And Colorectal Metastatic Tissue by Combined NanoString Technology and DESI-MSI**

Abstract ID: **1036**

**Presenting author: Yasmin Shanneik, University of Manchester**

### **Introduction**

Tumour-promoted inflammation is an enabling characteristic of cancer. Inflammatory cells secrete growth factors and cytokines to support tumour growth and survival. Tumour-associated macrophages play an important role in tumour progression. Altered metabolism is a feature of cancer cells that not only impacts cancer progression but also the regulation of macrophage phenotypes.

The NanoString nCounter provides specific and sensitive genetic profiling data, identifying up to 800 genes simultaneously from traces of RNA. For molecular analysis, mass spectrometry imaging (MSI) provides rapid identification of phenotype-dependent MS signatures from tissues with little sample preparation.

This study explores the link between fatty acid (FA) metabolism/synthesis, FA oxidation and macrophages in human colorectal and colorectal cancer metastasis using immunohistochemistry, NanoString nCounter analysis and Desorption Electrospray Ionization (DESI) MSI.

### **Methods**

Fresh-frozen human colorectal adenocarcinoma and metastasis samples were cut into 12  $\mu\text{m}$  sections. DESI-MSI (Xevo G2-XS, Waters, UK) was performed in both polarities with 50  $\mu\text{m}$  spatial resolution. Masslynx (Waters, UK) and SCiLS Lab (Bruker, Germany) software were used for data analysis. Histologically-stained (H&E) adjacent sections were used for microscopy.

For genomic analysis, RNA was extracted from 20  $\mu\text{m}$  sections. At least 10 sections/sample were required. RNA abundance was measured using Qubit fluorimeter. mRNA hybridisation, detection and scanning was performed on a NanoString nCounter mRNA Gene Expression system and analysed using the Rosalind platform (San Diego, CA).

### **Preliminary data (results)**

Histological differences within the tumour sections were identified and visualised with the H&E staining. The histological regions were annotated as cancerous and the surrounding non-cancerous region containing mucin, muscles and connective tissue. Using DESI-MSI in negative ion mode, we identified multiple phospholipid species, at  $m/z$  865.5,  $m/z$  1012.7, and  $m/z$  1028.7, linked to inflammation. In addition, several FA species were identified and seen at high intensity in the cancerous region of the tumour biopsies. Positive ion mode DESI-MSI revealed multiple carnitine species including palmitoylcarnitine at  $m/z$  400.33 oleylcarnitine at  $m/z$  426.35, and stearoylcarnitine at  $m/z$  428.37, which were also primarily present in the cancerous region of the tumour biopsies.

Gene expression analysis using NanoString nCounter tumour signalling 360 gene expression panel revealed an abundance of various cell type populations in the metastatic compared to the primary tumours. Particularly macrophages were seen in high abundance in the metastatic samples compared to the primary tumours. Gene expression data also showed higher IL-10 expression in the metastatic samples compared to the primaries.

Using a p-value threshold of 0.05, a total of 26 genes were identified with significant differences between the samples. Of those, 22 genes were upregulated in the metastatic samples and only 4 in the primary tumours. Genes linked to inflammation and specifically to macrophages were only found in those upregulated in the metastatic samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

Within the multimodal approach used, the genetic data are put in the context of spatially resolved metabolic data and lipid inflammatory markers from the DESI-MSI measurements.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



**Co-authors:**

*Emrys A. Jones, Waters corporation*  
*Michal Smiga, University of Manchester*  
*Bipasha Chakrabarty, The Christie NHS Foundation Trust*  
*Steven Pringle, Waters corporation*  
*Kaye J. Williams, University of Manchester*  
*Omer Aziz, The Christie NHS Foundation Trust*  
*Adam McMahon, University of Manchester*

## Session: Lipidomics

Poster number: **LS-PB-092**

### **FDR-CONTROLLED LIPID ANNOTATION FOR MASS SPECTROMETRY-BASED LIPIDOMICS**

Abstract ID: 57

**Presenting author: Christer Ejsing, University of Southern Denmark and EMBL Heidelberg**

#### **Introduction**

Correct and confident lipid identification is of paramount importance for the overall quality and reproducibility of lipidomics studies. This, however, has so far not been supported by a universal statistics-based approach that estimates the probability of whether individual lipid annotations are correct or not. We report LipidProphet, a framework for false discovery rate (FDR)-controlled lipid annotation in high-throughput MS/MS data. We demonstrate that LipidProphet is able to compute probabilities that have a high power to discriminate between correct and incorrect lipid molecule annotations.

#### **Methods**

The performance of the LipidProphet framework is benchmarked using several well-annotated direct infusion- and LC-MS-based datasets; where true positives and false positives lipid annotations are confidently and explicitly annotated. Specifically, we made use of datasets of plasma, liver and brain recorded using direct infusion-based high-resolution MS<sup>ALL</sup> analysis as well as LC-MS/MS analysis. The MS<sup>ALL</sup> analysis was carried out using an Orbitrap Fusion Tribrid equipped with a TriVersa NanoMate nanoelectrospray ion source. The LC-MS/MS analysis was performed using an Orbitrap Velos Pro coupled to a Dionex Ultimate 3000 RS UHPLC system equipped with a BEH C8 column.

#### **Preliminary data (results)**

The LipidProphet framework entails three computational routines: 1) Import of database search results; 2) Calculation of search scores for lipid molecules using a scoring function; 3) Statistical modelling to estimate the probabilities that lipid assignments are correct and calculation of cutoff search scores to control the global FDR of a dataset. More specifically, lipidomics datasets were searched towards the comprehensive ALEX database using the ALEX<sup>123</sup> search engine for the direct infusion-based MS<sup>ALL</sup> data and Lipid Data Analyzer for the LC-MS/MS data. Search results were subsequently scored using an algorithm designed to assess the statistics of precursor and fragment ions in a dataset as well as the lipid structure coverage at the fragment-level. Statistical modelling was then used to account for incorrect and correct annotations, from which global FDRs for search score cutoffs were estimated.

We benchmark the performance of LipidProphet by its ability to discriminate between correctly and incorrectly assigned lipid molecules in high-throughput MS/MS datasets of blood plasma, liver and brain. Generally, we find that LipidProphet is able to accurately estimate and control the global FDR of lipid annotations across different lipidomic datasets. Overall, the LipidProphet framework makes it possible to filter large volumes of search results with false identification error rates and can serve as a common standard by which the results of different lipidomic workflows and studies are compared.

#### **Please explain why your abstract is innovative for mass spectrometry?**

We present a routine that safeguards the overall FDR of lipid annotations in lipidomic datasets

#### **Co-authors:**

*Jürgen Hartler, Institute of Pharmaceutical Sciences, University of Graz*

Poster number: **LS-PB-093**

## QUANTITATIVE ANALYSIS OF LIPIDS IN HUMAN PLASMA USING DERIVATIZATION WITH H5/D5-BENZOYL CHLORIDE AND RP-UHPLC/MS

Abstract ID: **85****Presenting author: Ondrej Peterka, University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry**

### Introduction

Lipids play an important role in all organisms, such as membrane components, energy storage, precursors for metabolic processes, and signaling, which predestines lipids for biomarkers of cancer, cardiovascular diseases, and neurodegenerative diseases. The chemical derivatization of lipid functional groups can improve an extraction efficiency, chromatographic separation, and sensitivity. Benzoyl chloride is a non-hazardous derivatization agent with high reactivity for several functional groups producing esters with hydroxyl groups, amides with amino groups, and anhydrides with carboxylic acid groups. Mass spectrometry is a key technique in the lipidomic analysis and coupled to a separation method is typically used in lipidomics. Moreover, reversed-phase liquid chromatography enables to resolve isomeric lipids, such as the fatty acyl level and fatty acyl positions on the glycerol skeleton.

### Methods

Deproteinized plasma was derivatized with benzoyl chloride in pyridine. The reaction mixture reacts at ambient temperature for 60 minutes. The reaction was stopped by applying a modified Folch extraction.

We used UHPLC connected to high-resolution mass spectrometer Xevo G2-XS QTOF for non-targeted analysis and Xevo TQD for targeted analysis. Acquity UPLC BEH C18 column (150 mmx2.1 mm, 1.7  $\mu$ m) was employed. The gradient elution using ACN/H<sub>2</sub>O (3:2, v/v) and IPA/ACN (9:1, v/v), both phases containing 8 mM ammonium formate and 0.1% formic acid, was set for the separation of lipid species.

### Preliminary data (results)

The reaction conditions, such as volume and ratio of reagents, reaction time, and temperature, were thoroughly optimized for 4 nonpolar and 8 polar lipid classes using spiked pooled plasma. The molar ratio 4:1 of pyridine and benzoyl chloride, and the reaction time of 60 min at ambient temperature provide the best yield. The reproducibility of reaction was investigated by 2 operators with reported RSD lower than 20 %. The derivatization and nonderivatization approaches were compared using spiked human plasma based on the calibration curves of 22 internal standards from 12 lipid classes. The new method decreased the limit of detection 9-fold for monoacylglycerols, 6.5-fold for sphingoid base, and 3-fold for diacylglycerols. The sensitivity was increased 2 to 10-fold for almost all investigated lipid classes and even more than 100-fold for monoacylglycerols. The derivatization method enables detecting more lipid species of monoacylglycerols, diacylglycerols, and sphingoid bases compared to the nonderivatized approaches.

Mixing H5 and D5 lipidomic extracts lead to the creation of doublets for all derivatized lipids, which simplifies the identification and provides internal standard per each compound. H5 and D5 derivatives coelute together ( $\Delta$ RT  $\pm$  0.1 min), which leads to the same matrix effect and ionization efficiency. This approach was fully validated, and the method was applied for relative quantitation of human plasma. Moreover, the method was also used for the investigation of lipidomic differences between healthy subjects and cancer patients.

This work was supported by Czech Science Foundation (GAČR) project No. 21-20238S.

### Please explain why your abstract is innovative for mass spectrometry?

We developed a new and highly reproducible derivatization method, which was used for quantitative analysis of human plasma based on pooling of extracts prepared by nonlabelled and labelled derivatization agents.

### Co-authors:

*Robert Jirasko, University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry*  
*Zuzana Vankova, University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry*  
*Michal Holcapek, University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry*

Poster number: LS-PB-094

## FLOW INJECTION ANALYSIS TANDEM MASS SPECTROMETRY FOR DETERMINATION OF SELECTED CLASSES OF SPHINGOLIPIDS IN PLASMA OF PATIENTS WITH COLORECTAL CARCINOMA

Abstract ID: 88

**Presenting author: Denisa Kolářová, Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice**

### Introduction

Imaging techniques followed by the subsequent biopsy are widely used for tumor detection. Although these procedures are relatively reliable, they require invasive surgery, and early-stage tumors may still go unnoticed. Non-invasive laboratory tests from blood plasma usually show less reliability than imaging techniques, and there is a lack of specific biomarkers for many cancer types. Therefore, the development of screening methods for the non-invasive and early detection of different types of cancer is essential. In this context, we focused on sphingolipids analysis in the case of colorectal cancer. Sphingolipids play an important role in the death and survival of cancer cells and have recently attracted attention because their levels are usually altered in the biofluids and tissues of cancer patients compared to healthy volunteers.

### Methods

Modified Folch protocol was used for plasma samples extraction. The Agilent LC system consisting of 1260 Infinity binary pump and autosampler, and 1290 Infinity capillary pump was applied for direct infusion of 50  $\mu$ l of a sample at 3.5  $\mu$ l/min flow into the source of QTRAP mass spectrometer (Sciex). The running phase was chloroform/methanol/2-propanol (1:2:4, v/v/v) mixture. Ceramides, hexosyl-, and dihexosylceramides were quantified based on the precursor ion scan 264.3, and sphingomyelins 184.1, respectively. The obtained data were processed with LipidView and LipidQuant softwares.

### Preliminary data (results)

We have optimized and validated the flow injection analysis (FIA) - tandem mass spectrometry (MS/MS) -based method to analyze selected sphingolipids, including sphingomyelins and low abundant lipids, such as ceramides, hexosylceramides, and dihexosylceramides, in human plasma samples. The method was used to quantify the sphingolipids in the standard reference material NIST SRM 1950 and to measure a small cohort of plasma samples collected from colorectal cancer patients and healthy volunteers. The quantitation was performed using precursor ion mass scans (PIS 264.3 and 184.1, respectively), and samples were measured in the positive polarity mode. Based on the validation results, it was verified that the method is applicable for the quantification of selected sphingolipids, e.g., the accuracy and precision were <20 % for most of the standards at the medium concentration level. In NIST SRM 1950 human plasma, 39 sphingolipids were identified and quantified, and the concentrations were comparable with previously published data. Then, 36 sphingolipids were identified in a small cohort of plasma samples, and 6 of them showed statistically significant differences in concentration, Cer 18:1;O2/24:0, SM 39:1;O2, SM 41:1;O2, SM 42:1;O2, and SM 43:1;O2 were downregulated and Hex2Cer 18:1;O2/16:0 was upregulated. This work was supported by the Czech Science Foundation (GAČR) project No. 21-20238S.

### Please explain why your abstract is innovative for mass spectrometry?

We developed the simple FIA-MS/MS approach for robust sphingolipids' quantitation in plasma. The method was also applied to determine if colorectal cancer influences selected sphingolipids levels.

### Co-authors:

*Jakub Idkowiak, Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice*  
*Robert Jirásko, Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice*  
*Michal Holčapek, Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice*

Poster number: **LS-PB-095**

## DEEP LIPIDOMICS OF PHOSPHOLIPID *sn*-ISOMERS USING OZONE-INDUCED DISSOCIATION COMBINED WITH A HIGH-THROUGHPUT DATA-INDEPENDANT ANALYSIS WORKFLOW

Abstract ID: 103

**Presenting author: Jesse Michael, School of Chemistry and Molecular Bioscience, University of Wollongong**

### Introduction

Mass spectrometry-based shotgun lipidomics workflows enable the detection of several hundreds of lipids present in complex biological extracts. However, conventional MS/MS methods cannot accurately resolve lipids varying in the location of acyl chains on the glycerol backbone (*sn*-isomers). As these isomers reflect different biosynthetic processes, this underestimates sample complexity and limits insight into lipid metabolism.

Sequential Collision-Induced Dissociation and Ozone-induced Dissociation (CID/OzID) exploits the high reactivity of ozone towards the headgroup loss fragment of ionised glycerophospholipids, producing further fragments that allow unambiguous assignment of *sn*-isomers. However, CID/OzID has primarily been deployed in targeted analyses of selected precursors and not comprehensively in lipidomics studies. We overcome this limitation by developing a high-throughput DIA-based CID/OzID workflow for deep lipidomics of *sn*-isomers.

### Methods

Porcine brain and liver lipid extracts were analysed by nano-electrospray ionisation on an Orbitrap Fusion with ozone supplied to the linear ion trap. In methanolic ammonium formate, sequentially-isolated 1 Da windows in both positive- and negative-ion modes were subjected to HCD. In methanolic sodium chloride, each 1 Da mass window (collision energy 40%, activation time 15ms) was subjected to CID with subsequent exposure of headgroup loss fragments to ozone (collision energy 0%, activation time 800 ms). MS<sup>2</sup>/MS<sup>3</sup> spectra were acquired at resolution 240,000@200 m/z. Automated lipidomics data analysis was performed using a modified version of ALEX123.

### Preliminary data (results)

High-confidence *sn*-isomer annotations were provided by characteristic CID/OzID aldehyde and criegee ions detected to  $\pm 3$  ppm accuracy with validation between sodium and ammonium solutions. PC and PE classes were targeted for CID/OzID. Crucially, the high mass resolving power of the Orbitrap Fusion allows assignment of fragments to specific precursors among an isobaric mixture, thus diacyl, alkyl and alkenyl (plasmalogen) ether lipids are readily identified within the same spectra. Demonstrating the rich data generated, 218 individual *sn*-isomers belonging to 57 sum-composition PC species were identified (Figure 1).

Informative CID/OzID spectra were generated from phospholipid precursors with abundances <0.1% in the MS<sup>1</sup> spectrum. The high sensitivity of the instrument and specificity of the CID/OzID technique allows us to report with confidence numerous minor isomers for each sum-composition species as well as a number of unusual lipids. For example, porcine brain was found to contain PC 46:2, shown here to be predominantly PC 28:1/18:1 (Figure 2). A series of similar PC lipids containing very-long-chain MUFAs predominantly at the *sn*-1 position, ranging from 24 to 30 carbon lengths suggests an unrecognised activity of elongase enzymes in the brain.

Modest acquisition times (approximately 20 minutes for wide coverage) allows simple incorporation into high-throughput shotgun lipidomics workflows. Deep isomer-resolved lipidomics should provide an important avenue to explore the specificities of acyl chain remodelling events occurring within tissue and cells.

### Please explain why your abstract is innovative for mass spectrometry?

The incorporation of CID/OzID into a shotgun lipidomics workflow enables the rapid, comprehensive, and *sn*-isomer-resolved analysis of glycerophospholipids, including diacyl and alkyl/alkenyl ether varieties, within complex biological extracts.

**Co-authors:**

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours

*Alan Maccarone, School of Chemistry and Molecular Bioscience, University of Wollongong*

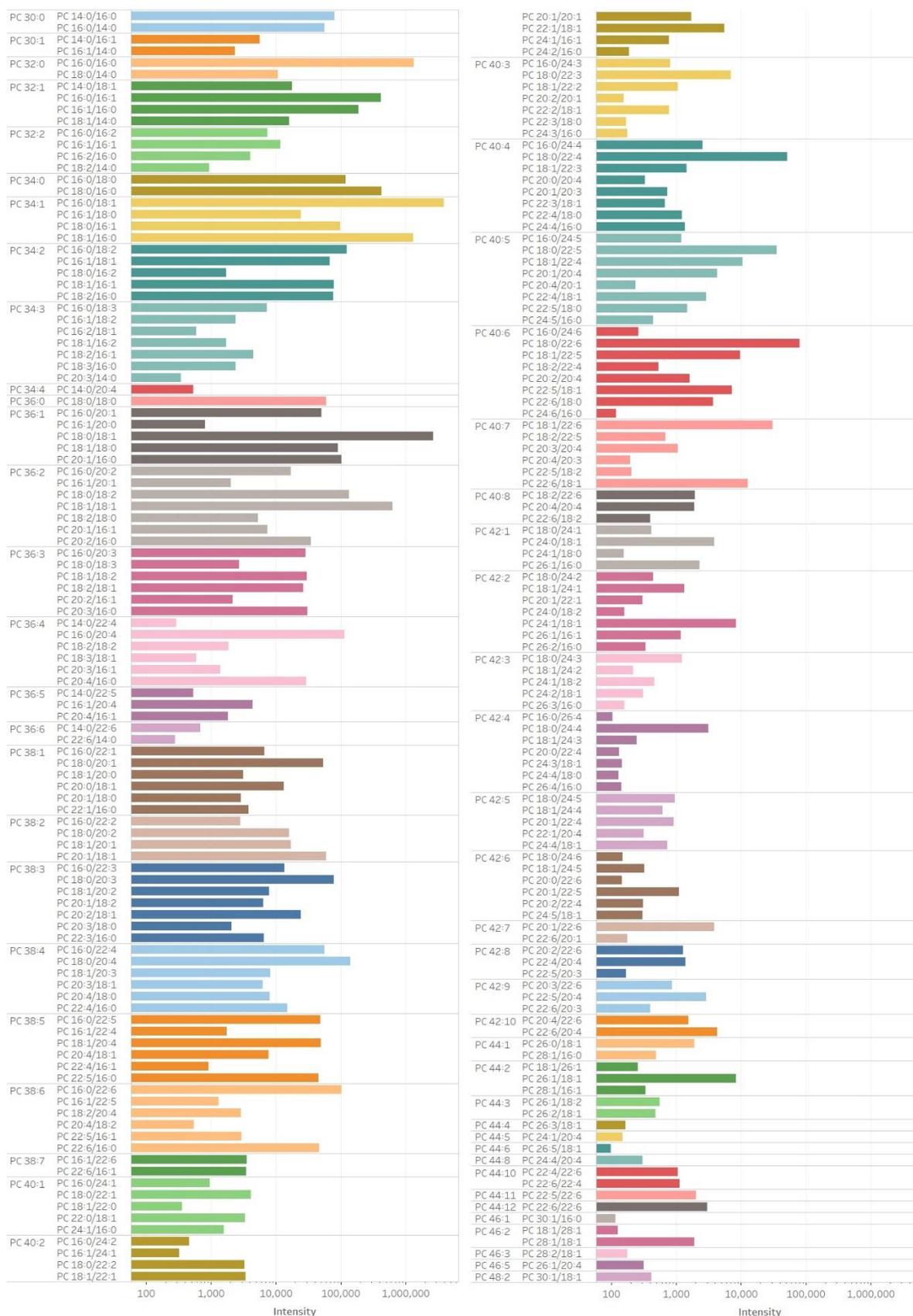
*Todd Mitchell, School of Chemistry and Molecular Bioscience, University of Wollongong, Illawarra Health and Medical Research Institute (IHMRI)*

*Christer Ejsing, Department of Biochemistry and Molecular Biology, VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, Cell Biology and Biophysics Unit, European Molecular Biology Laboratory*

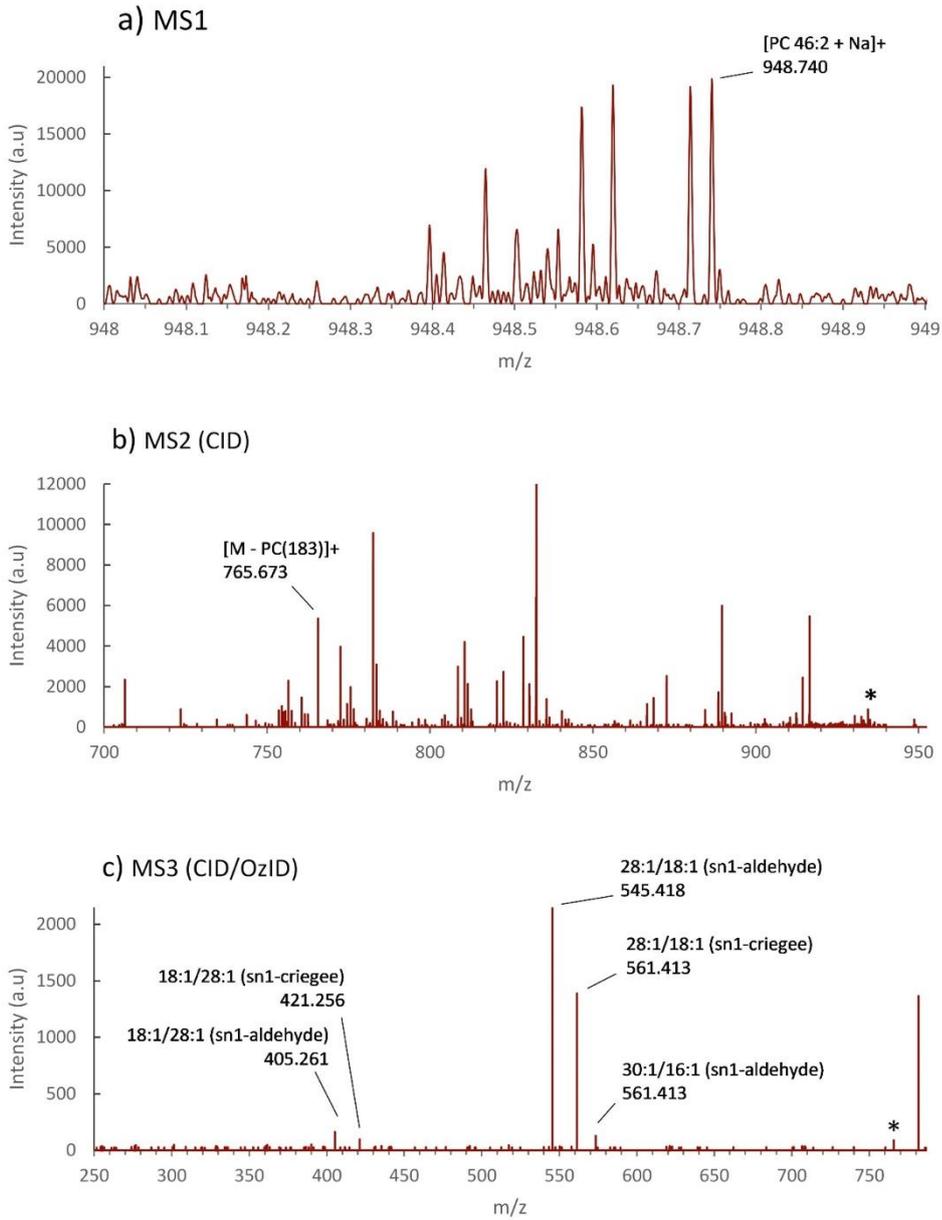
*Shane Ellis, School of Chemistry and Molecular Bioscience, University of Wollongong, Illawarra Health and Medical Research Institute (IHMRI)*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



Identified PC *sn*-isomers with intensities of *sn*-1 aldehyde fragment.



MS1, CID and CID/OzID analysis of PC 46:2.

Poster number: **LS-PB-096**

## **NANOLC-MS/MS AS A TOOL FOR VERIFICATION OF CHLORINE EXPOSURE**

Abstract ID: 121

**Presenting author: Pernilla Lindén, Swedish Defence Research Agency**

### **Introduction**

The use of chemical weapons is prohibited under the chemical weapons convention. Enforcement of the ban is supervised by the Organisation for the Prohibition of Chemical Weapons (OPCW), through a network of inspectors and designated analysis laboratories.

Chlorine is a toxic chemical that has been repeatedly used as a chemical weapon in recent armed conflicts. The aim of this work was to develop forensic analytical methods for the verification of chlorine gas exposure that would greatly enhance the probability of bringing the perpetrators of such attacks to justice. It was previously shown that inhaled chlorine gas reacts with unsaturated phospholipids in the pulmonary surfactant forming phospholipid chlorohydrins (PL-HOCl) and palmitoyl oleoyl phosphatidylglycerol chlorohydrin (POPG-HOCl) was selected for analysis.

### **Methods**

Here we use two alternative methods to analyse PL-HOCl in broncho-alveolar lavage fluid (BALF) samples from chlorine exposed mice.

Method 1: After extraction of BALF, POPG-HOCl was analysed by nanoLC-MS/MS. Peaks were detectable up to 72h post exposure. There were issues with carry-over and sample solubility and/or stability.

Method 2: PL-HOCl were extracted using a transesterification protocol on a Waters HLB-SPE. The derived biomarker originate from all of the diverse PL-HOCl derivatives carrying the same unsaturated oleic acid. Samples were analysed by nanoLC-MS/MS with a C4 column. Contamination from the SPE and ion suppression hampered the method.

### **Preliminary data (results)**

Mice (n=8/group) was exposed to three different chlorine exposure regimes, with equal calculated toxic load. Group A was exposed to a high chlorine load (150 ppm) for a short period of time (15 min). Group B was exposed to a lower but twice repeated load (106 ppm, 2 x 15 min) with a 30 min time gap. Group C was exposed to a low chlorine load (75 ppm) for a longer period of time (60 min), **figure 1**. BALF was collected from all three groups, and a control group, 24 hours after exposure.

The result implies that analysis Method 1 give lower variation within groups and larger variation between groups than Method 2. Group A, treated with short but high chlorine load showed the highest levels of POPG-HOCl, followed by the group treated with a low load for a longer period of time (Group C), while the group that was treated with a repeated low exposure (Group B) had the lowest level of POPG-HOCl, **figure 2**.

In the future we aim to show that these methods also can be used for different biofluids such as nasal lavage fluid (NLF), which in turn can be used for verification of chlorine exposure of humans.

### **Please explain why your abstract is innovative for mass spectrometry?**

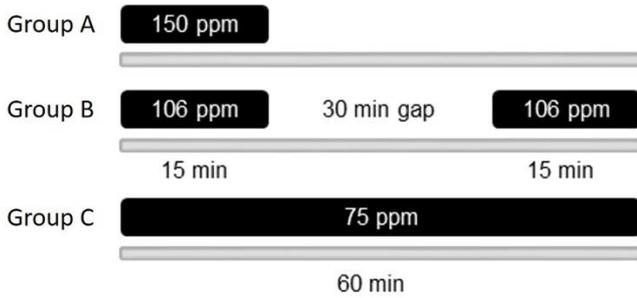
We present two methods for targeted lipid analysis using nanoLC-MS/MS.

### **Co-authors:**

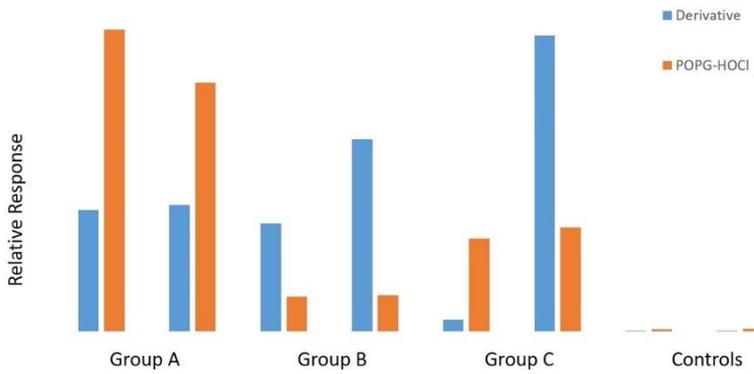
*Lovisa Ålander, Swedish Defence Research Agency*  
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Linda Elfsmark, Swedish Defence Research Agency  
Crister Åstot, Swedish Defence Research Agency

Chlorine Exposure Regimes



Design of exposure experiment.



Measured levels of POPG-HOCl and derivate for different exposure regimes.

Poster number: LS-PB-097

## LIPID ANALYSIS BY MEANS OF LC-IM-QTOF-MS AND THE EFFECT OF DATA PRETREATMENT IN TARGETED AND NON-TARGETED ANALYSIS

Abstract ID: 127

**Presenting author: Sven W. Meckelmann, Applied Analytical Chemistry, University of Duisburg-Essen**

### Introduction

Coupling IM with LC-MS increases the overall peak capacity and leads to the separation of co-eluting lipid isomers. In addition, IM offers an additional identification parameter and allows lipid annotation by their retention time, exact mass, and the collision cross-section (CCS). However, the drift dimension adds complexity to the data and the problems described for LC-MS data analysis can be transferred. It is well known that results are strongly influenced by the program settings and the type of instrument as well as the parameters chosen. In addition, processing errors, false negatives, false positives, and misalignments lead to incorrect interpretations of the results.

### Methods

Here the influences of method parameters, data pretreatment, and feature analysis parameters were investigated to highlight possibilities and challenges. Therefore, an RPLC method was used with the Agilent 6560 drift tube ion mobility time-of-flight mass spectrometer (IM-qTOF-MS). The initial study examined the influences of different data acquisition modes and pretreatment strategies for various lipids from major lipid classes. Complex sample assessment includes pooled human blood plasma and serum, as well as HepG2 cells. Different data pretreatment strategies were tested before feature analysis was performed. Furthermore, the influence on the subsequent feature annotation was investigated.

### Preliminary data (results)

Different data pretreatment strategies (e.g. smoothing in chromatographic and drift dimension, a signal intensity lower threshold, and spike removal function that filters points with less than one or two adjacent points in each drift and m/z dimension) were tested. Furthermore, the influence on the subsequent feature annotation was investigated. Results show a reduction of peak variation and background by optimizing the sampling rate and subsequently the use of data pretreatments. The sampling rate and data pretreatment can increase LOD and LOQ by lowering background noise whereas, non-optimal data sampling rates and preprocessing can lead to adverse effects such as a loss of chromatographic resolution when not optimized correctly. Moreover, it also plays an important role to improve the detection and annotation of lipids from complex biological samples in feature analysis. Adequate settings can help to improve the quality of the detected features and to reduce the false-positive discovery rate. In all omics disciplines as well as in IM-based lipidomics and metabolomics, it is very important to have a detailed look at the data (e.g. peak height and width, number of data points). Optimizing these parameters along with adequate settings for data pretreatment is often not well addressed. However, optimal parameters can help to improve feature analysis for a non-targeted as well as automated integration for a targeted analysis hence increasing the confidence in the results.

### Please explain why your abstract is innovative for mass spectrometry?

The results highlight possibilities and challenges in data pretreatment strategies. Correctly optimized parameters can improve LODs and LOQs and the confidence in the results by reducing the false-positive discovery rate.

### Co-authors:

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Poster number: **LS-PB-098**

## HOW MULTIPLEXING AND POST-PROCESSING CAN INCREASE THE SEPARATION POWER AND LIPIDOME COVERAGE FOR A NON-TARGETED LIPIDOMICS WORKFLOW

Abstract ID: 133

Presenting author: **Sven W. Meckelmann, Applied Analytical Chemistry, University of Duisburg-Essen**

### Introduction

Omics approaches aim to analyze a large number of samples with complex compositions. To face these analytical challenges, fast, sensitive, and high-resolution analyses are needed. Currently, the coupling between liquid chromatography, ion mobility, and mass spectrometry (LC-IM-MS) can provide a high separation power because of the multiple dimensions. However, the application of multiplexing mode acquisition in combination with post-processing in the IMS field has shown a new opportunity to increase the separation and resolution of complex analysis even further.

### Methods

In this work, LC-IM-MS using single-pulse and multiplexing modes with high-resolution demultiplexing tools have been compared and applied. Therefore, an LC-IM-QTOF-MS method was applied for the analysis of a mixture of lipid standards containing all relevant families of lipids as well as different samples such as cells, serum, and plasma. The analysis was carried out using an Agilent 1290 LC coupled with an Agilent 6560 Drift Tube IM-QTOF-MS. Lipids were separated using a C18 column and detected in both, single-pulse and multiplexing modes for comparison. Multiplexing data was then post-processed with a method called High-Resolution demultiplexing (HRdm)

### Preliminary data (results)

Single-pulse and multiplexing IMS modes were compared and high resolution demultiplexed data showed an increase in the IM separation demonstrating the enhanced performance. The multiplexing lipid analysis using the HRdm tool revealed an increase in peak capacity of ~32% in comparison to a single pulse. Besides, the signal-to-noise ratio is improved in multiplexing mode, resulting in a higher sensitivity when using this IMS mode for some of the analyzed lipids like lysophosphatidylcholines, phosphatidylcholines, triglycerides, and sphingomyelins. Moreover, the dynamic range for these lipids was increased by using the multiplexing mode enabling quantification over a larger range. The lipidomic analysis of biologically relevant samples (cells, serum, and plasma) in the multiplexing mode and in particular after the use of the post-processing HRdm tool, allowed the separation of isobaric compounds that used to coelute. The use of multiplexing mode in IMS together with HRdm post-processing enhanced the quality parameters of the analysis of lipids. In particular, the separation power (peak capacity), sensitivity (LODs and LOQs), and dynamic range were improved using this analytical workflow. Moreover, in complex samples like cells, serum, and plasma, it was possible to separate isomeric lipids that were not possible to separate by LC, MS, or single-pulse IMS.

### Please explain why your abstract is innovative for mass spectrometry?

The analytical platform using multiplexing in combination with High-Resolution demultiplexing as post-process Method showed to be a promising tool for lipidomic analysis by increasing the separation power and lipidome coverage.

### Co-authors:

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Poster number: **LS-PB-099**

## **USING KENDRICK PLOT AS A RAPID VISUALIZATION TOOL FOR LIPIDOMICS IN COMPLEX SAMPLE USING DIRECT INFUSION MASS SPECTROMETRY AND REVERSE PHASE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY**

Abstract ID: **148**

**Presenting author: Justine Hustin, Mass Spectrometry Laboratory - ULiège**

### **Introduction**

Lipidomics is an emerging field of growing interest that has developed rapidly over the past decade. Lipidomics is the study of lipids from a qualitative and quantitative perspective. Non-targeted lipidomics from biological samples is still a challenge due to the high diversity of lipids in terms of structures, isomerisms, functional groups, and the complexity of biological origin matrices usually investigated. For example, cells contain tens to hundreds of thousands of different lipids at concentrations ranging from  $\mu\text{mol}$  to  $\text{nmol}/\text{mg}$  protein.

The present work, developed in the context of the Eurlipids project (Euregio Meuse-Rhine Interreg, <http://eurlipids.com/>) introduces the use of Kendrick plots as a rapid visualization tool for specifically extracting the signals of lipids from complex mixtures.

### **Methods**

The approach is demonstrated on lipids extracted from dry yeast with the MTBE method, from data generated by direct infusion electrospray mass spectrometry (ESI-FT-ICR) obtained from a Solarix XR 9.4T coupled to the nanoflow ion source NanoMate HD (Advion BioSciences, Ltd., Ithaca, NY) and liquid chromatography-ESI MS (LC-MS) performed on a Waters Acquity UPLC I-Class system (Milford, MA, USA) coupled with a 7T LTQ-FT Ultra mass spectrometer (LTQ-FT Ultra 7T, Thermo Finnigan, Bremen).

### **Preliminary data (results)**

Each lipid family is identified according to its theoretical Kendrick Mass defect in the Kendrick plots (using  $-\text{CH}_2-$  scale). Lipids sharing the same unsaturation level but variable  $-\text{CH}_2-$  units are aligned horizontally in the Kendrick plot while they are parallel in the case of different unsaturation levels. To the contrary, the lipids are plotted with specific angles if the nature of the repeating units and end-chains is affected (of different nature). The  $m/z$  and the intensity of all the lipids from these patterns can be extracted with our in-house Python software and assigned on the basis of one of the most intense member of the series using exact mass measurements and the LIPID MAPS database (Nature Lipidomics Gateway, [www.lipidmaps.org](http://www.lipidmaps.org)).

The comparison between the Kendrick plot of the yeast lipid extracts collected by direct infusion and LC-MS using a C18 column was performed by the superposing the Kendrick plots using an in-house Python software called MSKendrickFilter. The analysis of lipids/adducts specific to direct infusion or LC-MS was also performed. Finally, the comparison of the LC-MS method and the direct infusion of lipids with respect to the Kendrick plot will be discussed.

### **Please explain why your abstract is innovative for mass spectrometry?**

The use of Kendrick combined with liquid chromatography and the comparison between Kendrick plots extracted from direct infusion and LC-MS are the innovations presented.

### **Co-authors:**

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Poster number: **LS-PB-100**

## **STRUCTURAL ELUCIDATION OF THE POLAR LIPID CLASSES N-ACYLPHOSPHATIDYLETHANOLAMINES, N-ACYL-LYSOPHOSPHATIDYLETHANOLAMINES AND ACYL-MONOGALACTOSYLDIACYLGLYCEROLS IN CEREALS WITH ESI-MS AND ESI-MS/MS**

Abstract ID: **163**

**Presenting author: Svenja Schneider, University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstraße 30, 48149 Münster, Germany**

### **Introduction**

Lipids have different biochemical functions in plants, which are not fully understood yet. Due to the methods used in previous studies, not all lipids were covered or not investigated in detail. The aim of this work is the structural elucidation of polar lipid classes in cereals that have received less attention so far, such as *N*-acylphosphatidylethanolamines (NAPE), *N*-acyl-lysophosphatidylethanolamines (NALPE) and acyl-monogalactosyldiacylglycerols (acyl-MGDG). While MGDG and digalactosyldiacylglycerols (DGDG) represent a major fraction of membrane lipids in amyloplasts, structural derivatives, such as acyl-MGDG, which are acylated at C-6 position, are somewhat neglected. In addition, NAPEs show a relatively high contribution to the total phospholipid content in wheat.

### **Methods**

Given the high complexity of the cereal lipidome, comprehensive characterization of these compounds is a challenging task. For precise assignments of lipid classes, a chromatographic separation method, such as hydrophilic interaction liquid chromatography (HILIC) or supercritical fluid chromatography (SFC) is used. Online coupling with high-resolution ESI-MS and data-dependent MS/MS experiments in positive and negative ionization mode were utilized to determine lipid species of different lipid classes.

### **Preliminary data (results)**

Lipid species distributions within the identified lipid classes NAPE, NALPE, PE and acyl-MGDG were determined by measuring the accurate masses of lipid species using SFC- or HILIC-MS. MS/MS fragmentation experiments in negative ionization mode allowed the identification of lipid species based on the length and degree of saturation of bound fatty acid residues, whereas MS/MS in positive ionization mode enabled further in-depth characterization. Especially acylated galactose head groups in acyl-MGDG and *N*-acylated headgroups of NAPE could be identified by fragmentation experiments in positive mode. In addition, the two lipid classes NALPE and PE can be differentiated from each other due to different fragmentation behaviour in positive ionization mode.

### **Please explain why your abstract is innovative for mass spectrometry?**

Characterisation of *N*-acyl-lysophosphatidylethanolamines using ESI-MS/MS experiments in positive and negative ionization mode.

### **Co-authors:**

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Poster number: LS-PB-101

## ADDRESSING STRUCTURAL DIVERSITY OF CARDIOLIPINS AND THEIR OXIDATION PRODUCTS BY HYPHENATED TECHNIQUES

Abstract ID: 174

Presenting author: Heiko Hayen, University of Münster

### Introduction

Cardiolipin is an important phospholipid (PL) subclass which is found in mitochondrial membranes and has specific properties in the biochemistry of organisms, i.e. the respiratory chain. In addition to that, oxidized CL can occur due to oxidative stress and are linked to apoptotic events. Due to the dimeric structure of CL, it is the only phospholipid, which comprises four fatty acyl moieties. The already high structural diversity due to differing lengths and degrees of saturation of the fatty acyl chains is further increased by oxidation. Therefore, not only powerful separation techniques based on HPLC and IMS are required, but hyphenation to high-resolution MS and MS/MS as well.

### Methods

A separation of different PL classes based on their polar head group was achieved by hydrophilic interaction liquid chromatography (HILIC). A selective transfer of a distinct PL class to the second dimension with a heart-cut setup was carried out. In the second dimension, a reversed phase (RP)-LC column was utilized for the separation of PL species based on their fatty acyl moieties. Subsequent to the RP-LC separation, the transferred CLs were analyzed by HRMS and further characterized by MS/MS experiments. Furthermore, the use of trapped ion mobility-mass spectrometry (TIMS) shed light on the occurrence of isomeric oxidation products.

### Preliminary data (results)

The hyphenation of two chromatographic techniques offers the possibility to separate interfering matrix components such as other PL classes or triacylglycerides. First, the individual PL classes have been separated by their head group using HILIC in the first dimension. Non-polar lipid classes such as triacylglycerides or sterols and esters thereof were removed. The CL fraction was transferred to a RP-LC column in the second dimension by the developed heart-cut setup. There, CL species were separated according to their chain length and degree of unsaturation. Due to less matrix effects regarding the chromatographic separation and ion suppression during electrospray ionization, the online two-dimensional-LC/MS method showed a higher sensitivity compared to the conventional one-dimensional LC-MS method. The identification and structural elucidation of different CL species and their hydroxy and hydroperoxy counterparts was carried out by means of accurate mass and MS/MS fragmentation. Isomeric oxidation products like hydroperoxylated vs. dihydroxylated CLs could be differentiated by mobility-resolved MS/MS fragmentation experiments.

### Please explain why your abstract is innovative for mass spectrometry?

Differentiation of isomeric cardiolipin oxidation products by hyphenation of two-dimensional HPLC and trapped ion mobility spectrometry (TIMS) to HR-MS/MS.

### Co-authors:

Vera Schwantes, University of Münster  
Edward Rudt, University of Münster  
Patrick O. Helmer, University of Münster

Poster number: LS-PB-102

## LIPID QUANTIFICATION BY HILIC-MS/MS UTILIZING TRAPPED ION MOBILITY SPECTROMETRY

Abstract ID: 205

Presenting author: Edward Rudt, University of Münster

### Introduction

The sum of all lipids in an organism, also called lipidome, is characterized by a variety of important functions at cellular level such as stabilizing the cell membrane and the formation of mediators. Therefore, a change in lipid composition and concentration often correlates with various neurodegenerative and cardiovascular diseases such as Alzheimer disease and atherosclerosis. An important role is assumed by polar phospholipids as a major component of the cell membrane. Due to the high progress of lipidomics research in the last decade the number of biomedical applications with lipids as biomarkers has increased significantly. Nevertheless, quantification of lipids remains analytically challenging due to the large number of lipids and their structural diversity.

### Methods

In this work, a method for lipid quantification of polar phospholipids in human plasma was developed using ion mobility mass spectrometry after chromatographic lipid class separation. Lipid class separation was achieved by hydrophilic interaction liquid chromatography (HILIC). The additional use of the orthogonal, postionization separation technique trapped ion mobility spectrometry (TIMS) hyphenated to mass spectrometry (MS) allowed an unambiguous assignment of lipids based on mobility and retention time. For quantitative profiling of phospholipids in human plasma, an isotopically labeled internal standard per lipid class was used, which coeluted with the corresponding phospholipid class applying HILIC separation conditions.

### Preliminary data (results)

The ion mobility spectrometry (IMS) technique TIMS has the advantage of an adaptable mobility resolution according to the analytical problem. At high ramp times, and thus higher IMS resolution, isobaric and isomeric interferences can be identified and separated via mobility. In particular, for type-II overlap<sup>[1]</sup> caused from lipids with increasing double bond number due to the natural isotopic distribution of carbon isotopes, TIMS-MS is a potential alternative to ultra-high resolution MS.

A variety of phospholipids were detected in human plasma using the MetaboScape<sup>®</sup> identification software based on accurate mass, specific fragmentation, natural isotope distribution and collisional cross section (CCS) values. The lipid annotation could be confirmed by the lipid class-specific retention time windows and by trends in 4D Kendrick mass plots.

For these reasons, the presented HILIC-TIMS-MS method has the potential to become a versatile tool for lipid quantification. Especially the high sample throughput of this method could find application in clinical studies for the investigation of pathophysiological biomarkers.

[1] M. Höring, C. S. Ejsing, S. Krautbauer, V. M. Ertl, R. Burkhardt, G. Liebisch "Accurate quantification of lipid species affected by isobaric overlap in Fourier-transform mass spectrometry", *Journal of lipid research* **2021**, 62, 100050.

### Please explain why your abstract is innovative for mass spectrometry?

This is the first study on the quantification of lipids using TIMS after HILIC separation. Concentration falsifying overlaps can be separated and the sensitivity of TIMS can be utilized.

### Co-authors:

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Heiko Hayen, University of Münster

Poster number: **LS-PB-103**

## **ZONATED LIVER DISEASES ANALYSIS VIA LASER CAPTURE MICRODISSECTION AND MASS SPECTROMETRY**

Abstract ID: **236**

**Presenting author: Silvia Radrezza, MPI-CBG**

### **Introduction**

Due to their incidences and gravity, hepatic diseases (i.e. non-alcoholic fatty liver disease, hepatocellular carcinoma etc.) represent a considerable worldwide health problem. Nevertheless, their etiopathology and development are still not fully clear.

If the knowledge of intra- and extra-cellular profile alterations at organ level can inform about the disease's main impact, a better look can be achieved by isolating the pathological tissue sections.

To do that, we applied, laser capture microdissection (LCM) technique to cryodeselected liver tissues isolating specific liver pathological cell types with the main focus on their lipidome and proteome composition. Approaching complex matrixes, by reducing its complexity thanks to LCM could help a deeper characterization of the pathological area and may assist in the biomarker discovery for a more specific treatment procedure.

### **Methods**

The extracted lipids and proteins from the micrometer-sized isolated tissue regions identified based on immunostaining were then analyzed by mass spectrometry applying the shotgun approach for lipids analyses (Q-Exactive™ equipped with a robotic nanoflow ion source TriVersa NanoMate™) and liquid-chromatography MS (Ultimate 3000 nano-UPLC system coupled online to a LTQ Orbitrap Velos™) for proteins ones. The acquired spectra were processed through the repetition rate filtering PeakStrainer software, the lipids identified by LipidXplorer software and accurate quantified based on the spiked internal standard. The proteins identification and quantification were instead performed through MaxQuant software.

### **Preliminary data (results)**

Compared to the other imaging technologies (i.e. MALDI), the here applied LMC-MS allowed us both to quantitatively analyze irregular-shaped features and reducing at the minimum the matrix manipulation achieving a noticeable benchmark by the offer in once of a broad spectrum of lipids and proteins peculiar information.

Indeed, the hepatic zonation followed by the comparison of both characteristics lipids and proteins in pathological vs normal tissue sub-sections offered us a unique multi-omics vision of the biological features with (at least partial) the associated molecular mechanism bringing the knowledge related to hepatic diseases to a deeper and more specific level.

### **Please explain why your abstract is innovative for mass spectrometry?**

The combination of LMC and MS analysis showed an excellent sensitivity considering the low amount of needed tissue and a large coverage of zonated lipids and proteins.

### **Co-authors:**

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*Andrej Shevchenko, MPI-CBG*

Poster number: LS-PB-104

## DEFINITION OF BIOMARKER CLASS FOR DIAGNOSIS OF PARKINSON'S DISEASE FROM SEBUM USING PAPER SPRAY IONISATION ION MOBILITY MASS SPECTROMETRY

Abstract ID: 250

Presenting author: Depanjan Sarkar, University Of Manchester

### Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder and identification of robust biomarkers to complement clinical diagnosis will accelerate treatment options. Here we demonstrate the use of direct infusion of sebum from skin swabs using paper spray ionisation coupled with ion mobility mass spectrometry (PS-IM-MS) to determine the regulation of molecular classes of lipids in sebum that are diagnostic of PD.

### Methods

A PS-IM-MS method for sebum samples that takes three minutes per swab was developed and optimised. The method was applied to skin swabs collected from 150 people and elucidates ~ 4200 features from each subject which were independently analysed. The data included high molecular weight lipids (>600 Da.) that differ significantly in the sebum of people with PD. Putative metabolite annotations of several lipid classes, predominantly triglycerides and larger acyl glycerides, were obtained using accurate mass, tandem mass spectrometry and collision cross section measurements.

### Preliminary data (results)

- 1) The development of a fast (data generated in 3 minutes) and facile method for analysing a complex biofluid (sebum).
- 2) The combination paper spray ionisation with ion mobility mass spectrometry to elucidate species from the complex biofluid that is sebum.
- 3) The detection of large ( $m/z$  700-1800) lipid molecules that are not readily detectable with more common analytical techniques (primarily LC-MS)
- 4) Identification of lipid classes that are present in sebum and have a statistically significant relative abundance difference between PD and control samples.

### Please explain why your abstract is innovative for mass spectrometry?

The detection of large ( $m/z$  700-1800) lipid molecules that are not readily detectable with more common analytical techniques (primarily LC-MS).

### Co-authors:

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Poster number: LS-PB-105

## ANALYSIS OF SPHINGOMYELINS IN CAENORHABDITIS ELEGANS VIA 2D-LC-MS/MS AND SFC-TIMS-MS

Abstract ID: 307

**Presenting author: Johannes Scholz, Institute of Inorganic and Analytical Chemistry, University of Münster**

### Introduction

Sphingomyelins (SMs) are found in eukaryotic cells as a phosphorylated subclass of sphingolipids in the cell membrane. They stabilise the outer leaflet lipid layer and in addition promote the formation of lipid rafts. Recently the varying abundance of SMs was identified as a biomarker for e.g. non-alcoholic fatty liver disease (NAFLD). The nematode *Caenorhabditis elegans* (*C. elegans*) is often used as a model organism for the research on diseases or drugs and is especially well suited because of the simplicity of cultivation and the fast life cycle. The correct identification and the mapping of the species distribution across the development of the worm are therefore essential for the research on the worm.

### Methods

For this project, we employed a heart-cut two-dimensional liquid chromatography (2D-LC) method coupled to electrospray ionisation-high resolution mass spectrometry (ESI-HRMS) to analyse lipid extracts of varying development stages of *C. elegans*. As a second method of analysis the extracts were analysed by supercritical fluid chromatography (SFC) hyphenated to trapped ion mobility spectrometry (TIMS) in combination with a quadrupole-time of flight-mass spectrometer (Q-TOF-MS).

### Preliminary data (results)

After the lipid extraction via the preferred protocol (e.g. Folch, Bligh and Dyer, Matyash *et al.*, ...), the usual processing in the analysis of sphingolipids includes an alkaline depletion of the glycerophospholipids to exclude these lipids from the analysis and avoid ion suppression in the later ESI. To work around this laborious sample preparation step, the lipid extract was separated in the first dimension by hydrophilic interaction liquid chromatography (HILIC). Thereby, lipid class separation was achieved according to the polar headgroup. The SM fraction was cut out and transferred onto the second dimension, where the SMs were separated via reversed phase-liquid chromatography (RP-LC) according to the chain length and within the class, even enabling the chromatographic separation of SM isomers. The identification was based on retention time, accurate mass and matching MS/MS spectrum, which assured the correct annotation. As an alternative method, TIMS was employed for the analysis. The separation thereby depends on the mobility of the ions in an electric field respectively the collision cross section (CCS) of the analyte and was already employed for the identification of isobaric and isomeric lipid species. For the first time, we combined the TIMS technology with a class specific separation via SFC. By this approach, the chromatographic separation could be reduced to a less complex 1D separation while at the same time generating structural information through the CCS values and the Parallel Accumulation Serial Fragmentation (PASEF) MS/MS experiments, which were performed in the high-resolution Q-TOF-MS.

### Please explain why your abstract is innovative for mass spectrometry?

The confident identification of the SMs was achieved employing two different HRMS systems (Orbitrap and Q-TOF-MS) and fragmentation experiments.

### Co-authors:

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Heiko Hayen, Institute of Inorganic and Analytical Chemistry, University of Münster

Poster number: **LS-PB-106**

## **SPIDERMASS: A NOVEL THERAGNOSTIC TOOL FOR OVARIAN CANCER PATIENT CARE**

Abstract ID: **358**

**Presenting author: Lucas Roussel, PRISM, INSERM U1192, Université de Lille**

### **Introduction**

Patient survival of ovarian cancer is closely related to the precision of the surgery, especially in ovarian cancer. As these cancers are diagnosed at a late stage (III or IV), the quality of the debulking surgery is central. However, there are different ovarian cancer subtypes associated to different evolution and patient survival. To assist the surgeon in taking the best decision for the patient care, novel tools must be developed. Here, the objective is to develop SpiderMass technology developed for *in vivo* MS analysis as a theragnostic tool ovarian cancer. We thus studied both excised tissues and established models thanks to bioprinting reconstruction to enable diagnostic and prognostic.

### **Methods**

A cohort of ovarian cancer excised tissue from Lille Cancer Center were sectioned on a cryostat. The first section (7 $\mu$ m) was stained and annotated by the pathologist. A Second section (20 $\mu$ m) was analyzed using the SpiderMass to create classification models (LDA) on AMX software. The last (12 $\mu$ m) was analyzed by MALDI-MSI using norharmane (sprayed with HTX M5) to cross-validate the SpiderMass results. Discriminative lipids were identified by MS<sup>2</sup> to determine their role in the pathophysiology. Different ovarian cancer cell lines (PEO4, Healthy, THP1) were grown and bioprinted (BioX system) with 3 mg/mL of collagen matrix and analyzed by SpiderMass.

### **Preliminary data (results)**

The SpiderMass MS spectra of the 79 ovarian and 68 of endometrium cancers acquired in positive and negative ion modes were used to build supervised classification models based on pathologist annotation. The LDA showed a clear discrimination of the different cancer subtypes (endometrioid, mucinous, serous high grade, serous borderline) and the healthy tissues with a correct classification of about 95%. To get better understanding of the pathophysiological mechanisms involved the discriminative lipids to each subtype were identified by MS/MS. Then the tissues were analyzed by MALDI-MSI to cross-validate the result and study the tissue heterogeneity. In parallel, different cell lines, ovarian cancer (SKOV3, PEO4, THP1, SW626, PA1), normal ovary cells plus macrophages in different phenotypic state (MO, M1, M2) and fibroblasts were studied by SpiderMass to get a complete molecular description of the different cells composing the tumor microenvironment. The classification models obtained from these different cells showed about 98% correct classification. The lipids that were discriminative for the different cells were then searched in the MS spectra obtained from the tissues to identify the presence of the different specific cell types in the different area of the tissue. Finally, co-culture of macrophages, healthy and cancer cells were bioprinted in collagen to create a margin model that was studied using SpiderMass. All these data will be used as the ground to establish an *in vivo* intraoperative diagnostic thanks to robust classification models and gain prognostic information by quantifying the tumor associated macrophages in relation to patient overall survival data.

### **Please explain why your abstract is innovative for mass spectrometry?**

Development of *in vivo* analysis of the tumor microenvironment to obtain prognostic information in real-time.

### **Co-authors:**

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Poster number: **LS-PB-107**

## LIPIDOMIC PROFILE OF BASAL CELL CARCINOMA OF HUMAN SKIN SAMPLES BY MASS SPECTROMETRY IMAGING

Abstract ID: **508**

**Presenting author: Fernanda Endringer Pinto, Department of Dermatology, Copenhagen University Hospital-Bispebjerg and Frederiksberg, Department of Pharmacy, University of Copenhagen**

### Introduction

Keratinocyte cancer (KC), which includes basal cell carcinoma (BCC), is among the cancers with the most incidences per year, but fortunately with a relatively low mortality rate. The non-lethality of this cancer is ideal for introducing a new approach to cancer surgery based on tumor tissue identification by mass spectrometry. Therefore, it is essential to know the lipidomic profile of BCC and healthy skin tissue. We aim to identify if the tumor exhibits endogenous lipids that show compositional differences from healthy skin tissue. Using mass spectrometry imaging (MSI), we identified lipids from KC that were upregulated compared to healthy skin tissue, which will be used to build a skin database and apply in REIMS (Rapid evaporative ionization mass spectrometry) surgery for KC diagnostics and treatment.

### Methods

Cryo-sections of healthy and BCC human tissue (n=160) were dried in a vacuum desiccator prior to MSI analysis. The MALDI analysis was performed in negative ion mode with an AP-SMALDI10 ion source connected to a QExactive Orbitrap mass spectrometer with 1,5-diaminonaphthalene as the matrix. The DESI analysis was carried out on a custom-built DESI imaging ion source mounted on a QExactive Orbitrap. Raw data were converted with RAW+UDP to imzML Converter. MSI data was viewed by MSiReader. Various statistical methods were used to identify differences between tissue types and generate predictive models capable of classifying independent samples.

### Preliminary data (results)

The preliminary data of MALDI and DESI imaging analysis clearly show differences in the profile of lipids species between tumor area and healthy tissue (Figure 1). The hematoxylin and eosin-stained specimens histologically verified for BCC were compared to the mass spectrometry images. In the tumor area, the ion of  $m/z$  857.5185, tentatively assigned as phosphatidylinositol (PI 36:4), is present in significantly elevated levels compared to healthy tissue (Figure 1a). Another molecular ion abundant in the tumor area was phosphatidylethanolamine (PE 36:4) at  $m/z$  722.5130. Compared to the tumor area, healthy skin exhibit a lower relative abundance of phosphatidylserine (PE 38:4). The ion of  $m/z$  465.3043, identified as cholesterol sulfate, was distributed in the stratum corneum and in hair follicles. The colored overlay distribution of compounds PI 36:4 (green), cholesterol sulfate (blue), and PE 36:4 (red) can be seen in figure 1b. Indeed, several other lipid species follow similar distribution patterns while others primarily, but not always exclusively, are found in healthy tissue. Our preliminary data show promising results. In addition to the univariate analysis showing significantly different ion intensities, we have constructed supervised classification models. These models demonstrate accuracies for tumor vs. normal detection in excess of 95%. All  $m/z$  features, identified by uni- and multi-variate methods, that differentiate between the tissues will be part of the lipidomic skin database that will be further used to build a classifier used in REIMS surgery to diagnose the investigated tissue.

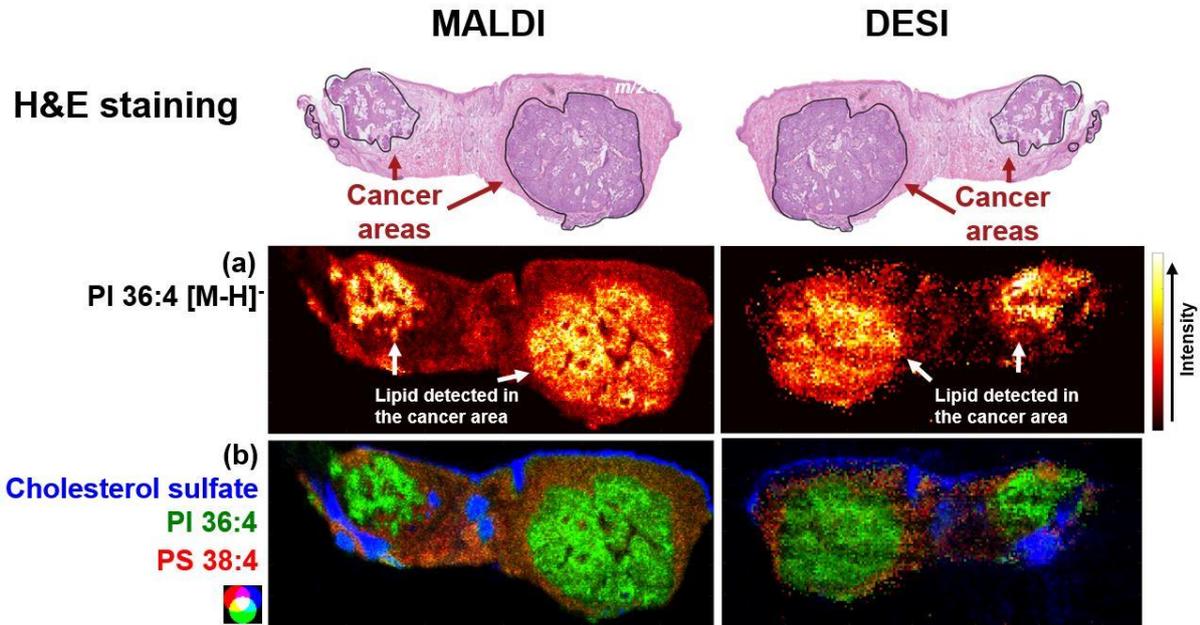
### Please explain why your abstract is innovative for mass spectrometry?

Use of high-resolution MALDI and DESI imaging to build a lipidomic BCC and healthy skin tissue database to diagnose cancer by REIMS surgery.

### Co-authors:

*Lauritz F. Brorsen, Department of Dermatology, Copenhagen University Hospital-Bispebjerg and Frederiksberg, Department of Pharmacy, University of Copenhagen*  
*Martin Glud, Department of Dermatology, Copenhagen University Hospital-Bispebjerg and Frederiksberg*  
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Lipids that show compositional differences between cancer and healthy tissue

Poster number: **LS-PB-108**

## MULTIMODAL FRAGMENTATION, 1- AND 2-DIMENSIONAL TANDEM MASS SPECTROMETRY OF LIPIDS EXTRACTED FROM HUMAN PLACENTAL TISSUE

Abstract ID: **578****Presenting author: Basim R. Hussain, University of Warwick**

### Introduction

Lipids are a vastly diverse class of complex biomolecules that serve as principal components of cellular membranes. Lipids provide cell adhesion, energy storage, migration, signal transduction, cellular apoptosis, and gene expression. However, their biophysical architecture can provide an insight into the dissociation mechanism in adverse obstetrics, where the lipid pathophysiology can have tremendous implications on a developing foetus, and consequentially on a new-born and its mother. The aim of this study was to develop a 1- and 2-dimensional tandem mass spectrometry method, with multiple fragmentation methods with positive and negative nano-electrospray ionisation to accurately identify the structural lipidomic signatures in relation to lipid species, the polar head groups, sn-positional fatty acyl chain lengths and double-bond position.

### Methods

Direct infusion of the placental tissue extracts was performed on a Bruker 12T FT-ICR MS. 1DMS spectral assignments was then performed by matching each precursor mass against a database to identify the lipid classes based on the individual elemental composition. Multimodal fragmentation was then performed for the detailed structural characterisation of the various lipid species. 2DMS on FT-ICR with multimodal fragmentation enabled multiple species to be identified and characterised without the need for any prior chromatographic separation or quadrupolar isolation. Each precursor species was then grouped by common fragments, displayed by the precursor and neutral loss lines, enabling accurate identification.

### Preliminary data (results)

Combining complementary fragmentation methods with 1D- and 2D FT-ICR MS provided an invaluable platform for the comprehensive characterisation of each lipid precursor ions. The lipid species identified within the healthy, diabetic, and obese Human placental tissue samples were predominantly comprised of phosphatidylcholine and lysophosphatidylcholine species. The low energy CAD experiment provided limited diagnostic information, with fragment ions corresponding to the loss the sn-positional ketene, glycerol backbone, fatty acid chain, and the polar phosphate head groups. Correspondingly, IRMPD and UVPD at a wavelength of 193 nm yielded limited diagnostic information, due to some lipid precursor ions having no points of unsaturation, or IR chromophores on the diacyl chains. However, IRMPD with a 10.6 $\mu$ m laser enabled identification of the lipid polar head groups with fragment ions corresponding to the loss of the glycerol backbone and sn-positional fatty acid chain. 1D and 2D-EID-MS displayed distinctive and sequential identification/assignments of the odd- and even-number of electron product ions, providing fatty acyl chain length, localisation of the double bonds, and identification of the polar head group moieties. Additionally, 2DMS which is a true which is a data independent analysis offered an alternative to chromatographic methods, allowing for a more accurate and high-resolution identification of each lipid precursor ion within the patient human placenta tissue extracts. 2DMS with EID and IRMPD enabled site-specific localisation of the double-bonds on the fatty acyl chains, sn-positional chain length/information of the diacyl branching points on the glycerol backbone, along with the identification of the various polar head groups.

### Please explain why your abstract is innovative for mass spectrometry?

Positive and Negative mode nESI 2DMS of Human Placental Lipids.

MSn with Multimodal fragmentation (CAD, EID, UV-, and IR-Photon Dissociation).

Ultra-High Resolution, with Sub ppm Mass Accuracy.

### Co-authors:

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Edyta Carrion Paczkowska, Swansea University*  
*Bryan P. Marzullo, University of Warwick*  
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*Cathy Thornton, Swansea University*  
*Mark P. Barrow, University of Warwick*  
*Peter B. O'Connor, University of Warwick*

Poster number: **LS-PB-109**

## NOVEL WORKFLOW PROVIDING IMPROVED SENSITIVITY AND ANNOTATION QUALITY FOR EICOSANOID ANALYSIS BASED ON HEATED ESI SOURCE AND PASEF DATA ACQUISITION

Abstract ID: **622**Presenting author: **Sven Meyer, Bruker Daltonics GmbH & Co. KG**

### Introduction

Eicosanoids are important signaling molecules and are commonly correlated with pathological processes like inflammations. In the human body, they can be detected for example in urine or lipid extracts. The hydroxyeicosatetraenoic acids (HETE) are a subgroup of the eicosanoids and they are key precursors to many bioactive metabolites. Due to their chemical structures, hydroxyeicosatetraenoic acids like 15(S)-HETE or 20-HETE are relatively labile and profit from optimized methods for a gentle desolvation in LC-MS analyses.

Here, we present enhanced sensitivity for eicosanoid analysis using a new heated ESI source. Additionally, the benefit of TIMS separated QTOF data will be discussed with respect to data quality and annotation confidence.

### Methods

Eicosanoid standards were diluted in a range of 0.1-1000 ppb to acquire dilution series. Also, they were spiked into urine at different concentration levels. Samples were separated by 5-minute RP chromatography in combination with standard ESI and heated ESI sources. MS data was acquired using a TIMS-MS setup in PASEF acquisition mode. Data processing was performed using preliminary versions of TASQ 2023 and MetaboScape 2023 software. Annotations of urine metabolites were performed with target lists containing compound name and molecular formula as basic input. Additional structural information (InChI) was used for automatic *in-silico* fragmentation and CCS value prediction.

### Preliminary data (results)

To improve the sensitivity of the ionization of hydroxyeicosatetraenoic acids, we optimized the LC-MS method parameters using a heated ESI (VIP-HESI) source for 12(S)-, 15(S)- and 20-HETE standards in negative ion mode. The chromatography was optimized to provide rapid profiling within 5 minute gradient times. The standards were diluted in solvent mixtures (0.1-1000 ppb) and also spiked into urine at different concentration levels.

4D-data was processed using the client-server based softwares TASQ and MetaboScape. These provided CCS-enabled screening / quantitation and untargeted profiling / ID workflows on the same raw data sets. For both approaches, the TIMS separation provided enhanced results by separating coeluting compounds (targets and background ions) and hence generating cleaner MS and MS/MS spectra.

Quantitative data evaluation revealed improved sensitivity and dynamic ranges with the heated ESI source. For untargeted profiling of endogenous compounds in urine, a target list compiled from the human metabolome database (HMDB) including structural information was used for annotation. The structures enabled the use of automatic *in-silico* fragmentation and CCS prediction for metabolites lacking reference MS/MS and/or CCS information. This CCS-enabled annotation process improved confidence in annotations compared to approaches matching exact mass, isotopic pattern quality and MS/MS information only. In summary, we developed improved methods for the analysis of eicosanoid compounds providing higher sensitivity for screening, profiling, quantitation and ID capabilities. Additionally, we demonstrate the benefit of automatic *in-silico* fragmentations and CCS predictions for increased confidence in tentative compound annotations.

### Please explain why your abstract is innovative for mass spectrometry?

Increased sensitivity and annotation quality for eicosanoid analysis using rapid chromatographic separation in combination with 4D PASEF data acquisition.

**Co-authors:**

Ansgar Korf, Bruker Daltonics GmbH & Co. KG  
Aiko Barsch, Bruker Daltonics GmbH & Co. KG  
Nikolas Kessler, Bruker Daltonics GmbH & Co. KG  
Viola Jeck, Bruker Daltonics GmbH & Co. KG  
Mohamed Elsadig, Bruker Daltonics GmbH & Co. KG

Poster number: LS-PB-110

## WORKFLOW FOR HIGH THROUGHPUT LIPIDOMICS SCREENING AND RELATIVE QUANTIFICATION OF HUMAN PLASMA AND SERUM UTILIZING MALDI-TIMS-TOF

Abstract ID: 713

**Presenting author: Sebastian-Alexander Tölke, University Medical Center Mainz - Institute for Physiological Chemistry - Clinical Lipidomics Unit**

**Introduction**

High throughput lipidomic of patient-derived samples has become increasingly important for clinical applications. During the recent years of analytical development matrix assisted laser desorption ionization (MALDI), a fast and versatile ionization method was coupled to high-resolution mass spectrometry (HR-MS). The significant advantage of IM-HR-MS is its ability to separate isobaric and isomeric analytes by ion-neutral collision cross-sections. The improved sensitivity and resolution of MALDI-IM-HR-MS compensates for the lack of front-end chromatographic separation by allowing conformational separation of molecules post-ionization. With the added dimension of ion mobility to the MALDI-IM-HR-MS application, 3D descriptors: i) accurate  $m/z$  of molecular ions; ii) intensity of molecular ions and iii) mobility (CCS) are used to annotate structural diversity in a complex mixture, lacking the dimension of time (4-D-data).

**Methods**

In order to develop a 3D MALDI-tims-TOF rapid plasma lipidomic profiling, first a matrix-sample preparation procedure suitable for analysis of a broad range of lipids in both polarities, e.g. "+" and "-" was developed. Then a standard library containing CCS and  $m/z$  of MALDI-specific ions was created with known standard mixtures. The quantification was carried out by multi-point calibration utilizing class-specific internal standards. As a proof of concept, a plasma sample has been quantified with LC-tims-TOF and MALDI-tims-TOF. In the case of MALDI-tims-TOF, a triplicate measurement was performed on three spots, resulting in 9 total data points.

**Preliminary data (results)**

A processing pipeline was implemented in order to allow a database matching of 2D features with 3D lipidomic features curated with LC-tims-TOF data. The calibration exhibits acceptable linearity, exemplified by the relative quantification of Phosphoinositide 16:0\_18:1. Both, the mobilogram intensity and area result in acceptable linearity ( $> 0.0992$ ). The results are exemplified by the relative quantification of Phosphoinositide 16:0\_18:1, which concurrence within 20% with the one obtained by 4-D lipidomics on an LC-tims-TOF instrument. The results and the cross-validation with LC-tims-TOF do show that 3D-MALDI is well suited for relative quantification of major lipid classes in clinical plasma samples. This process will be confirmed by measuring complex mixtures of membrane lipids containing overlapping isomers. To increase the specificity of structure identification, a cross-validation with LC-tims-TOF data of the identification and quantification performance across multiple lipid classes will be performed.

**Please explain why your abstract is innovative for mass spectrometry?**

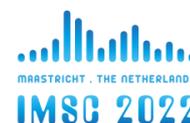
3D-MALDI is suitable for the identification and relative quantification of major lipid classes. By applying this strategy, the instrument time can be reduced drastically while also reducing sample uptake.

**Co-authors:**

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



*Julia Maria Post, University Medical Center Mainz - Institute for Physiological Chemistry - Clinical Lipidomics Unit*  
*Sarah Neuhaus, University Medical Center Mainz - Institute for Physiological Chemistry - Clinical Lipidomics Unit*  
*Kritika Chauhan, University Medical Center Mainz - Institute for Physiological Chemistry - Clinical Lipidomics Unit*  
*Laura Bindila, University Medical Center Mainz - Institute for Physiological Chemistry - Clinical Lipidomics Unit*

Poster number: LS-PB-111

## A SENSITIVE LC-MS/MS METHOD FOR THE SIMULTANEOUS DETERMINATION OF HEXOSYLCERAMIDES AND HEXOSYLSPHINGOSINES IN CELLS

Abstract ID: 753

**Presenting author: Tine Loomans, Discovery and Exploratory Bioanalysis – DMPK, Janssen Research and Development a division of Janssen Pharmaceutica NV**

### Introduction

Parkinson disease's (PD) is a multifactorial neurodegenerative disorder characterized by progressive dopaminergic neurodegeneration and formation of insoluble aggregates of  $\alpha$ -synuclein.

Recently, it was proposed that hypofunction of ATP10B, a glucosylceramide/phosphatidylcholine (GluCer/PC) flippase, as well as glucocerebrosidase 1, encoded by the PD risk gene *GBA1* cause lysosomal dysfunction and GluCer accumulation. These evidences trigger the necessity to understand the role of GluCer and related compounds in PD. Thus, we are presenting herein the development, validation and application of a sensitive liquid chromatography - tandem mass spectrometry (LC-MS/MS) analytical method for the simultaneous analysis of ceramide, lactosylceramide, glucosylceramides, galactosylceramides, sphingosine, glucosylsphingosine and galactosylsphingosine in different cell models with the aim to correlate the level of this target with the ATP10B and GBA1 activities.

### Methods

Sample pre-treatment, starting from 400 K of cells, is articulated in two consecutive liquid-liquid extractions, the first with methanol and acetone and the second with methanol and HCl 1 N, and a subsequent solid phase extraction (Waters Oasis MCX® 96 well-plate). The chromatographic separation is achieved on a Halo HILIC column and the mass spectrometry analysis is performed using a triple quadrupole operating in selected reaction monitoring mode. The method was validated in terms of selectivity, linearity, limit of quantification (LOQ), recovery, matrix effect and carry over, using different cell lines and phospholipids solutions.

### Preliminary data (results)

Eleven target compounds (ceramide C18, sphingosine, lactosylceramide (LacCer), glucosylceramide (GluCer) 16:0, GluCer 18:0, GluCer 24:1, galactosylceramide (GalCer) 16:0, GalCer 18:0, GalCer 24:1, glucosylsphingosine (GluSph) and galactosylsphingosine (GalSph)) and five internal standards (ceramide C17, LacCer C17, GluCer C17, GluCer C18-d5 and GluSph-d5) were selected based on the application field.

During the method development and optimization steps, to reduce the number of cells from 2 M to 400 K and to improve and guarantee the separation of each isomeric species (e.g., GluCer 16:0 and GalCer 16:0) having the same MS/MS fragmentation pathway, different combinations of liquid-liquid extraction solvents, different SPE 96 techniques (Waters OASIS MCX® cartridges, Water OASIS MCX® 96 well-plates, Waters OASIS HLB® cartridges, Waters OASIS HLB® well-plates) and different chromatographic columns were tested. The best conditions for the identification and quantification of the target compounds are obtained after two consecutive liquid-liquid extractions (1<sup>st</sup> MeOH and Acetone and 2<sup>nd</sup> acidified MeOH + 1N HCl), and a solid phase extraction (SPE) performed with Waters Oasis MCX® 96 well-plates. The best chromatographic resolution was achieved using the Halo HILIC column 4.6 x 150 mm, 2.7  $\mu$ m.

LLOQ values are between 0.05 and 1 ng/mL depending on the analyte while linear dynamic ranges are in accordance with the expected concentration in the various cell models (upper limit of quantification (ULOQ) are between 1000 and 10000 ng/mL). No carry over neither matrix effect were observed.

**Please explain why your abstract is innovative for mass spectrometry?**

## POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

To the best of our knowledge, this is the first developed LC-MS/MS method for the simultaneous analysis of ceramide, sphingosine, glucosylceramides, galactosylceramides, glucosylsphingosine and galactosylsphingosine in cell models.

### **Co-authors:**

*Michele Iannone, Discovery and Exploratory Bioanalysis – DMPK, Janssen Research and Development a division of Janssen Pharmaceutica NV, Research & Development, Neurosciences, Janssen Pharmaceutica NV*

*Farid Jahouh, Discovery and Exploratory Bioanalysis – DMPK, Janssen Research and Development a division of Janssen Pharmaceutica NV*

*Brian Hrupka, Research & Development, Neurosciences, Janssen Pharmaceutica NV*

*Diederik Moechars, Research & Development, Neurosciences, Janssen Pharmaceutica NV*

*Rob J Vreeken, Discovery and Exploratory Bioanalysis – DMPK, Janssen Research and Development a division of Janssen Pharmaceutica NV, Maastricht MultiModal Molecular Imaging Institute (M4I), Faculty of Health, Medicine and Life Sciences, Maastricht University*

Poster number: **LS-PB-112**

## **HIGH THROUGHPUT HDMSE BLOOD PRODUCT LIPIDOMIC SCREENING USING A DESI INLET**

Abstract ID: **825**

**Presenting author: Lisa Reid, Waters Corporation**

### **Introduction**

In biomedical research, to profile blood products for large cohort lipidomic studies may consist of long LC/MS analyses requiring several weeks of instrument time. Consequently, increasing the potential for instrument failure, unwanted batch effects and sample degradation.

DESI imaging is traditionally used to investigate compound localization within tissue where, by visualizing the structure and molecular composition of tissues, a greater understanding of organ structure and changes due to disease may be obtained. Here we have examined the feasibility of using DESI as an alternative high throughput direct analysis method for performing lipid profiling. This technique has been applied to screen sera obtained from a UK human population as part of the Human Serum Metabolome study (HUSERMET).

### **Methods**

Serum samples ( $n=500$ ) from the HUSERMET study (Stockport, UK) were protein precipitated 1:4 v/v with IPA, and 2 $\mu$ L of the supernatants pipetted onto Teflon spots on a glass microscope slide.

The slides were analyzed by DESI-HDMS<sup>e</sup> using a QToF mass spectrometer, performing a single line pass through the center of each Teflon spot. Data acquisition was performed in full scan HDMS<sup>e</sup> in both positive and negative ESI.

The resulting data were assessed using MassLynx<sup>TM</sup> software before being exported into a statistical software package for multivariate analysis, highlighting potential "biomarkers" and reveal phenotypic differences in the study population.

### **Preliminary data (results)**

This feasibility study demonstrates the potential of DESI to be used as a high throughput basic screening technique for the lipidomic analysis of small volumes of serum. Individual sample analysis was achieved at a rate of <15 seconds per sample, representing a significant reduction in instrument time compared to typical LC/MS. Benefits include: reduced potential for time-related batch effects or sample degradation during analysis and drastically reduced file size, thus improving both storage demands and processing time. DESI pre-screening can also be used to indicate potential issues such as contamination or the presence of incorrect sample type, which could have devastating effects on subsequent LCMS analysis.

The data generated via this workflow, although not as comprehensive as could be obtained in a typical 10-20 minute LC/MS analyses (particularly regarding isomers only separated chromatographically), nevertheless provided an overview of sample quality, revealed fold changes in lipid profiles and could be used to facilitate a more in-depth subset analysis by LC/MS. If performed using a high mass resolution or ion mobility capable instrument improved separation/identification of lipid species could be possible, enabling greater information extraction from each sample.

The full workflow described, employs minimal sample preparation maintained as identical to the method for LCMS, enabling storage and subsequent LC analysis if desired.

We highlight that good data quality can be achieved using rapid data acquisition and show how these data can be imported into peak processing and/or statistical software packages depending upon application goal.

### **Please explain why your abstract is innovative for mass spectrometry?**

DESI can provide a high throughput method for blood product lipidomic screening thereby enabling the rapid analysis of large cohort studies such as HUSERMET.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



**Co-authors:**

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*Ian Wilson, Department of Biochemistry and Systems Biology, Institute of Systems, Molecular, and Integrative Biology, University of Liverpool*

Poster number: LS-PB-113

## HIGH-THROUGHPUT $\mu$ LC-MS/MS LIPIDOMICS OF 3D IN VITRO DISEASE MODELS TO INVESTIGATE LIPID DYSREGULATION

Abstract ID: 867

Presenting author: Darshak Gadara, RECETOX, Faculty of Science, Masaryk University

### Introduction

Microflow liquid chromatography interfaced with mass spectrometry is increasingly applied for the high-throughput profiling of biological samples, demonstrating an acceptable tradeoff between sensitivity and reproducibility. However, a limited number of applications developed in lipidomics. This work aims to establish a sensitive, high-throughput, and robust  $\mu$ LC-MS/MS lipidomics to elucidate the lipid metabolism using 3D in vitro disease models. Consecutively, we injected 303 samples over ~75 hours to prove the robustness and reproducibility of microflow separation. As a proof of concept, we studied lipid metabolism of APOE phenotypes of AD iPSC cerebral organoid. Also, we characterized the hepatic lipidome profile after repeated exposure of amiodarone to human liver 3D-hepatospheroids cell culture.

### Methods

The lipid extract was analyzed using 1290 Infinity II UHPLC (Agilent) system coupled with the 6495 Triple Quadrupole MS (Agilent). Lipids were separated using a reverse-phase microbore column (CSH, 1 mm \*100 mm, 1.7 $\mu$ m, Waters) at 100 $\mu$ l/min flow rate over 15 min. Mobile phase A was 10 mM ammonium formate in acetonitrile: water (60:40), and mobile phase B was 10 mM ammonium formate in Isopropanol: acetonitrile (90:10). Data were acquired in positive mode using the dynamic multiple reaction monitoring (MRM) mode, 2 min retention time window for each transition.

### Preliminary data (results)

The  $\mu$ LC-MS/MS lipidomics shows an average 3.7-fold increase in response compared to a conventional high-flow method. To benchmark the quantitative performance, 303 samples were acquired over 75 hours. The median coefficient of variation (CV) of 351 lipid species was 12.95%, and only 26 lipid species show CV higher than 30%. We demonstrated that microflow separation coupled to three-stage quadrupole MS substantially increases the sensitivity and allows for robust multiplexed lipid profiling (including cholesterol determination) in a single cerebral organoid. As a proof of concept, lipidomics analysis was performed for the lipid extract of APOE 3/3 and APOE 4/4 (n=10 biological replicates) CO samples. Our results reveal that the APOE4 phenotype causes lipid perturbations, including altered cholesterol and sphingolipid metabolism in a 3D neural tissue model, likely relevant to the pathophysiology of Alzheimer's disease. Further, we quantified ~ 210 lipid species from a single 3D-hepatospheroid sample, which elucidates lipid dysregulation associated with amiodarone.

### Please explain why your abstract is innovative for mass spectrometry?

First systematic report demonstrating  $\mu$ LC-MS/MS lipidomics enables sensitive, high-throughput, and robust measurement of global lipidome. This paves the way for routine application of microflow lipidomics separation.

Poster number: **LS-PB-114**

## REVEALING THE COMPLEXITY OF THE HAIR LIPIDOME

Abstract ID: **920**

**Presenting author: Maria Lavoir, University of Antwerp**

### Introduction

Hair is a new, promising matrix for clinical analyses to assist in diagnosing and guiding the management of a chronic or recurrent condition. The extended detection window of weeks to months provides the opportunity to monitor metabolic alterations over a longer timeframe with the potential to identify small molecules that play a key role early in chronic conditions. However, up to date, there is no comprehensive, accurate identification of the constituents of the hair lipidome. Current untargeted hair metabolomic studies rely on forensic hair analysis methodologies detecting polar exogenous compounds. Nevertheless, lipids play an essential role in various chronic diseases.

### Methods

The presented study aimed to determine a robust preanalytical and analytical workflow for the characterization of the hair metabolome and lipidome. First, the potential of human hair as a novel matrix in lipidomics is proved by an exhaustive extraction experiment. Furthermore, a Fractional Factorial Design was performed to determine sample preparation factors that significantly impact lipidome coverage and signal intensity. The optimized workflow was applied to establish the global composition of the hair metabolome. The 0-3 cm proximal segment of the hair samples, representing three consecutive months, from 32 healthy volunteers were examined using LC-IM-QTOF-MS.

### Preliminary data (results)

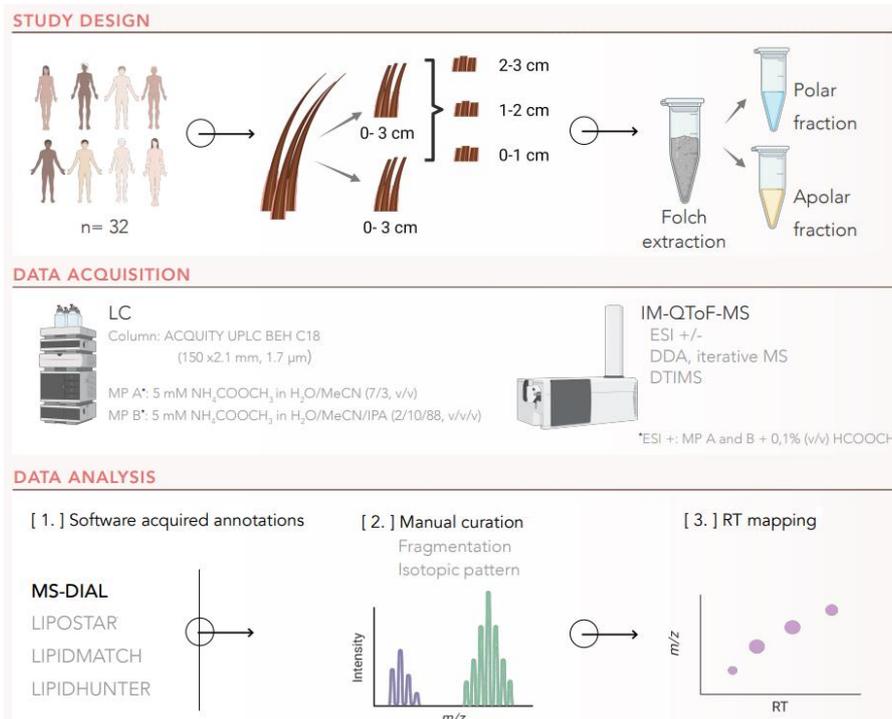
A significant increase in the percentage of extractable lipids is evidenced using a lipidomics-based sample preparation strategy. Results indicate *incubation technique* and *sample-to-solvent ratio* as factors with a significant impact on the lipidome coverage, while the *type of extraction solvent* affects lipid signal intensities. Deep profiling analysis allowed the detection of fatty acyls, glycerolipids, glycerophospholipids, sphingolipids and sterol lipids in human hair for the first time, using an untargeted approach.

### Please explain why your abstract is innovative for mass spectrometry?

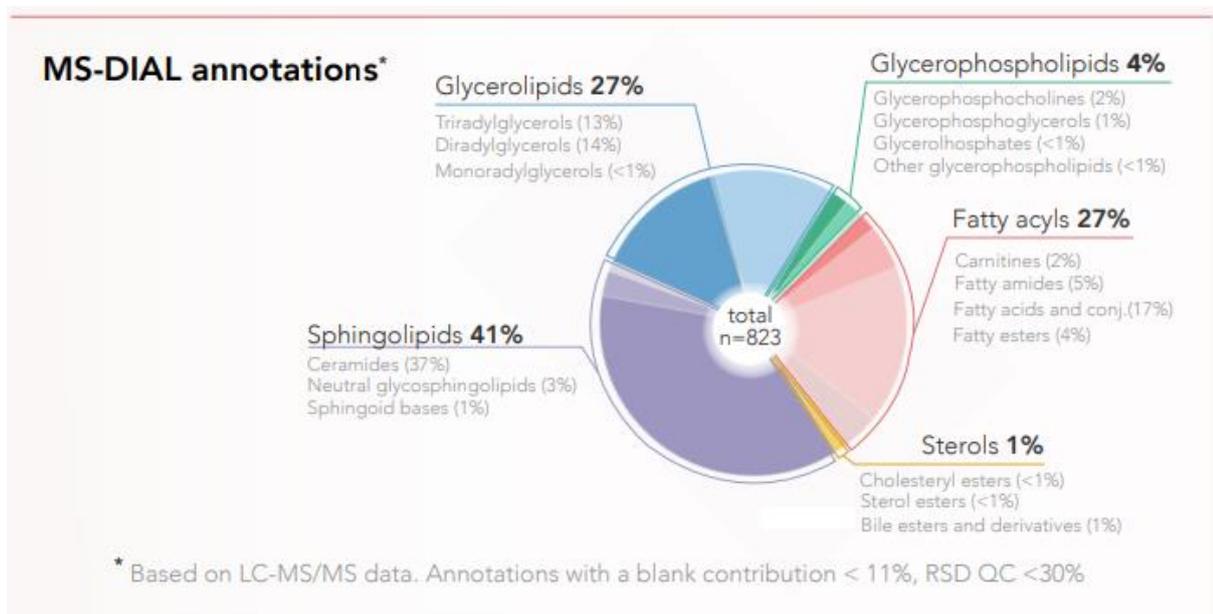
The multi-dimensional, LC-IM-MS workflow used in this study, facilitates the detection and annotation of novel lipid species in human hair.

## POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Study design: Establishing the global composition of the hair lipidome.



Preliminary results: Overview of detected lipids in human hair.

Poster number: LS-PB-115

## THE ASSESSMENT OF ELECTRONIC-ACUPUNCTURE TREATMENTS AS AN ELECTROCEUTICALS ON HYPERTENSION BASED ON LIPIDOMICS USING MASS-SPECTROMETRY

Abstract ID: 954

Presenting author: Sarah Shin, Korea Institute of Oriental Medicine

### Introduction

Hypertension is the most important risk factor for the global burden of disease, implicated in ischemic heart disease, stroke, and chronic kidney disease. Electronic acupuncture (EA) as an electroceutical is a one of the treatments that could lower blood pressure. However, its specific mechanism of action has not yet been fully elucidated. This study aimed to investigate changes in cardiovascular functions related to hypertension induced by consecutive EA treatment by using conventional *in vivo* methods and mass-spectrometry based on lipidomics in spontaneously hypertensive rats (SHR).

### Methods

The experimental animals were divided in to three groups as follow: Normal control group (WKY, Wistar-Kyoto rats); Disease induced group (SHR); Treatment group (SHR+EA, Consecutive EA treatment for 6 weeks). The hypertension related factors cardiac function, systolic blood pressure, and vascular reactivity were measured by using a conventional *in vivo* methods. Changes in aortic lipid composition among experimental groups were analyzed by using ultra-high performance liquid chromatography (UHPLC) connected to a quadrupole time of flight (Q-TOF) mass spectrometry system.

### Preliminary data (results)

The consecutive EA treatment restored the impaired acetylcholine- and sodium nitroprusside vasodilation and cardiac dysfunctions in SHR. The PLS-DA score plot of the experimental groups with EA treatment for consecutive weeks revealed that each cluster (WKY, SHR, and SHR+EA) were clearly separated. In particular, the SHR group was clearly separated from the WKY group by the first component, and the SHR + EA group was slightly shifted from the WKY cluster to the SHR cluster in both positive and negative modes. Moreover, there has been shown the markedly different lipid composition patterns in aortic lipidome between SHR- and EA-treated SHR group. Especially, sphingomyelins and phospholipids were significantly changed by EA, showing a significant correlation with systolic blood pressure and cardiac function indicators. To the best of our knowledge, this is the first and most comprehensive study to investigate that the EA treatment can change the aortic lipid composition in hypertension.

### Please explain why your abstract is innovative for mass spectrometry?

These findings demonstrate that lipidomics/metabolomics based on LC-MS techniques can be used as a useful tool to elucidate the therapeutic mechanism of EA treatment on hypertension.

### Co-authors:

*You Mee Ahn, Korea Institute of Oriental Medicine*  
*Jeeyoun Jung, Korea Institute of Oriental Medicine*

Poster number: LS-PB-116

## 4D-LIPIDOMICS PROFILING IN ADRENOLEUKODYSTROPHY USING VACUUM INSULATED PROBE HEATED ESI AND TRAPPED ION MOBILITY TIME-OF-FLIGHT MASS SPECTROMETRY

Abstract ID: 957

**Presenting author: Michel Van Weeghel, Laboratory Genetic Metabolic Diseases, Amsterdam UMC, University of Amsterdam, Amsterdam Gastroenterology Endocrinology Metabolism, Amsterdam, Netherlands, Core Facility Metabolomics, Amsterdam UMC, Amsterdam, Netherlands**

### Introduction

Adrenoleukodystrophy (ALD) is a genetic progressive metabolic disorder which results in the accumulation of saturated very long-chain fatty acids (VLCFA) in plasma and all tissues of the body. The disease is characterized by a striking clinical spectrum and is unpredictable in disease course and severity, with no established genotype–phenotype relationship. Currently it is unknown what underlies the disease mechanisms that result in the multiform pathology of ALD. We used an in-depth 4D-lipidomics approach to characterize the complex lipidomic changes in ALD.

### Methods

A timsTOF Pro-based (Bruker) 4D-lipidomics approach was used to analyze fibroblasts from ALD patients and healthy controls. The 4D-lipidomics method utilizes four different LC runs on two analytical columns in positive and negative ionization modes. Following LC separation, compounds were ionized using a recently developed Vacuum Insulated Probe – Heated ESI source (VIP-HESI), which increases sensitivity dramatically compared to ESI. Detection and fragmentation of ions was performed using Parallel Accumulation Serial Fragmentation (PASEF). Annotation of lipids was done by an established bioinformatics workflow in combination with a rule-based annotation tool included in MetaboScape (Bruker).

### Preliminary data (results)

Using the timsTOF Pro 4D-lipidomics approach we acquired data in PASEF mode. This DDA MS/MS routine separates ions by ion mobility and subsequently fragments these at an almost 100% duty cycle. Lipids were annotated based on the four-dimensional data, which includes retention time,  $m/z$ , ion mobility and intensity. By utilizing ion mobility, the PASEF workflow provided increased confidence in lipid annotation in addition to the collection of high quality MS/MS data. We used automatic outlier detection based on retention time and predicted CCS values for a first flagging of likely false-positives. These were manually reviewed and annotations deleted if false annotation was confirmed. Investigating MS/MS spectra and annotations in 4D Kendrick mass defect plots helps to further pinpoint false annotations. This workflow allowed for in-depth profiling of the systemic changes in the lipidome of ALD patients. Our results demonstrate that saturated VLCFAs are incorporated into multiple complex lipid species including phosphatidylcholines, lysophosphatidylcholines, cholesterol esters and triglycerides. Furthermore, a reduction of polyunsaturated variants of these lipids was observed in ALD. These systemic changes in the lipidome might underlie the disease mechanisms that result in the multiform pathology of ALD. Finally, we demonstrate the benefit of 4D-lipidomics using VIP-HESI for the analysis and annotation of lipids compared to our established lipidomics platform.

### Please explain why your abstract is innovative for mass spectrometry?

We used a 4D-lipidomics approach to characterize a complex genetic lipid disorder by using VIP-HESI, trapped ion mobility and PASEF.

### Co-authors:

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Eric Wever, Laboratory Genetic Metabolic Diseases, Amsterdam UMC, University of Amsterdam, Amsterdam Gastroenterology Endocrinology Metabolism, Amsterdam, Netherlands, Core Facility Metabolomics, Amsterdam UMC, Amsterdam, Netherlands*

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Poster number: LS-PB-117

## UTILIZING CID AND EAD FRAGMENTATION FOR GLOBAL LIPID PROFILING OF HUMAN AND RAT PLASMA

Abstract ID: 1013

Presenting author: Takashi Baba, SCIEX

### Introduction

Higher-throughput methodologies typically result in significant loss of species annotated due to reduced chromatographic resolution and/or an insufficient acquisition rate of the LC-MS for the increased elution concurrency. In addition, the strategy for determining in-depth structural information can involve multiple injections and methodologies. In this work, we utilized NIST 1950 human plasma and Sprague Dawley and Zucker fatty rat plasma to assess these throughput, sample volume and interpretation concerns.

### Methods

Human and rat plasma (1–20 $\mu$ L) were added to 100 $\mu$ L of ice-cold chloroform, followed by 200 $\mu$ L of methanol containing internal standards. 100 $\mu$ L of the supernatant was analyzed using Phenomenex Luna Omega Polar C18 (100mm) column with a 3 $\mu$ L injection volume with 5mM ammonium acetate in a mixture of water, acetonitrile, and methanol (3:1:1) and 5mM ammonium acetate in isopropanol over a 1%B–95%B gradient with variable run times (10.5–25min) coupled with ZenoTOF 7600 system operated in information dependent acquisition (IDA/DDA) mode using Zeno CID and Zeno EAD IDA.

### Preliminary data (results)

The impact of gradient length was assessed using 20 $\mu$ L of plasma and the run time was reduced linearly from 25min, 19min, 15.5min and 12.5min to 10.5min. In positive ion mode, the CID analysis shows a <3% drop in annotations, from 25min to 12.5min, and only a 12% drop at 10.5min. In negative ion mode, the annotations improved up to 13% with faster gradients. The analytical reproducibility was established across the 4 lowest plasma concentrations: 1 $\mu$ L, 2 $\mu$ L, 3 $\mu$ L and 5 $\mu$ L. Each volume of plasma was extracted in duplicate and injected with triplicate technical replicates. From the >500 lipids annotated for the NIST 1950 human plasma from a 20 $\mu$ L volume, a subset of high, medium and low abundant lipid species was selected for precision assessment. These included PC 34:2, SM 32:1;2O, LPE 20:4, Hex2Cer 34:1;2O and Cer 42:1;2O with 1.4e3–1.5e6 cps abundance. For the high abundant PC 34:2 across the 4 plasma volumes, the percentage coefficient of variation (%CV) was sub-2% on n=6, which is to be expected. For the 3 lowest abundant species, the %CV on peak area across all plasma volumes was less than 6%. From the initial analysis of the electron activated dissociation (EAD) spectrum acquired for the species PC 38:4 in positive ion mode, the information collected confirmed PC (m/z 224.10 and 226.08 for PC vs. 225.09 for SM) and the sn- position was determined as 18:0/20:4 and double bonds at 8Z, 11Z, 14Z and 17Z.

### Please explain why your abstract is innovative for mass spectrometry?

High scan rates coupled with CID and EAD fragmentation allows for deep lipid profiling.

### Co-authors:

Jason Causon, SCIEX  
Eva Duchoslav, SCIEX  
Jose Castro-Perez, SCIEX

Poster number: LS-PB-118

## THE ROLE OF APOEε4 IN BRAIN LIPID METABOLISM IN DEMENTIA

Abstract ID: 1030

**Presenting author:** Qi Zhong, UK Dementia Research Institute, Imperial College London, MRC-NIHR, Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London

### Introduction

Apolipoprotein E (ApoE) is responsible for lipid transport between cells. APOEε3 is the most common allele of the APOE gene among the population, while studies have shown a significantly increased risk of Alzheimer's disease (AD) in APOEε4-carriers compared to individuals with ε3. However, how APOEε4 contributes to disease pathogenesis remains incompletely understood. Regarding the role of ApoE in lipid transport and the role of lipid metabolism in AD highlighted by previous -omic studies, the aim of our study is to investigate the difference in lipid profile between humanised APOEε4 and APOEε3 knock-in mice.

### Methods

2-month and 6-month male homozygous APOEε3 and APOEε4 mice were used as the AD mice model. Lipids from the mice frontal cerebrum were double extracted by Bligh and Dyer method (2:1:1 methanol: chloroform: water). Untargeted lipid profiling was performed by ultrahigh-performance liquid chromatography (UHPLC) ion-mobility mass spectrometry (IMMS) using the dried organic extract. Mass correction, collision cross-section (CCS) calibration and feature extraction were performed using the vendor's software. Data pre-processing were done by KniMet workflow (Liggi et al, 2018). Finally, multivariate and univariate statistical analyses were conducted in SIMCA and the GraphPad Prism package.

### Preliminary data (results)

Results showed a decrease in phospholipids abundance including phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) in the 2-month APOEε4 mice compared to APOEε3 mice in the negative ionisation mode. In the 6-month mice group, we also observed downregulation of phospholipids, mainly PCs, in APOEε4 related to APOEε3 in both ionisation modes (Figures 1 and 2). Additionally, sphingolipids such as sulfatides and hexosylceramides were significantly decreased in 2-month APOEε4 mice compared to APOEε3 mice. Sphingolipids, in particular, lactosylceramides, showed significant association with AD SNPs in our previous epidemiology study. Our results suggest that APOEε4 contributes to AD pathology via lipid metabolism modulation.

### Please explain why your abstract is innovative for mass spectrometry?

we used the KniMet workflow which conducted data deconvolution, blank and/or quality control-based filtering, missing value imputation, normalisation and feature annotation (utilising the LIPIDMAPS database) for untargeted lipidomic analysis.

### Co-authors:

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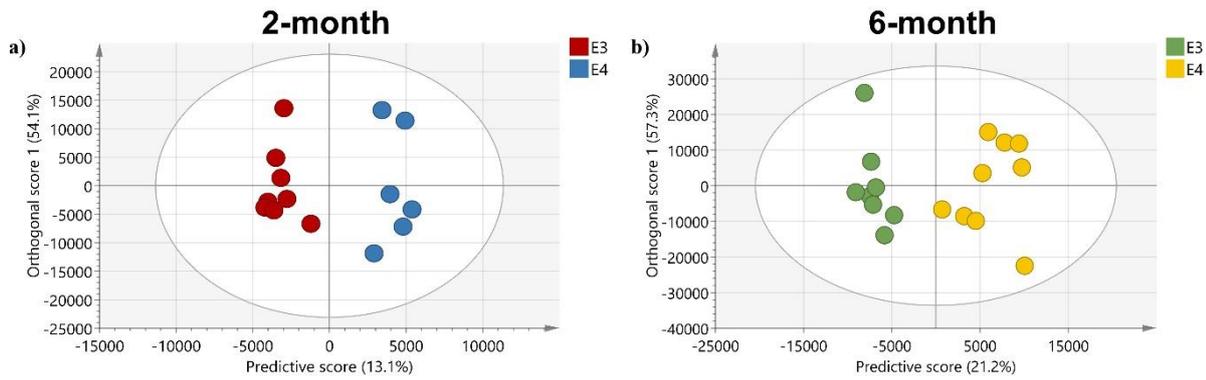
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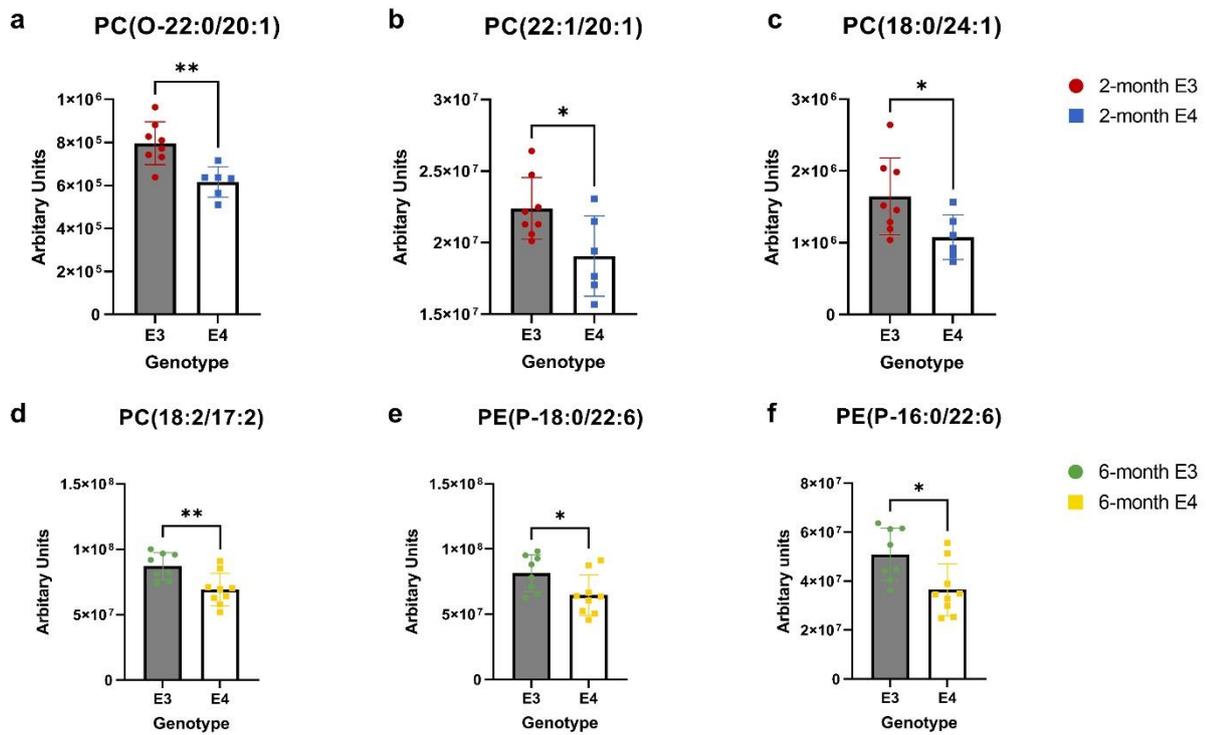
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OPLS-DA scores plot of APOEε3 and APOEε4 mice brain lipidome



Box plots of lipid changes between APOEε3 and APOEε4 mice

## Session: MS in Structural biology - Native MS, HDX-MS

Poster number: LS-PB-119

### PROBING THE CONFORMATIONAL DYNAMICS OF ALPHA-SYNUCLEIN BY ION MOBILITY MASS SPECTROMETRY

Abstract ID: 83

Presenting author: Emily Byrd, University of Leeds

#### Introduction

$\alpha$ -Synuclein ( $\alpha$ S) is an intrinsically disordered protein implicated in Parkinson's disease (PD), due to its high propensity to aggregate into amyloid fibrils. Monomeric  $\alpha$ S populates a diverse conformational ensemble, and it is not yet understood whether particular conformations promote aggregation. In this work, ion mobility mass spectrometry (IM-MS) has been applied to structurally interrogate  $\alpha$ S, along with sequence variants of  $\alpha$ S whereby crucial regions controlling have been removed, to determine whether particular conformations present in the ensemble are more aggregation prone. Following this, regional specific data has been obtained through cross-linking (XL), HDX MS and electron capture dissociation (ECD) to identify residues crucial for stabilising specific conformations and for metal ion binding. Elucidating aggregation prone species may identify viable targets for the treatment of PD.

#### Methods

IM-MS experiments were performed on a Waters Synapt G1 Instrument using 20  $\mu$ M  $\alpha$ S in 20 mM ammonium acetate at pH 7.5 with the addition of 500  $\mu$ M zinc acetate, manganese acetate or calcium acetate. Aggregation kinetics were measured using Thioflavin-T (ThT) fluorescence using samples containing 100  $\mu$ M  $\alpha$ -syn in 20mM ammonium acetate, pH 7.5. For XL-MS, DMTMM in HEPES buffer was added to 2.5  $\mu$ M  $\alpha$ S and incubated at 4°C for 1.5 hr. Proteins were digested using trypsin and GluC overnight at 37°C and analysed using an Orbitrap Exploris 240, Thermo Scientific.

#### Preliminary data (results)

The native mass spectrum of  $\alpha$ S shows a multimodal charge state distribution whereby a lowly charged population (more compact) and a highly charged (extended) population are present. IM-MS data has clarified that  $\alpha$ S populates an extended conformation as well as three distinct partially compact conformations for the +8 charge state, present in equilibrium confirming the conformational diversity detected in the native mass spectrum. Using IM-MS in combination with ThT aggregation assays aimed to be able to correlate the conformational fingerprint of  $\alpha$ S with an increase or decrease in aggregation kinetics. Initially, we could correlate wild-type  $\alpha$ S compaction with faster aggregation in the presence of divalent ions: Mn<sup>2+</sup>, Ca<sup>2+</sup> and Zn<sup>2+</sup>, all of which are present with  $\alpha$ S in Lewy Body deposits in PD patients. However, our finding identified a variant of  $\alpha$ S whereby a critical sequence in the N-terminal region is deleted, of which compacts in the presence of divalent metal ions but does not aggregate. This suggest that specific intra-molecular interactions that govern the assembly of particular conformations might influence the ability of  $\alpha$ S to assemble into amyloid fibrils. Thus far we can confirm that the degree of structural plasticity of  $\alpha$ S can determine aggregation kinetics. IM-MS and ThT kinetics have been used to establish a role that metal ions may play in PD whereby intra-molecular interactions within  $\alpha$ S monomers are highly important for stabilising conformations and predisposing the  $\alpha$ S to aggregate.

#### Please explain why your abstract is innovative for mass spectrometry?

These findings, achieved by the unique capability of IM-MS to capture transient, heterogeneous species, offer promise to understand how  $\alpha$ S aggregates and causes disease, potentially identifying targets for intervention.

#### Co-authors:

Sheena Radford, University of Leeds  
Frank Sobott, University of Leeds

Poster number: LS-PB-120

## HDX MASS SPECTROMETRY FOR THE STUDY OF BIOTHERAPEUTIC FORMULATION EFFECTS ON THE STRUCTURAL DYNAMICS OF MONOCLONAL ANTIBODIES

Abstract ID: 96

**Presenting author: Emilia Christofi, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology, University of Manchester**

### Introduction

Monoclonal Antibody (mAb) biotherapeutics, constitute a growing proportion of the drug catalogue for virtually all major pharmaceutical companies. Although these therapeutics can be utilized to treat serious diseases, they are also associated with a number of challenges in their manufacturing and formulation. One of the main challenges is their tendency to aggregate or self-associate in solution through weak protein-protein interactions (PPIs). Aggregation has an impact both on the production of a consistent drug product and also on human health. Effects include product recovery loss, storage limitations, and unwanted immune responses when infused in patients. The first stage of assembly would be an aggregation competent conformer and therefore studying the conformational variation at the monomer level is the first stage in understanding this process.

### Methods

The structural dynamics of a model mAb taken across 4 different sample points of its production line, provided by FUJIFILM Diosynth Biotechnologies, were examined using HDX-MS. We evaluate how different storage solutions can impact the tertiary fold and flexibility of the mAb by studying the deuterium uptake on the peptide level. This is achieved with the use of a Waters Nano-Acquity UPLC system with ESI-MS detection (Waters Synapt-G2S) coupled to a LEAP-Technologies dual-armed robot. Consequently, any intrinsic dynamics changes will be correlated to the aggregation propensity of the antibody by looking into the Aggregation Prone Regions (APRs) of the sequence.

### Preliminary data (results)

From preliminary results, we were able to show how buffer composition affects the conformational flexibility of the model mAb. A comparison was made between the original formulation and when buffered exchanged in a smaller and simpler buffer such as Ammonium Acetate, with the use of a butterfly plot for each chain.

On the butterfly plots, we examine the overlapping peptides for each chain with respect to their deuterium uptake. In these experiments, significant deuterium uptake is defined as a mass shift above 1 Da. By comparing the peptides' deuterium uptake it was established that in the original formulation, mAb4 retained its high stability as only a few peptides presented a significant deuterium uptake. However, in the ammonium acetate buffer, higher flexibility was revealed as many peptides presented a significant uptake. The same behaviour was also obtained for the heavy chains of the model mAb.

By mapping the uptake on the crystal structure of the mAb we were able to visualize that in the case of the light chain in the Original Formulation, the uptake is localized just above the hinge region. However, in Ammonium Acetate the variable region of the light chain also presents significant uptake; suggesting increased flexibility.

### Please explain why your abstract is innovative for mass spectrometry?

Developing a method to detect these aggregation tendencies as early on as possible in the production line will be beneficial, as early interventions to prevent/minimize them will be enabled.

### Co-authors:

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*Robin Curtis, Manchester Institute of Biotechnology, University of Manchester*

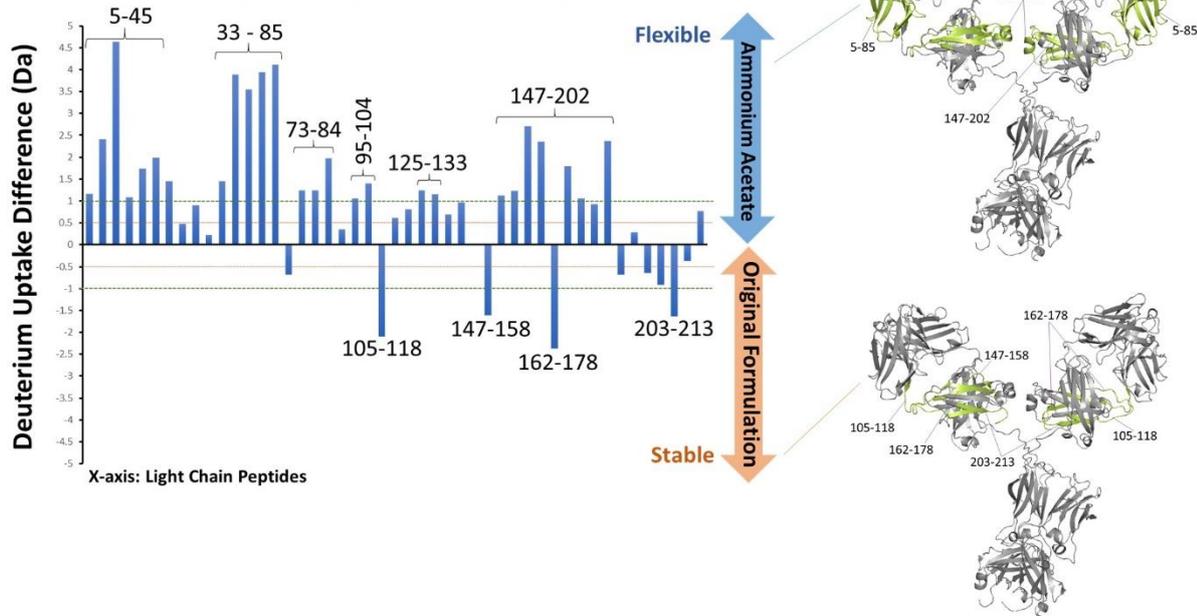
*Jim Warwicker, Manchester Institute of Biotechnology, University of Manchester*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

Jeff Keen, FUJIFILM Diosynth Biotechnologies  
Arghya Barman, FUJIFILM Diosynth Biotechnologies  
Tibor Nagy, FUJIFILM Diosynth Biotechnologies  
Perdita Barran, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology, University of Manchester

Uptake ( $\Delta m$ ) in AmAc compared to Original Formulation in triplicate measurements



Butterfly plot for the light-chain of the model mAb.

Poster number: **LS-PB-121**

## **OXIDATIVE FOOTPRINTING AND HYDROGEN-DEUTERIUM EXCHANGE TO STUDY DIFFERENT PROTEIN-LIGAND SYSTEMS**

Abstract ID: **162****Presenting author: Thomas Nehls, Clemens-Schöpf-Institute, Department of Chemistry, Technical University of Darmstadt**

### **Introduction**

Mass spectrometry is used in drug discovery to study the binding pockets of target proteins to develop or improve ligands. Both top-down and bottom-up methods can be used to obtain information about the fold. In footprinting methods, a chemical modification is introduced under native conditions, which provides information about the conformation. Thus, the relevant information is "stored" and a bottom-up approach can be taken. Hydroxyl radicals react with amino acid side chains and not with the peptide bond. Moreover, any solvent-accessible function (except glycine) can be oxidatively modified. In contrast, in hydrogen-deuterium exchange (HDX) the exchange is modulated by different interactions and accessibility of the peptide backbone.

### **Methods**

For the oxidative footprinting we used hydroxyl radicals resulting from a Fenton reaction of hydrogen peroxide with  $\text{Fe}^{2/3+}$ -EDTA. The catalyst was regenerated with ascorbic acid. The reaction was quenched with thiourea after 15 seconds. For bottom-up analysis, a tryptic digest followed. Digests were analyzed using a  $\mu\text{LC}$  combined with a Thermo LTQ Orbitrap XL. The exact details can be found in our publication (Nehls T et al., Int J Mol Sci., 2021, 22, 9927). HDX experiments were performed with the same samples. For HDX, we used a Waters Synapt XS with an HDX-robot and online pepsin digest.

### **Preliminary data (results)**

When studying the active site of myoglobin, we found that the heme group can also catalyze the Fenton reaction, which was manifested in a variety of modifications in the binding pocket. In particular, site-specific oxidation was observed that reflected the known binding mode of molecular oxygen to the heme group. In another set of experiments, we investigated the binding domain of FK506-binding proteins (FKBPs), which are chaperones with prolyl isomerase activity and which are targets of immunosuppressive drugs. We were able to study the conformational change of FKBPs with selected ligands. We observed a clear difference in the global extent of modification of ligand-free and -bound states from intact-mass measurements. From peptide-level analysis, we observed site-specific differences between the ligands in the labelling pattern after relative quantification. Finally, we performed complementary HDX experiments to gain further insight into the solution-phase structure and dynamics of these proteins.

### **Please explain why your abstract is innovative for mass spectrometry?**

Oxidative footprinting and HDX in combination provide a better insight into the conformation of the protein in addition, oxidative footprinting is an inexpensive method that can be widely implemented.

### **Co-authors:**

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*Christian Meyners, Clemens-Schöpf-Institute, Department of Chemistry, Technical University of Darmstadt*  
*Felix Hausch, Clemens-Schöpf-Institute, Department of Chemistry, Technical University of Darmstadt*  
*Frederik Lermyte, Clemens-Schöpf-Institute, Department of Chemistry, Technical University of Darmstadt*

Poster number: **LS-PB-122**

## UNDERSTANDING THE MECHANISM OF NATIVE SUPERCHARGING OF NUCLEIC ACIDS BY ION MOBILITY MASS SPECTROMETRY

Abstract ID: 191

**Presenting author: Debasmita Ghosh, ARNA Laboratory, Inserm U1212, CNRS UMR5320, Université de Bordeaux, France**

### Introduction

For proteins, bimodal charge state distributions (CSD) indicate the coexistence of two structural ensembles in solution, as globular ensembles ionize via the charge residue mechanism (CRM) and unfolded ensembles ionize via the chain ejection mechanism (CEM). In contrast, for nucleic acids, the CRM/CEM branching depends more on the ionic strength than on the folding in solution [JASMS (2019) 30, 1069]. CSDs can be modified by “supercharging” agents (SCA). The underlying mechanism of supercharging, however, is still unclear for nucleic acids. Our goal is to understand the mechanism of native supercharging for nucleic acids in physiological conditions. To this aim, we used G-quadruplex nucleic acid structures (G4), formed by guanine-rich sequences and stabilized by the coordination of specific cations.

### Methods

Structures of intermolecular, intramolecular G4s and non-structured oligonucleotides, as well as synthetic polymers and proteins were probed by IM-MS in a 6560 DTIMS-Q-TOF (Agilent) in helium. The pre-IMS conditions were kept soft. Step-field experiments were performed to convert arrival time to collision cross sections (CCS). Arrival time distributions were converted to CCS distributions (CCSD). Experiments were done in NH<sub>4</sub>OAc at various ionic strengths, and using SCAs such as sulfolane, m-nitrobenzyl alcohol (mNBA) or polypropylene carbonate (PC).

### Preliminary data (results)

The following results were obtained on oligonucleotides. At high ionic strength (100-150 mM NH<sub>4</sub>OAc), the CSD is similar for structured and non-structured oligonucleotides. ESI follows CRM ionization. In presence of SCA, the CSD and collision cross sections distribution (CCSD) changes, depending on the structure of the G4. The most stable (intermolecular) G4 shows unimodal CSD in presence of SCA, indicating mostly CRM. For intramolecular G4, a bi-modal CSD (CEM and CRM) was seen, despite the structure being 100% folded in solution, and cation loss is observed at high charge state. Broadening of the CCSD and population of extended structures at high charge states was observed in presence of mNBA, while narrower and less extended CCSD was observed in PC or sulfolane. For non-G4 sequences, mNBA shifts the CSD fully to the CEM pathway at high ionic strength. mNBA adducts were found in very soft instrumental conditions. We hypothesize that long-lived adducts are still present in the IM cell, and get lost afterwards. This explains the broadening of CCSD in mNBA.

In summary, PC and sulfolane possess lower supercharging ability, but fully preserve native-like ion conformations at physiological ionic strength. In contrast, mNBA showed highest supercharging ability, but caused unwanted unfolding of some solution-folded G-quadruplex structures. We speculate that direct interaction could displace buffer ions and promote unwanted unfolding. Experiments are under way to generalize these findings to other ionization modes, additives and analyte classes.

### Please explain why your abstract is innovative for mass spectrometry?

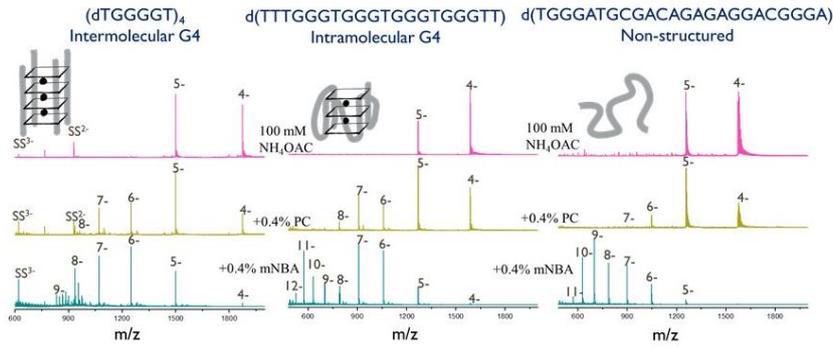
Native supercharging is investigated for nucleic acids in negative ion mode, and results are interpreted according to CRM/CEM partitioning.

### Co-authors:

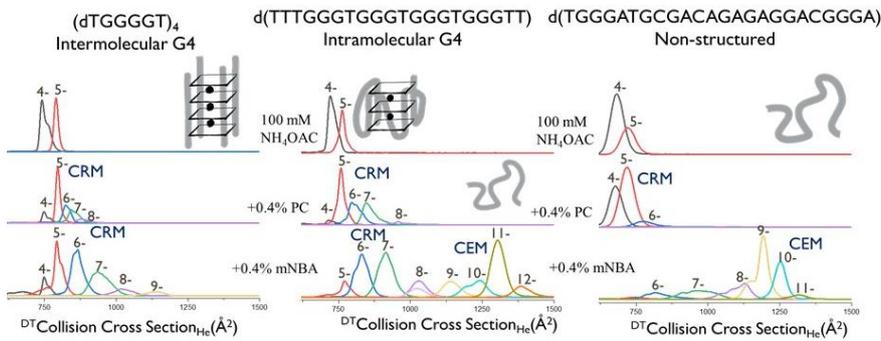
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*Valérie Gabelica, ARNA Laboratory, Inserm U1212, CNRS UMR5320, Université de Bordeaux, France, IECB, CNRS UAR3033, Inserm US01, Université de Bordeaux, France*

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
 Thursday 1 September 2022 from 14:00 to 15:30 hours



Comparison of charge state distributions of oligonucleotides



Comparison of collision cross section distributions of oligonucleotides

Poster number: LS-PB-123

## NATIVE MASS SPECTROMETRY TO ELUCIDATE HOW FOLDAMER LIGANDS SELECTIVELY TARGET NON-CANONICAL DNA STRUCTURES

Abstract ID: 195

Presenting author: Alexander König, University of Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, IECB

### Introduction

Native Mass Spectrometry seeks to preserve the solution structure of a molecule upon transfer into the gas phase and the analyzers. In this work, we focus on DNA G-quadruplexes, which are non-canonical nucleic acid structures enriched in the telomeres and oncogene promoters, and involved in key biological processes. This sparks scientific interest towards developing selective G4-targeting ligands to regulate these processes. The main objectives are to 1) screen new foldamer-based ligands with promising G-quadruplex selectivities to discover original scaffolds able to target specific DNA sequences, and 2) understand the structural determinants of these selectivities, opening avenues for rational hit-to-lead optimizations.

### Methods

The quadruplex formation and complexation experiments are carried out on an Agilent 6560 IMS-QTOF. To ensure biologically relevant G-quadruplexes are formed, 10  $\mu$ M of DNA strand are doped with an excess of 1 mM KCl, with 100 mM of volatile trimethylammonium acetate to mimic the physiological ionic strength. The different G4-ligand complexes are separated by mass, allowing to determine distinct formation constants for all ligand stoichiometries, and the Ion Mobility spectrometry (IMS) measurements provide an insight into the effects of ligand binding on the secondary structure of the G-quadruplex.

### Preliminary data (results)

The chosen DNA sequences were selected for their ability to form a large range of G4-quadruplex conformers (Figure 1) for which we have determined that they are formed in native MS conditions. Alternative secondary DNA structures (duplex strand, i-motif) and unfolded single strands of varying nucleobase compositions were also used as controls. This diversity serves to characterize the true selectivity of ligands, and to provide more information on the structural determinants of binding. Four foldamers were chosen as G4-quadruplex targeting ligands. Foldamers are molecules that assume high-order structures through intramolecular folding, mimicking biomolecules in both structure and biochemical behavior. The foldamers in these experiments consist of chains of units of quinoline (Q) and pyridine (P) derivatives (Figure 2).

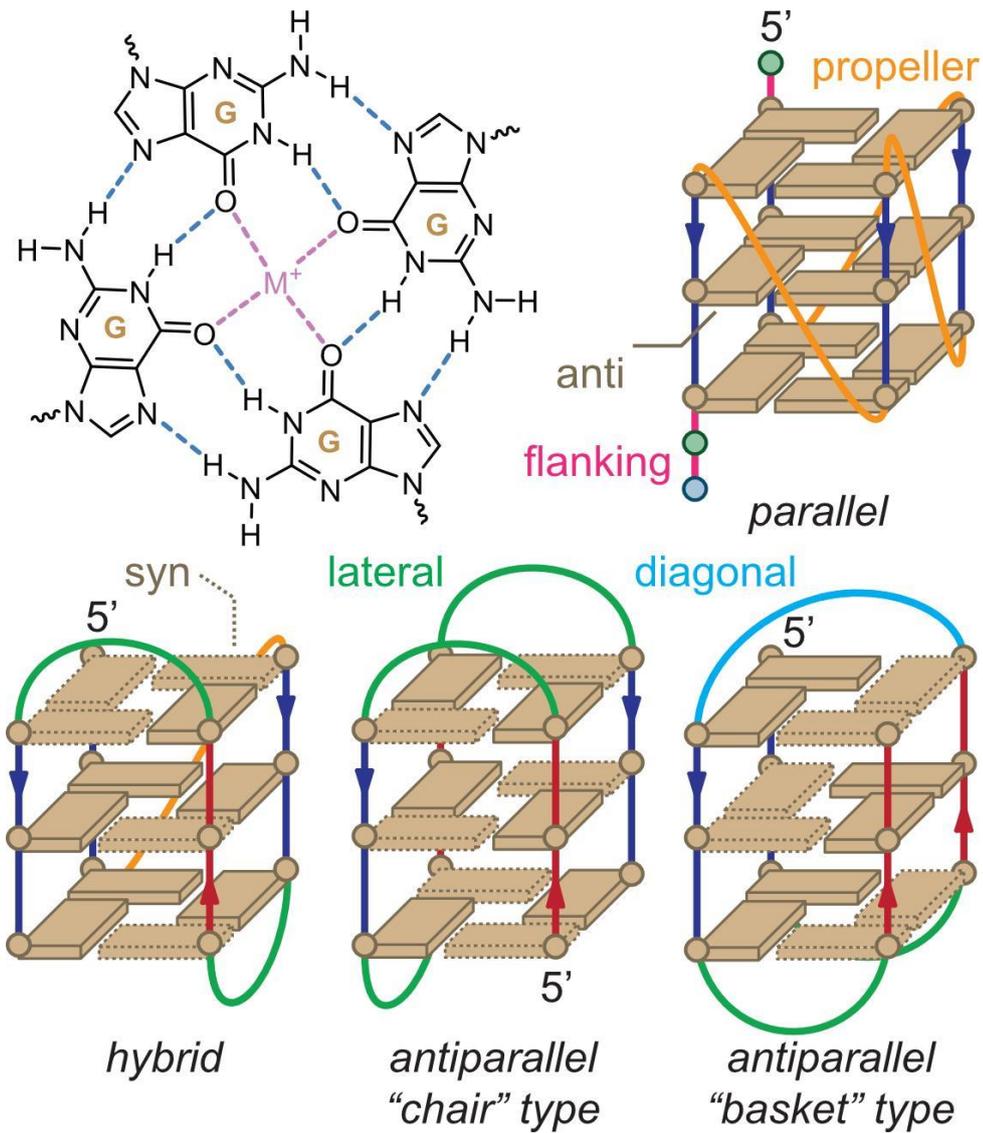
From a panel of 20 DNA sequences, we extracted several G4-forming sequences that are selectively targeted by the foldamer ligands. These sequences all form parallel G-quadruplexes with thymine-rich loops, which we determined from Circular Dichroism (CD). The  $K_D$  values for the 1:1 and 2:1 complexes that were obtained from the mass spectra are on the  $\mu$ M scale, with the  $K_{D2}$  value being 2-10 times that of the  $K_{D1}$  value. Comparing the  $K_D$  values among different ligands suggests that the choice of foldamer sequence makes room for a trade-off between affinity (i.e. lower  $K_D$  values) and nucleobase selectivity. The IMS results reveal different shifts of collisional cross sections (CCS) for the first and second binding event, indicating that the two binding sites/mechanisms are not chemically equivalent.

### Please explain why your abstract is innovative for mass spectrometry?

Native MS highlighted the specificity of foldamer ligands for a DNA target. Association constants and stoichiometries were obtained from mass spectra. IMS correlated ligand binding to potential structural effects.

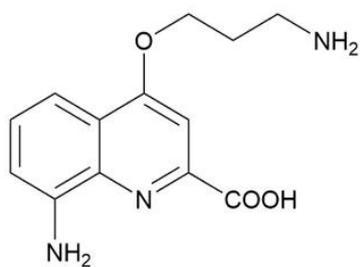
### Co-authors:

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Yann Ferrand, University of Bordeaux, CNRS, IPB, CBMN, UMR 5248, IECB  
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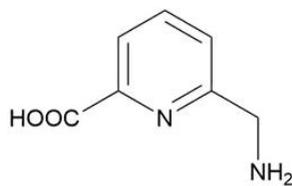


G-quartet (top left) and the four main topologies of G-quadruplexes.

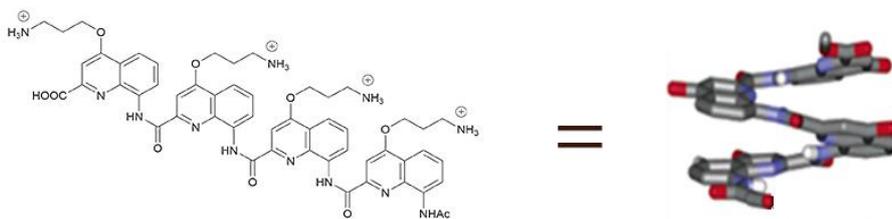
Quinoline subunit (Q)



Pyridine subunit (P)



## Foldamer: QQQQ



Subunits of the oligomeric foldamers (top), example: QQQQ (bottom)

Poster number: **LS-PB-124**

## **DYNAMIC INTERPLAY BETWEEN THE PERIPLASMIC CHAPERONE SURA AND THE BAM COMPLEX IN OUTER MEMBRANE PROTEIN FOLDING STUDIED BY STRUCTURAL MASS SPECTROMETRY**

Abstract ID: **204**

**Presenting author: Antonio Calabrese, Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, University of Leeds**

### **Introduction**

Correct folding of outer membrane proteins (OMPs) into the outer membrane of Gram-negative bacteria requires unfolded OMPs to be delivered to the beta-barrel assembly machinery (BAM). The periplasmic chaperone SurA is thought to play a key role in this process, but the mechanism of this remains unknown. Here, we have used hydrogen deuterium exchange mass spectrometry (HDX-MS), chemical crosslinking-MS (XL-MS) and computational modelling to determine the structural basis of the interaction between SurA and BAM. We reveal that binding causes changes in BAM and SurA conformation and/or dynamics distal to the sites of binding, suggesting concerted changes in conformational dynamics upon binding.

### **Methods**

HDX experiments were conducted using an automated robot (LEAP) that was coupled to an Acquity M-Class LC with HDX manager (Waters). Data analysis was performed using PLGS and DynamX (Waters). Peptides with statistically significant increases/decreases in deuterium uptake were identified using Deuterios. For XL-MS experiments, the BAM-SurA complex was assembled *in vitro* and DSBU was added. The crosslinking reaction was allowed to proceed for 30 min. The proteins were digested with trypsin and the resultant peptides were then analysed by liquid chromatography-mass spectrometry (LC-MS) on an Orbitrap Exploris 240 mass spectrometer (Thermo Fisher). Cross-link identification was performed using MeroX.

### **Preliminary data (results)**

Here, we have used both HDX-MS and XL-MS to map regions mediating the interaction between BAM and SurA. These data have revealed, for the first time, the regions on both SurA and BAM responsible for binding. Changes in deuterium uptake in the BamA beta-barrel upon SurA binding were also observed using HDX-MS, indicating that the interaction with SurA modulates the structure and/or dynamics of BAM distal to the SurA binding site. We have used AlphaFold-multimer to generate a model of the SurA-BAM complex that is consistent with all of our experimental data and previous literature reports. Combined, our data suggests that SurA targets substrates to BAM and that binding drives conformational changes in BAM which are essential for its catalytic function. These insights could only be gained by exploiting the powers of structural mass spectrometry.

### **Please explain why your abstract is innovative for mass spectrometry?**

Integrating information from structural proteomics methods reveals the mechanism by which the periplasmic chaperone SurA delivers unfolded proteins to the outer membrane-embedded BAM complex for folding.

### **Co-authors:**

*Bob Schiffrin, Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, University of Leeds*

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Poster number: **LS-PB-125**

## **PULLING APART HEAVY METAL BINDING TO PHYCOBILIPROTEINS WITH NATIVE MASS SPECTROMETRY**

Abstract ID: **209****Presenting author: Jeddidah Bellamy-Carter, University of Birmingham**

### **Introduction**

Pollutants, such as heavy metals, are toxic to microalgae, disrupting the ecosystem within which microalgae's role is essential. Cyanobacteria and other microalgae utilise phycobilisomes, large protein complexes, to light harvest through chained transfer of fluorescence. The constituents of these complexes, phycobiliproteins, seem to be particularly affected by heavy metals, which quench these vital fluorescence properties. However, the binding mechanism, stoichiometry of binding, and how the complexes are disrupted remains largely unexplored. We show using high-resolution mass spectrometry, how different phycobiliproteins respond to various heavy metals (including copper, silver, iron and zinc). We show that some metals bring about oligomeric changes to these phycobiliproteins, affecting the proper assembly of the phycobilisome and therefore photosynthesis.

### **Methods**

Phycobiliproteins (phycocyanin and allophycocyanin) were extracted from microalgae and purified through ion exchange, hydroxyapatite and size exclusion chromatography. The purified phycobiliproteins were exchanged into 100 mM ammonium acetate (pH 6.8) for native mass spectrometry (MS) experiments.

The phycobiliproteins were incubated variously with volatile salts of heavy metals (including Cu, Fe, Zn and Ag) and complexes analysed by native MS. Native MS measurements were performed by nano-electrospray ionisation with gold-coated borosilicate emitters on Orbitrap Eclipse Tribrid (Thermo Fisher Scientific) mass spectrometer. Protein-bound metals were localised using MS2 and (pseudo)MS3.

### **Preliminary data (results)**

Using high-resolution native mass spectrometry, we show the stoichiometry of heavy metal binding to phycobiliproteins that correlates with their loss of function. Fe, Cu, Zn and Ag show varied affinity for the two phycobiliproteins, with Ag showing the greatest overall affinity followed by Cu. Interestingly, the stoichiometry of binding between Ag and Cu differs dramatically. Additionally, upon addition of Ag, the phycocyanin complex architecture changes dramatically from a hexameric to tetrameric conformation. This is in sharp contrast to allophycocyanin whereby no structural rearrangement is observed. The different behaviours show the location of binding differs for the metals.

Overall, native MS provides key insight into heavy metal binding that is critical to understand the mechanisms behind how different metals have different toxicities within algae. In vivo, allophycocyanin forms the stable core of the photosynthetic complex while phycocyanin builds into rods to shuttle light towards it; the rods are believed to be dynamic, adjusting to environmental conditions. Our results suggest that structurally 'locking up' phycocyanin through the binding of specific heavy metals would disrupt the formation of these rods, hindering photosynthesis that ultimately kills the microalgae.

### **Please explain why your abstract is innovative for mass spectrometry?**

Native mass spectrometry reveals the mechanism by which different heavy metals prevent photosynthesis in microalgae.

### **Co-authors:**

*Jaspreet Sound, University of Birmingham*  
*Aneika Leney, University of Birmingham*

Poster number: **LS-PB-126**

## **ION-MOBILITY MASS SPECTROMETRY OF ALPHA-1 ANTITRYPSIN: STRUCTURAL AND METHODOLOGICAL INSIGHTS.**

Abstract ID: **225**

**Presenting author: Sarah Vickers, University College London, Birkbeck, University of London**

### **Introduction**

Alpha-1 antitrypsin (AAT) is a serine protease inhibitor essential to the control of proteolytic pathways. AAT has a complex mechanism for inhibition involving a conformational change, leaving it vulnerable to misfolding. Consequently, patients suffer from AAT deficiency, caused by mutant AAT, leading to two complications: The mutants can misfold and polymerise causing cirrhosis. AAT thus fails to reach the lungs and inhibit elastase, leading to emphysema. Using ion mobility mass spectrometry (IMMS) we examine the difference in structure and stability of plasma samples from healthy and diseased patients. We also have begun developing a method for analysing ex-vivo liver samples using IMMS.

### **Methods**

Wild type and mutant samples of AAT were purified from plasma and polymeric mutant samples were purified from human liver, as described in literature. Samples were buffer exchanged into ammonium acetate and diluted to 5  $\mu$ M. A Waters HDMS Synapt G1 was used to analyse all samples. Mobility and collision activation experiments were performed to assess conformational stability and compare the CCS of both samples. A SELECT SERIES Cyclic IMS QToF (Waters Corp.) was then used to further separate conformers and study their unfolding. Fragmentation was performed using electron capture dissociation to gain detailed interface information.

### **Preliminary data (results)**

The glycosylation and unfolding patterns of wild type and mutant samples were analysed using IMMS. The glycoforms of the samples differed slightly, with one glycoform being less prevalent in the mutant sample, suggesting its glycosylation was not as mature. The mutant sample was also less stable when activated in the trap suggesting it forms the active intermediate (and therefore polymerises) more readily, as is reported in literature. When analysed using cyclic IMMS, multiple unstable intermediates were observed, which could be linked to the reactive intermediate in the polymerisation pathway. Further experiments employed a small molecule that blocks polymerisation by selectively binding to the intermediate to compare its structure to activated monomer samples.

Investigation of liver polymer samples using IMMS has produced extremely promising preliminary results, with high order polymers and multiple conformations observed. Further experiments will analyse the liver polymers using cyclic ion mobility mass spectrometry to separate out these conformations and gain insight into the formation of the toxic polymers.

A reproducible IMMS method has been developed for the structural characterization of monomeric samples of wild type and mutant AAT which lays the groundwork for future investigations of the serpins using mass spectrometry. The application of this workflow to biologically relevant liver polymer will provide exciting insight into the structural basis for this disease.

### **Please explain why your abstract is innovative for mass spectrometry?**

New mass spectrometry methods have been developed to analyse these ex-vivo samples, many of which have never been seen on a mass spectrometer before.

### **Co-authors:**

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*Hannah Britt, University College London, Birkbeck, University of London*  
*James Irving, University College London*  
*David Lomas, University College London*  
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Poster number: LS-PB-127

## WHAT CALCULATION LEVEL IS REQUIRED FOR THE STRUCTURAL INTERPRETATION OF ION MOBILITY MASS SPECTROMETRY EXPERIMENTS IN THE 1000-12000 DA RANGE?

Abstract ID: 229

Presenting author: Frederic Rosu, University of Bordeaux, CNRS

### Introduction

Folding and self-assembly are key for molecular function, and native ion mobility mass spectrometry can help the structural assignment via collision cross sections (CCS). However, there is still debate on the right level of calculation required to structurally interpret CCS values. To assess different calculation levels, we measured CCS values by drift tube IMS in helium on very rigid and monomorphic model structures (oligoquinoline foldamers, G-quadruplex nucleic acids), on ubiquitin and on oligourea foldamers assemblies, and compared the experimental CCS distributions with different theoretical CCS model (PA, EHSS, TM) using structures generated at different calculation levels.

### Methods

Native electrospray ionization mass spectrometry has been performed on an Agilent 6560 DTIMS-QTOF. The drift tube was operated in helium with custom modification to improve robustness (additional pump, valves and vacuum manometers). We studied ubiquitin and peptidomimetic oligourea foldamers in positive ion mode and nucleic acids and oligoquinoline phosphonate foldamers in negative ion mode. For the theoretical CCS calculations, we compared TM, EHSS and PA using mobcal and other popular software. We produce the structures candidates using force field (amber, Charmm, OPLS) and ab-initio molecular dynamics (semi-empirical level PM7 or DFT level (M062X)).

### Preliminary data (results)

Drift tube experiments in helium are preferred to test the effect of structure generation, because of lower uncertainties on the parameterization of TM and EHSS. Here we will review results obtained on model systems of various chemical nature.

For ubiquitin, the <sup>TM</sup>CCS of PM7-optimized structure matches the experimental values better than the structures generated by force fields. For proteins, force fields had been widely adopted to generate structure in-vacuo, but our results show a systematic overestimation of the theoretical CCS values compared to the experiments if the gas-phase structure is modeled with force fields (whether Amber, Charmm, or OPLS). We highlight the caveats in adapting CCS calculation methods based on ill-optimized structures. For example, PA underestimates the CCS values but combined with force-field generated structures, the values agree fortuitously.

For nucleic acids, we have demonstrated the failure of force field to generate in-vacuo structures (*ACS Cent.Sci.*,201,3,454) and propose now to use high level molecular dynamics where the electrons are treated at the DFT level or semi-empirical level depending of the size of the system and the nuclei are treated using classical formalism.

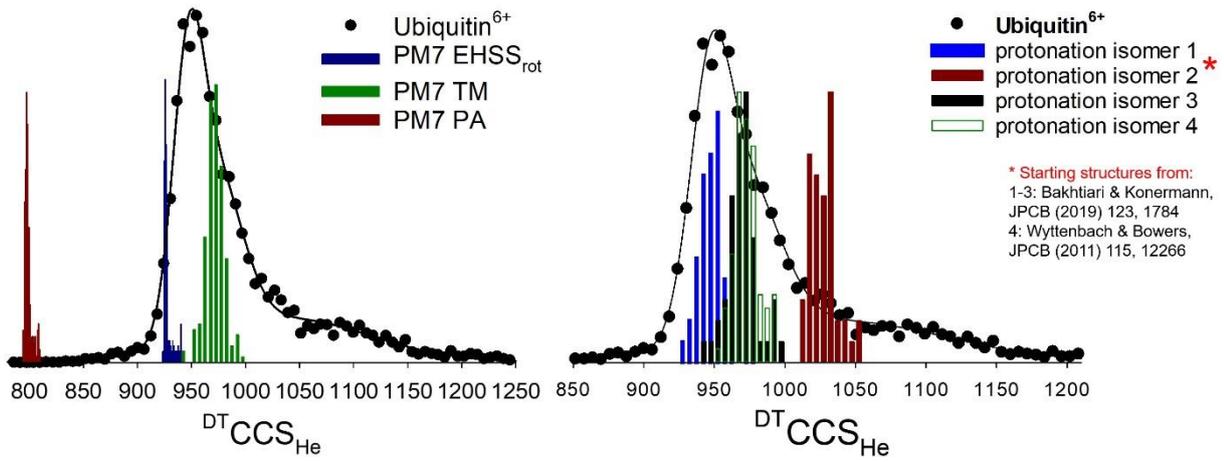
We also tested synthetic folded molecules (foldamers). Rigid oligoquinolines were used as benchmark because they are monomorphic, rigid and the width of their experimental CCS distribution is close to the diffusion limit. Again, PM7 molecular dynamics and CCS calculation with TM or EHSS matches very well with the experiment. Finally, we studied large oligourea hexameric (*Nat.Chem.*,2015,7,871) and octameric complexes.

### Please explain why your abstract is innovative for mass spectrometry?

In summary, DFT or semi-empirical calculations perform better than force field MD in the gas phase, and lead to better structural assignment based on CCS values.

### Co-authors:

Valerie Gabelica, University of Bordeaux, inserm



Collision cross sections distribution (DTCCS<sub>He</sub>) of ubiquitin.

Poster number: **LS-PB-128**

## **INSIGHTS INTO THE CONFORMATIONAL DYNAMICS OF THE DISORDERED PROTEIN TDP-43 FROM STRUCTURAL PROTEOMICS**

Abstract ID: **247**

**Presenting author: Thomas Minshull, University of Leeds**

### **Introduction**

Membrane-less organelles (MLOs) are multicomponent structures that form by liquid-liquid phase separation (LLPS) of proteins/RNA. They play key roles in organising cells, signalling and stress, whilst aberrant LLPS is associated with several neurodegenerative diseases (e.g. Amyotrophic lateral sclerosis (ALS)). TDP-43 is a key RNA-binding protein that undergoes LLPS and is implicated in the development of ALS.

TDP-43 is a 414 residue protein consisting of an N-terminal domain (NTD), two DNA/RNA binding domains (RRM1 & RRM2) and the C-terminal domain (CTD) which is mostly intrinsically disordered. This domain is the main site for disease causing mutations, and it is hypothesised to be key in the ability of TDP-43 to undergo LLPS, form higher-order oligomers and amyloid-like fibrils in vitro and in-vivo

### **Methods**

Here we have used powerful mass spectrometry (MS) techniques such as hydrogen-deuterium exchange-MS (HDX-MS) performed on an automated HDX robot coupled to an Acquity M-class LC system with HDX manager and G2-Si mass spectrometer (Waters) and cross-linking-MS (XL-MS) performed on an Exploris 240 mass spectrometer (Thermo) to interrogate the conformational dynamics of monomeric full-length TDP-43 and its assemblies with RNA.

### **Preliminary data (results)**

Our results from HDX-MS show that, alongside the expected protection from deuterium exchange due to the binding of RNA to the RRM domains, we observe long range allosteric protection from exchange in the NTD. This protection is supported by an increase in the number of crosslinks identified between the RRM domains and the NTD detected by XL-MS. Combined, this suggests involvement of the NTD in RNA binding. Most interestingly, HDX-MS reveals the presence of a protected species within the unstructured CTD upon RNA binding, concomitant with deprotection within the short alpha helix motif within the CTD upon RNA binding. These allosterically altered regions are known to influence the ability of TDP-43 to self-assemble, and can fine-tune its RNA binding repertoire.

Taken together, these data reveal new insights into the structure and dynamics of TDP-43, alongside the intra-protein and protein-RNA interactions which likely play a key role in fine tuning TDP-43 self-assembly into both condensates and amyloid fibrils. Understanding the molecular mechanism of these processes is key to elucidating the role of TDP-43 the pathology of ALS and FTL, and may lead to new therapeutics to treat these diseases.

### **Please explain why your abstract is innovative for mass spectrometry?**

Moreover, this work highlights the power of an integrative structural proteomics approach to interrogate the structure and dynamics of intrinsically disordered proteins and their interactions with nucleic acids.

### **Co-authors:**

*Antonio Calabrese, University of Leeds*

Poster number: LS-PB-129

## DEVELOPMENT OF A TEMPERATURE-CONTROLLED ELECTROSPRAY - CYCLIC ION MOBILITY METHODOLOGY TO INVESTIGATE PROTEIN AGGREGATION.

Abstract ID: 305

Presenting author: Julian Harrison, Department of Chemistry and Applied Biosciences, ETH Zürich

### Introduction

The development of ion mobility (IM) settings for mass spectrometry (MS) is important for the structural investigation of proteins. Incorrect settings can lead to loss of structural information, either by poor transmission of ions or unfolding due to the conditions being too harsh. However, when effective transmission can be achieved, the extra dimension IM adds to MS data can provide deep insight into the structure of a biomolecule. This can be seen when IM is coupled with another technique, such as temperature-controlled electrospray ionization mass spectrometry (TC-ESI-MS). The goal of this work was to develop instrument settings for IM and TC-ESI-MS for the analysis of large proteins and their aggregates.

### Methods

Protein samples were buffer exchanged into 200 mM ammonium acetate using size exclusion chromatography on a Superdex Increase 200 10/300 GL column (Massachusetts, USA). Samples were sprayed under non-denaturing conditions on a SELECT SERIES Cyclic IMS mass spectrometer (Manchester, UK). Several key IM parameters were examined for a range of proteins across a broad mass range to determine the effect of each setting on ion transmission.

### Preliminary data (results)

The IMS mass spectrometer used in this work has three phases during standard IM experiments: an 'inject' phase, where ions are moved on to the cyclic array located in the cyclic IM cell, a 'separate' phase, where the ions are pushed off the array for IM separation, then finally, the 'eject and acquire' phase where ions pass around the cyclic IM cell, and are guided out of the system by the cyclic array.

The two settings which had the greatest effect on ion transmission were the array offset and the so-called "racetrack bias" during the 'separate' phase. We found that similar voltages are required for effective ion transmission, otherwise ions would get caught in the array, and not move around the cyclic IM cell, which is evident in the IM chromatogram as a peak with a drift time starting just after the 'separate' phase. The degree of these effects was dependent on the mass of the protein being analyzed.

Optimized IM settings were then used in the TC-ESI-MS analysis of Jack bean urease. This analysis was performed to investigate temperature-induced effects on the structure of this protein. Prior to heating, the two highest oligomeric states of this protein were the hexamer (~ 550 kDa, native) and a dodecamer (~ 1.1 MDa, nonspecific). An increase in temperature led to urease forming octodecamers. Three distinct gas-phase conformations were observed for the dodecamers, the prevalence of two of which were affected by temperature.

### Please explain why your abstract is innovative for mass spectrometry?

This work presents a detailed investigation of parameters affecting ion transmission of proteins using cyclic IM, as well as the power of combining IM and TC-ESI-MS for structural analysis.

### Co-authors:

Adam Pruška, Department of Chemistry and Applied Biosciences, ETH Zürich  
Renato Zenobi, Department of Chemistry and Applied Biosciences, ETH Zürich

Poster number: **LS-PB-130**

## PROTEIN DYNAMICS MODULATE SUBSTRATE SWITCHING IN TYPE III SECRETION SYSTEM

Abstract ID: **424**

**Presenting author: Rinky Parakra, KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Molecular Bacteriology**

### Introduction

Many pathogenic Gram-negative bacteria deploy the type III secretion system (T3SS) as “molecular syringes” to inject virulence factors into their host eukaryotic cells. Enteropathogenic *E. Coli* (EPEC) uses a T3SS to colonize the intestinal tract. T3SS is a highly coordinated process that ensures hierarchical delivery of 3 classes of substrate: early, middle & late. The substrate specificity switch from middle to late in EPEC is regulated by the SctW protein forming trimeric complex with its chaperones SepD and CesL. SctW and the levels of  $Ca^{2+}$  dictates the type of substrate secretion: high levels promotes middle and suppress late substrates secretion, whereas low levels does the reverse job. How SctW protein senses the  $Ca^{2+}$  signals to trigger the substrate switching is still not known.

### Methods

To investigate protein dynamics of SctW and SctV using hydrogen-deuterium exchange mass spectrometry (HDX-MS) and correlate protein dynamics to substrate switching phenomenon, specifically, in response to calcium levels.

Briefly for HDX-MS experiments, protein was mixed with D<sub>2</sub>O buffer incubated at various time intervals before being added to quench solution. The labelled-protein was protease digested on-line, followed by peptide desalting and reversed-phase HPLC separation are performed prior to infusion into the ESI ion source of the mass spectrometer.

### Preliminary data (results)

Our data shows that,

1. SctW is not the calcium receptor protein but may mediate calcium secretion switching as seen by Trypsinolysis and HDX experiments.
2. The mutant derivatives of SctW (point mutation R333D and C-terminal truncation- 11 residues) failed to suppress effector secretion at high Calcium concentration suggesting need of functional Cterminus of SctW.
3. The mutant derivatives of SctW display enhanced dynamics in their C-terminal domain as seen from HDX-MS experiments (Fig 1).
4. Calcium alters intrinsic dynamics of the C-terminal domain of SctV (Fig2).

SctV, a part of the export apparatus, forms a nonameric ring based on structural data<sup>2</sup> and acts as a high affinity membrane receptor for SctW/SepD/CesL. We hypothesise that the  $Ca^{2+}$  and SctW regulates intrinsic dynamics of SctV which comprises of the conformational switch that controls translocator to effector affinity and secretion.

### Please explain why your abstract is innovative for mass spectrometry?

First time HDX-MS study on cytoplasmic domain of SctV (nonamer) and SctW (Trimeric complex with CesL & SepD). Both the proteins are an important part of the type III secretion system.

### Co-authors:

*Athina G Portaliou, KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Molecular Bacteriology*

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*Luit Barkalita, KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Molecular Bacteriology*

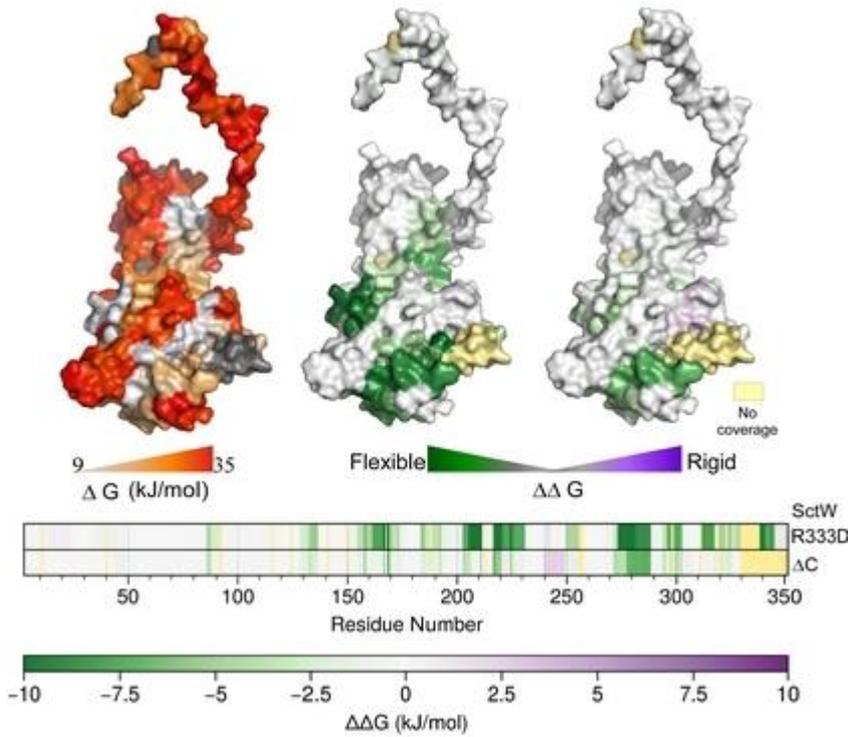
*Spyridoula Karamanou, KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research,*

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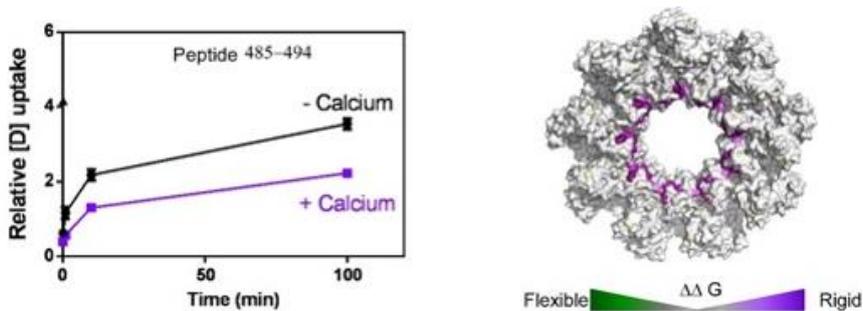
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Thomas C. Marlovits, Centre for Structural Systems Biology (CSSB), University Medical Center Hamburg-Eppendorf (UKE), Institute for Structural and Systems Biology, German Electron Synchrotron Centre (DESY)

Anastassios Economou, KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Molecular Bacteriology



Effect of C-domain mutants of SctW studied by HDX-MS



Effect of Calcium on SctV-C domain studied by HDX-MS

Poster number: LS-PB-131

## PROBING SIZE AND SHAPE HETEROGENEITY IN AAVS USING ION MOBILITY MASS SPECTROMETRY

Abstract ID: 427

**Presenting author: Ellen Liggett, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology, University of Manchester, UK**

### Introduction

AAVs are actively investigated viral vectors for gene therapy applications. They have low immunogenicity and do not transfer disease to the human host. Development of a robust characterisation method that quantifies the therapeutic gene content of the capsids is important for biopharmaceutical companies to get approval from medicine agencies. Differentially filled capsid species can be present in a downstream processing sample, adding to the complexity of the analysis. Additionally, the random assembly of capsid proteins into the mature capsid adds further heterogeneity. We present here characterisation of AAV species with both linear field DTIM and TWIM separators coupled ToF-MS. We suggest these as alternative methods for structural analysis and determining the empty:full ratio of viral capsids, and discuss structural variability in the mature capsids.

### Methods

The methodology includes buffer exchanging AAV samples, placing in fused silica tips applying a spray potential via inserted platinum wire and spraying into an ion mobility mass spectrometer. Ion transport in a linear field Drift Tube Ion Mobility separation is dominated by mobility and allows direct determination of Collision Cross Section (CCS). In contrast to DTIM, Travelling Wave Ion Mobility induced ion transport depends on both mobility and  $m/z$ . As such, TW parameters can be tailored to yield separation dominated by mobility or  $m/z$ .

### Preliminary data (results)

The observed Arrival Time Distribution (ATD) of the empty and full AAVs are not fully resolved, indicating similar CCS values. The ATD profile of the empty species (Figure 1A, red trace) is broader than the full species, suggesting many overlapping conformers, which may interconvert akin to a "breathing" motion. Interestingly, the full AAV species does not display this variation and exhibits slightly longer drift time, suggesting more rigid, "swollen" particles, perhaps due to the incorporated genetic cargo (Figure 1A, blue trace).

Since empty and full AAV capsids carry a similar average charge and vary in their mass due to being unloaded or loaded with DNA, they exhibit different  $m/z$  values and can be separated completely by a TWIM device (Figure 1B) and also ToF-MS.

The implications of these measurements to elucidate the differential states of manufactured AAVs will be discussed and instrumental operating characteristics expanded upon.

### Please explain why your abstract is innovative for mass spectrometry?

Investigation and separation of large megadalton viral species using Ion Mobility coupled to Mass Spectrometry

### Co-authors:

*Jakub Ujma, Waters Corporation*

*Keith Richardson, Waters Corporation*

*David Langridge, Waters Corporation*

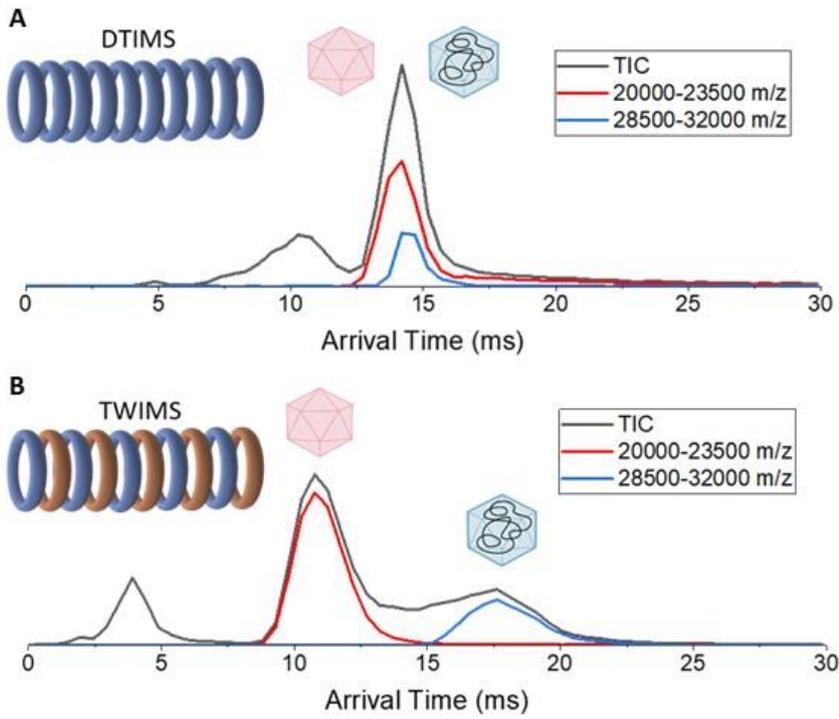
*Ian Anderson, Pharmaron*

*Kevin Giles, Waters Corporation*

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POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



DTIM vs TWIM separation of empty/full AAV capsids

Poster number: **LS-PB-132**

## **STUDY OF THE CAS9:SGRNA COMPLEX INTERACTION WITH TARGET DNA BY HDX MS**

Abstract ID: 441

**Presenting author: Alexander Chernonosov, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia**

### **Introduction**

The CRISPR-Cas9 system is widely used for genome editing, consisting only of Cas9 protein and single-stranded RNA guide (sgRNA).

However, these systems still suffer a systematic drawback: it cuts incompletely complementary DNA. The commonly used sgRNA/Cas9-based genome editing tool tolerates up to 6 mismatches within the sgRNA complementary region of tDNA, and Cas9 also cleaves dsDNA at sites in the absence of the extract 5'-NGG-3' PAM sequence in dsDNA.

Therefore, the elucidation of the mechanism of selectivity and specificity of cutting DNA with this protein is necessary to improve the accuracy of genomic editing using the CRISPR-Cas9 system. One of the analytical tools capable of detecting protein-ligand and protein-protein interactions is HDX-MS.

### **Methods**

In the current work, we studied the interaction of the Cas9:sgRNA complex with target DNA by the HDX-MS technique. Aliquots were taken at different time points after the result Cas9:sgRNA:DNA complex formation, samples were frozen in liquid nitrogen and stored at -70 °C up to analysis.

Online peptide digesting was performed on the immobilized pepsin column, follows by separation on the analytical column, and analysis using the HF QExactive mass spectrometer. Data analysis was carry out in the Proteome Discoverer v 2.2 and HDEaminer v. 3.0.3 software.

### **Preliminary data (results)**

Based on the results of the structural analysis performed by HDX-MS and molecular modelling, a mechanistic mechanism for the interaction of Cas9 endonuclease with sgRNA and DNA substrate has been proposed. The data obtained indicate a significant change in the conformation of the RuvC III, REC3, HNH, and CTD domains, linker sequences Arg and L-II upon binding of Cas9 to RNA and DNA. At the same time, the REC1-2 and RuvC II domains are more stable.

Comparison of the model structures and the nature of the exchange of amide protons in the sequence of the free protein and its complexes with sgRNA and DNA substrate made it possible to identify the regions involved in the interaction with the nucleic acid. In particular, peptides of the nuclease subdomains RuvC II and RuvC III, when passing from the free to the bound form of the enzyme, to a greater or lesser extent increase the availability of their amide protons for exchange. Most likely, the conformation of these domains changes significantly upon binding to sgRNA. At the same time, the structure becomes more "loose," and amide protons are more accessible to the solvent.

The work was supported by a Russian government-funded project for ICBFM SB RAS (20-14-00214).

### **Please explain why your abstract is innovative for mass spectrometry?**

The HDX-MS method allows studying structural changes in the Cas9 complex when interacting with DNA.

### **Co-authors:**

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Grigory Stepanov, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia

Vladimir Koval, Institute of Chemical Biology and Fundamental Medicine SB RAS, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia, Novosibirsk State University, Pirogova Str. 2, Novosibirsk, 630090, Russia

Poster number: **LS-PB-133**

## EPITOPE MAPPING BY LIMITED PROTEOLYSIS MASS SPECTROMETRY

Abstract ID: **451**

Presenting author: **Paul Boersema, Byondis B.V.**

### Introduction

Epitope mapping provides valuable information for an antibody in development and may help to elucidate its mode of action. High resolution epitope mapping can be done by X-ray crystallography or NMR, but these techniques come with the drawback of the need of large amounts of highly pure proteins and difficulties of generating crystals. H/D exchange mass spectrometry (HDX MS) has been successfully used to map binding sites. However, HDX MS requires specialized equipment to keep the HPLC at low temperature and to perform digestion at low pH. Here, we explored the use of limited proteolysis mass spectrometry (LiP-MS) to map binding sites between an antibody and its antigen.

### Methods

Purified antigen was added to an antibody in solution. LiP-MS was performed using various proteases (such as Proteinase K and trypsin) either on the antibody-antigen complex in solution or immobilized on Protein A-beads. The extent of proteolysis was then monitored by intact- and peptide mapping MS using high resolution MS instrumentation. The eventual epitope mapping was then achieved by comparing the LiP-patterns in peptide mapping of the antigen with and without the antibody.

### Preliminary data (results)

LiP-MS is based on the notion that under limiting conditions (low protease concentration, short incubation time) the sites of proteolysis are dictated by protease accessibility. This means that conformational changes of a protein will lead to different cleavage products. Binding to another protein will also locally limit protease accessibility. Drawing on this concept, we set out to map the binding sites of several antibodies in development on their respective antigens. We optimized the LiP-MS approach to achieve conditions in which protease cleavage is frequent but still solely dictated by structure and accessibility. We evaluated several proteases and digestion conditions, including double digestions and the use of glycosidases. We took advantage of the relative protease-resistance of the antibodies to focus the LC-MS analysis on the antigen. Also, the Fc tail of the antibodies provided a handle to immobilize the antibody-antigen complex. This allowed the separation of peptides that are part of the binding sites from peptides that are elsewhere in the protein structure.

The resulting epitope maps were matched with those obtained by HDX MS and showed remarkable overlap. Although HDX MS achieves a higher resolution, LiP-MS proved a technique that is more easily implemented and that does not require specific equipment. Furthermore, removal of the limit in choice of proteases gave the freedom to match the optimal protease with the protein of interest and to perform additional manipulations such as deglycosylation.

### Please explain why your abstract is innovative for mass spectrometry?

LiP-MS epitope mapping proved a versatile LC-MS approach that overcomes some of the limitations of HDX MS

### Co-authors:

*Lin Rietveld, Byondis B.V.*

*Benno Ingelse, Byondis B.V.*

Poster number: **LS-PB-134**

## **AGGREGATION OF HUMAN ISLET AMYLOID POLYPEPTIDE (HIAPP): AN INVESTIGATION INTO THE EFFECT OF METAL BINDING ON EARLY-STAGE OLIGOMERISATION**

Abstract ID: **486**

**Presenting author: Francesca O. Bellingeri, University of Warwick**

### **Introduction**

Aggregation of amyloid proteins within the body can act as biological disease markers, efforts to understand this process have yielded a variety of results. In the case of the human islet amyloid polypeptide, known as amylin, aggregation is indicative of type-2 diabetes, a risk factor in development of Alzheimer's disease and associated cognitive decline. Whilst this irregular oligomerisation process is not yet fully understood, analysis of the amyloid aggregate component of Alzheimer's brain tissue has revealed accompanying elevated levels of iron.

In order to understand how the presence of metal ions may stimulate protein misfolding and aggregation, we used a combination of TOF-MS quantification and TIMS-MS to monitor early-stage oligomerisation of hIAPP in the presence of several physiologically relevant metals.

### **Methods**

To monitor aggregation rates of hIAPP in the presence of metal ions, experiments using TOF-MS and TIMS-TOF-MS were designed. hIAPP was subject to incubation with a series of physiologically relevant metal ions, sampled at regular intervals and delivered into instruments natively using nano-electrospray ionisation. Quantification of monomer and dimer peaks was performed on TOF-MS spectra, with ratio change between the two used to track changes in aggregation rate.

TIMS-MS was implemented to resolve overlapping monomer, dimer and trimer peaks within the spectra, allowing accurate quantification values to be obtained and a number of different conformers identified and compared across samples.

### **Preliminary data (results)**

Data gathered during this study revealed several interesting features of the hIAPP aggregation and metal binding process.

Current theories suggest that amyloid protein aggregation proceeds through conversion of dimers to other early-stage oligomers; our hIAPP data supported this theory with amount of dimer decreasing at a faster rate than monomer, showing that monomer to dimer conversion is the rate limiting step and that incubation had triggered this aggregation process.

By monitoring the ratio between monomer:dimer using quantification, we were able to create a reproduceable method of tracking aggregation rate across different samples and observe unique values and corresponding species in each case. For example, in the case of copper, distinct species associated with binding were observed for both monomer and dimer throughout the duration of incubation, with an apparent retardation in aggregation rate. In contrast, cobalt displayed initial, but short-lived, binding to the monomer with little effect on aggregation rate. Ferrous iron displayed long-lived binding to the monomer only, however ferric iron was not observed at any stage of incubation.

TIMS-MS was optimised for the most abundant spectral signal, corresponding to overlapping 3+ monomer, 6+ dimer and 9+ trimer distributions. Under standard TOF-MS, the 9+ dimer was unable to be observed, however using TIMS, we were able to clearly separate the trimer from other overlapping peaks. Multiple conformations of the monomer and trimer were observed and separated as well, with consistent distributions achieved across the mass range observed, allowing for accurate quantification values to be obtained.

**Please explain why your abstract is innovative for mass spectrometry?**

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Separation and quantification of early-stage aggregates of the hIAPP protein using TIMS-TOF-MS and TOF-MS in the presence of metal ions allowed for discovery of novel aggregation measurements and metal impacts.

**Co-authors:**

*Meng Li, University of Warwick*  
*Yuko P. Y. Lam, University of Warwick*  
*Christopher A. Wootton, University of Warwick*  
*Mark P. Barrow, University of Warwick*  
*Joanna F. Collingwood, University of Warwick*  
*Peter B. O'Connor, University of Warwick*

Poster number: **LS-PB-135**

## AG INFLUENCE ON SILE, AN INTRINSICALLY DISORDERED PROTEIN

Abstract ID: **526****Presenting author: Clothilde Comby-Zerbino, Univ Lyon, Univ Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F-69622, VILLEURBANNE, France**

### Introduction

The antimicrobial properties of silver have been used for centuries. Its mode of action and toxicity to bacteria remain unknown. To counteract its toxic effect, efflux pumps exist to eject the metal from the cell. The Sil system, as it is currently thought to function, includes an ATPase (SilP) transporting silver from the cytoplasm to the periplasm, a periplasmic efflux pump complex (SilA,B,C), two periplasmic proteins (SilE,F) and a complex activating the production of other proteins in the presence of silver (SilR,S). In this system, SilE is particularly interesting since it is only produced in the presence of silver. However, its structure, function and interactions with the partners of the efflux system remain unknown to date [1].

### Methods

SilE solutions with gradients of silver concentration (0-12 equivalents) were prepared for analysis. NMR and Tandem Ion mobility (IM)-MS were combined to understand the conformational changes of SilE in the presence of Ag. In addition, the number of coordination sites (strong and weak) of silver ions (Ag<sup>+</sup>) in the SilE protein has been investigated by CE-ICP-MS and HRMS measurements. LC-HRMS after digestion of the protein are carried out to tentatively locate the silver binding sites.

### Preliminary data (results)

MS data show that SilE/Ag<sup>+</sup> adducts of different stoichiometries are present. The average number of Ag<sup>+</sup> bound to the protein increases globally with the relative silver concentration. The maximum observed number of Ag bound to SilE is 14. IMS measurements on these different adducts highlight two families of structures, both already observed for the protein alone. However, the association with silver seems to promote the more compact structure, as confirmed by NMR titration measurements. In addition, ICP-MS data indicates that only part of these SilE/Ag<sup>+</sup> complexes correspond to strong complexation. This is confirmed by HRMS measurements. IMS data shows that this binding is accompanied by a global compaction of the protein structure. Beyond this strong complexation, IMS data show that SilE can bind more Ag<sup>+</sup>, which is potentially the signature of different complexation sites, but may also result from non-specific complexes formed in the ESI source.

[1] Babel, L. *et al.*, NMR reveals the interplay between SilE and SilB model peptides in the context of silver resistance. *Chemical Communications* **2021**.

### Please explain why your abstract is innovative for mass spectrometry?

Combined ICP-MS, LC-HRMS, IMS-MS and NMR data strongly suggest that SilE modifies its conformation upon Ag<sup>+</sup> binding and open the way to the localization of Ag<sup>+</sup> binding sites.

### Co-authors:

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Poster number: **LS-PB-136**

## **MOLECULAR MASS DETERMINATION OF THE INTACT RIBOSOME-INACTIVATING PROTEINS BY LC-MS**

Abstract ID: **585**

**Presenting author: Anne Puustinen, VERIFIN, Department of Chemistry, University of Helsinki**

### **Introduction**

Ricin and abrin are highly poisonous plant toxins belonging to the type 2 ribosome-inactivating protein (RIP-II) family. These 60-68 kDa glycoproteins are composed of A and B - chains linked by a single disulfide bond. The European programme for establishment of validated procedures for the detection and identification of biological toxins (EuroBioTox, <https://eurobiotox.eu>) has produced ricin and abrin certified reference materials (CRMs) to enhance their detection from unknown samples. Characterization of these CRMs included molecular mass determination of the intact protein by liquid chromatography (LC) – mass spectrometry (MS).

### **Methods**

Buffer solution of CRMs was exchanged by MWCO-Ultrafiltration (0.5 mL Centrifugal Filter with a 30 kDa MWCO membrane (Millipore)) to water or to 10 mM ammonium acetate, pH 6.5.

LC-MS measurements were done with nanoLC (EASYnLC 1200) connected to Fusion Orbitrap (Thermo) with nanoHESI ion source. Both linear ion trap (LIT) and Orbitrap (OT) mass analysers were tested for data collection. Applied column was nanoEase M/ZProtein BEH C4 (Waters) with gradient from 30% to 80% of acetonitrile in ten minutes.

Mass deconvolution of charge states was performed either with ProMass (Novatia) or with Byos/Byonic (Protein Metrics).

### **Preliminary data (results)**

Direct infusion of analytes to MS was not preferred due to the high toxicity of the studied proteins. Instead nanoLC with protein C4 column was applied for data collection. NanoLCMS method testing was performed with 66kD BSA using 35 µg of protein per injection. It turned out that a low pressure mode was superior to standard pressure methods.

Charge state deconvolution of ricin CRM was successful both with LIT and OT analyzer data with both tested softwares. The most intensive LIT molecular masses were at 62956 and 62697 daltons, whereas for OT there were more equally intense mass peaks (62956, 62796 and 63043). The observed mass differences of 160 dalton among the deconvoluted mass peaks could arise from the loss of one sugar moiety from the glycan structure.

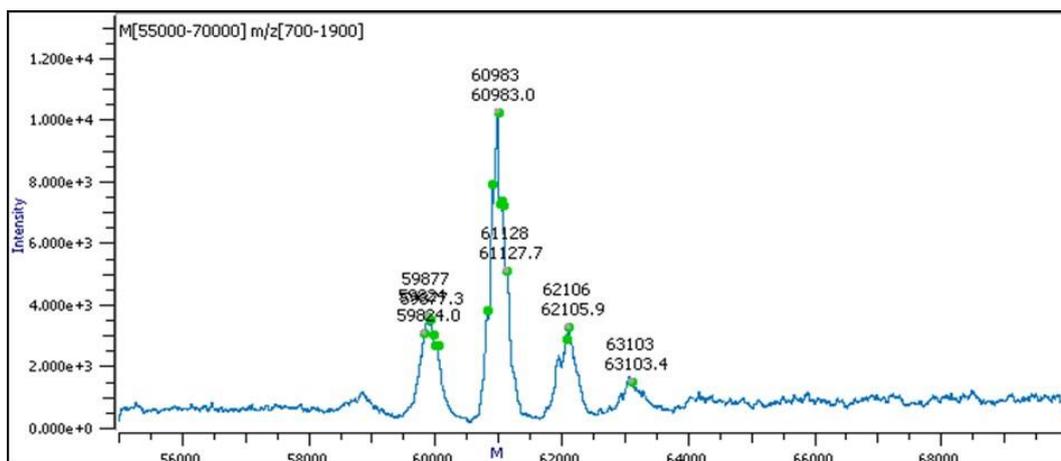
Abrin raw data was difficult to deconvolute as the sample contained four different proteins (A-D gene products) with several glycosylation sites. Deconvolution of the charge states worked out only from the raw data collected with LIT using intact protein workflow in Byos software. Four main molecular mass peaks at 59877, 60983, 62106 and 63102 Da (Figure 1) were calculated for the four glycosylated abrin proteoforms. Of these protein masses, 60983 Da was the most abundant mass with the occupancy of 44 %. The loss of one glycan sugar in the shoulder peaks is also observed here as in the ricin intact protein analysis.

### **Please explain why your abstract is innovative for mass spectrometry?**

Both OT and LIT mass analysers could be applied for molecular mass determination of ~65 kD intact proteins using nanoLC with low pressure mode of mass spectrometry.

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Deconvoluted intact protein molecular masses of abrin

Poster number: **LS-PB-137**

## **INVESTIGATION OF THE RIBONUCLEOCOMPLEX OF THE LASSA VIRUS BY NATIVE MS**

Abstract ID: **641**

**Presenting author: Lennart Sanger, Centre for Structural Systems Biology, Leibniz Institute of Experimental Virology, Bernard Nocht Institute for Tropical Medicine**

### **Introduction**

Arenaviruses are of high relevance for public health emphasized by periodic outbreaks of Lassa fever in West Africa with over 100.000 cases each year. At this point there are no antiviral drugs or vaccines available. The polymerase complex of the Lassa virus is not understood in sufficient mechanistic detail. Potential drug development requires more knowledge about how the polymerase complex is regulated and which binding partners are needed for the function. The Lassa virus consists of only four proteins that carry out the different functions of the virus: The RNA-dependent RNA polymerase (L) with ~250 kDa and a small RING finger protein Z with 11 kDa, as well as the viral glycoprotein (GPC) with 55 kDa and the nucleoprotein (NP) with 63 kDa.

### **Methods**

To gain insight into the Lassa replication machinery, the 3 proteins L, NP and Z are subjected to native mass spectrometry (native MS) revealing protein interactions, stoichiometry, and conformational changes upon complex formation. Furthermore, conformational changes upon RNA binding are observed in real time with native MS. Conformational changes upon complexation and binding interfaces are mapped by hydrogen deuterium exchange mass spectrometry (HDX-MS).

### **Preliminary data (results)**

L, NP and Z are recombinantly produced in *E.coli* (NP, Z) or insect cells (L) using established protocols. The suitability of the proteins for native MS was tested and purification strategies were optimized for this purpose. The 3 proteins were mixed in different combination to understand their complexation. NP and Z form complexes as well as NP and RNA. It was possible to map the interaction site of the NP-Z complex with HDX-MS. In conjunction with AlphaFold predictions, potential residues of the interaction were identified, and mutants created to prove their relevance to binding.

### **Please explain why your abstract is innovative for mass spectrometry?**

In an integrative approach, we combined structural mass spectrometry methods and structure prediction tools.

### **Co-authors:**

*Maria Rosenthal, Bernard Nocht Institute for Tropical Medicine*  
*Charlotte Uetrecht, Centre for Structural Systems Biology, University of Siegen*

Poster number: **LS-PB-139**

## **GLYCOPROTEIN CHARACTERIZATION COMBINING NATIVE AND CHARGE DETECTION MASS SPECTROMETRY.**

Abstract ID: **715**

**Presenting author: Claire Daully, Thermo Fisher Scientific**

### **Introduction**

Glycoproteins is gaining attention as they are actively involved in physiological functions as well as progression of certain diseases. However, glycoprotein characterization using intact and top-down mass spectrometry remains challenging as its heterogeneity leading to complex spectrum. Human Fetuin A. is a heterodimeric protein composed of A chain and B chain connected by an interchain disulfide bond. Heterogeneity of intact Fetuin results from its two N-glycosylation sites, seven O-glycosylation sites, and seven phosphorylation sites. Additionally, six disulfide bonds and sequence variants further contribute to the hurdle of top-down analysis. In this study, we evaluated a few novel techniques including individual ion mass spectrometry (I2MS) and proton transfer charge reduction (PTCR) to unravel Fetuin proteoforms comprehensively under native condition.

### **Methods**

Human Fetuin A (~40 kDa) was buffer exchanged into ammonium acetate with Amicon 10K-MWCO or 30K-MWCO. Native MS and I2MS were performed by direct infusion with Nanoflex ion source coupled to Thermo Scientific™ Q Exactive™ UHMR. PTCR and EThcD analyses were performed on Thermo Scientific™ Orbitrap Eclipse™. I2MS data were collected at 200K resolution by setting trapping gas pressure at 1 and injection time below 20ms. EThcD fragmentation was used to confirm sequence, determine glycosylation, and chain linkage. Data were analyzed using Thermo Scientific™ BioPharma Finder™ Software and I2MS processing software (Proteinaceous).

### **Preliminary data (results)**

To confirm sample identity and assess impurity present, we performed native top-down analysis of Fetuin. First, EThcD fragments from Fetuin prove the absence of signal peptide at N-terminus. Second, EThcD fragments demonstrate the B-chain is disulfide bonded to the N-terminal of A-chain through Cys32-cys358. After disulfide bond reduction with DTT to release the B-chain, top-down analyses could provide 95% sequence and identify partially O-glycosylated site at Ser346.

Initial native MS analysis of Fetuin shows a compact charge envelope across m/z 3000- 4000 from charge state +14 to +11. Numerous peaks crowded together reflect the sample complexity and heterogeneity. PTCR could reduce the charge state to +6 and extend the charge envelope to m/z 7500. By increasing the resolution to 200K, glycoforms can be isotopically resolved and deconvoluted. Accurate monoisotopic mass unambiguously discloses different numbers of phosphorylation, HexNAc, Hex, and Fucose on each glycoform. PTCR with 20 Th isolation width separates proteoforms which were previously overlapped in the full scan and increased further number of identified glycoforms.

Recently, I2MS emerged as another technique with high potential for heterogenous species characterization. In the next series of experiments, we used I2MS to analyze Fetuin. Interestingly, Fetuin cleaned by 30K-MWCO indicate main glycoforms distributed from 38 kDa to 44 kDa while the one cleaned by 10K-MWCO is from 42 kDa to 46 kDa. I2MS analysis, without any charge reduction, was extremely sensitive and can detect all forms in each preparation vs native MS analysis.

### **Please explain why your abstract is innovative for mass spectrometry?**

Multidimensional and complementary mass spectrometry analyses for intact and top-down glycoprotein characterization. By combining different MS methods such as I2MS, PTCR and EThcD, we could successfully characterize heavily glycosylated Fetuin.

### **Co-authors:**

*Weijing Liu, Thermo Fisher Scientific*  
*Ping Yip, Thermo Fisher Scientific*  
*Rosa Viner, Thermo Fisher Scientific*

Poster number: LS-PB-140

## RESOLVING DETAILS OF GROEL-ES COMPLEXES USING VARIABLE TEMPERATURE NATIVE MASS SPECTROMETRY

Abstract ID: 763

Presenting author: He Sun, Texas A&amp;M University

### Introduction

The GroEL-ES system is required for the survival of *E. coli*. by assisting the folding of many essential proteins. Though the structure and dynamics of the GroEL-ES complexes have been studied using cryo-EM, fluorescence, and many other traditional analytical methods, native mass spectrometry is able to provide information of individual species instead of the average signal of all species in the solution. Here, we report results obtained using the variable temperature nano-ESI source on the native MS that clearly display the dependence on solution conditions for the formation of the controversial bullet-shaped (BS) and football-shaped (FS) GroEL-ES complexes. We also observed distinctive adduct peaks of GroEL, which were assigned to be the hydration layer water molecules in the GroEL substrate-folding cavity.

### Methods

A vT-nESI source was used to control solution temperature in static-nESI emitter capillaries and the mass spectra were collected on an ultra-high mass range hybrid quadrupole orbitrap mass spectrometer. Unidec and Protein Metrics deconvolution programs were used to assign the charge states, mass, and abundance of each complex. Samples of GroEL-ES complexes were prepared in ethylenediamine diacetate (EDDA) solution to eliminate the ammonium adducts and lower the charge state of the complexes. The concentrations of Mg(OAc)<sub>2</sub> and ATP were varied to generate different GroEL-ES species. GroEL samples were prepared and examined in both EDDA and ammonium acetate.

### Preliminary data (results)

During our mass spectrometry analysis, the noncovalent interactions between and within GroEL and GroES subunits are largely preserved, and the charge state distribution indicates the detection of native GroEL-ES complexes. Our results showed that Mg and ATP are required for the formation of GroEL-GroES complexes, and their concentrations dominate the GroEL:GroES stoichiometry. In addition to the well-known BS and FS complexes, the binding of monomeric GroES subunits to GroEL was also observed at lower Mg-ATP concentrations, especially at temperatures below 10 °C and above 40 °C. At higher gas-phase activation energy, we also observed partial dissociation of the GroES cap and a lower charge state distribution of the BS complex. Due to the dynamic range of the Mg-ATP physiological concentration, it is hypothesized that both complexes are functional forms of the chaperonin system and *E. coli*. utilizes the BS complex at lower Mg-ATP concentrations and FS at higher concentrations. However, our data also suggest that the FS GroEL-ES is more rigid at low and high solution temperatures, indicating a higher energy barrier for releasing the GroES to finish the folding cycle. The adduct peaks we observed in the GroEL spectra correspond to the mass of the solvation layer of ~440 water molecules in the GroEL cavity, which is the first straightforward observation of the confined water layer in GroEL. The existence of confined cavity water will further strengthen the theoretical arguments of water-mediated effects in the substrate folding process of this chaperonin system.

### Please explain why your abstract is innovative for mass spectrometry?

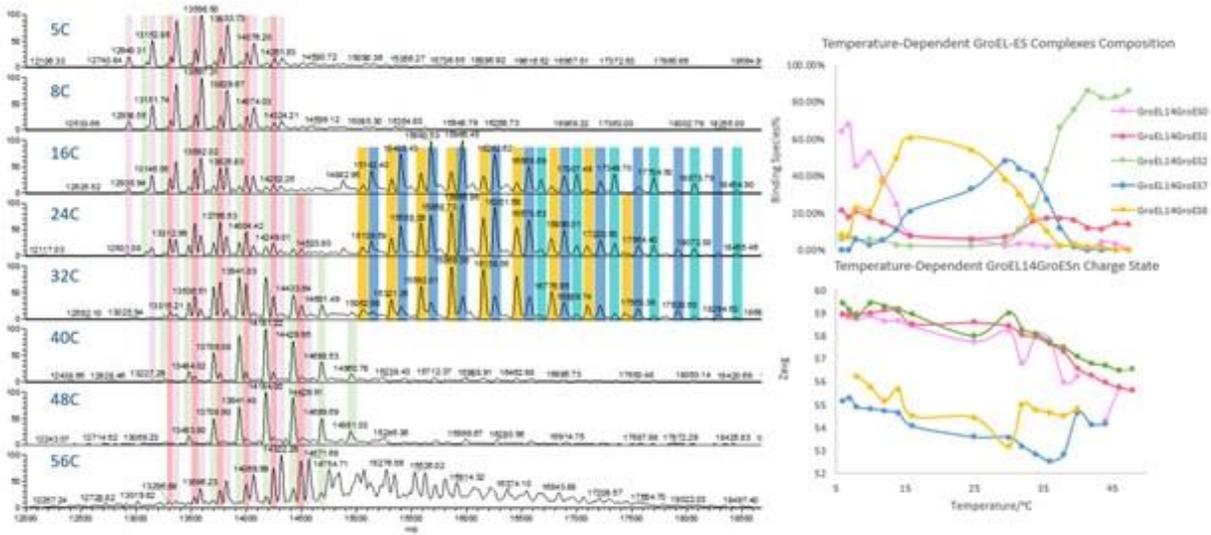
This study utilizes native vT-nESI MS to study the thermal stability of GroEL-ES complexes to further clarify their functioning mechanism.

### Co-authors:

Thomas Walker, Texas A&M University  
David Russell, Texas A&M University

POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



The variable-temperature mass spectra of the GroEL-GroES system.

Poster number: LS-PB-141

## NATIVE MASS SPECTROMETRY TOWARDS BIOFLUID SCREENING OF THE EFFECTS OF COVALENT PROTEIN MODIFICATIONS DUE TO ENVIRONMENTAL EXPOSURE

Abstract ID: 826

**Presenting author: Sara Jamshidi, Department of Material and Environmental chemistry, Stockholm University**

### Introduction

Reactions of electrophilic compounds that exist in the environment with nucleophilic sites of proteins and nucleic acids are ubiquitous and have given rise to the field of adductomics and the concept of exposomics. However, it is unknown how different adducts co-exist in native proteins or how they may perturb tertiary or quaternary structures. Native MS in combination with ion mobility mass spectrometry (IMS) has proven its ability to characterize a variety of protein-ligand interactions and their stoichiometries as well as discern structural information.

Harnessing these powers, we used native IM-MS to study the stability and conformational changes of hemoglobin (Hb) in human erythrocytes upon exposure to skin (contact) allergens.

### Methods

Hb was analysed in native IM-MS directly from human blood after separating the plasma from the erythrocytes. Generation of Hb adducts was performed by reaction of Hb with the skin allergens 1-Chloro-2, 4-dinitrobenzene (DNCB), 1, 2 epoxy-3-phenoxy-propane (PGE), and 2-methylenepentanedinitrile (2-MGN). After the reaction, the samples were subjected to buffer exchange to ammonium acetate followed by analysis on a Waters Synapt G2 instrument equipped with a Nano ESI source. The structure (oligomeric state) and stability (ability to withstand CIU) of Hb were studied with and without modifications by skin allergens.

### Preliminary data (results)

Hb consists of two  $\alpha$  and two  $\beta$  globin chains and could be detected in the folded native state when sprayed directly from the buffer exchanged erythrocyte samples. Under native conditions, a population of dimers and tetramers were detected, indicating that the solution state equilibrium for the protein could be monitored. By applying MS<sup>2</sup>, additional peaks were assigned to dissociated monomers ( $\alpha/\beta$ ) corresponding to adduction of the  $\beta$  chain for DNCB and adduction on both  $\alpha$  and  $\beta$  chains for 2MGN and PGE. The structure and stability of the oligomeric state of the Hb under collision-induced unfolding were studied with and without hapten modifications. DNCB and PGE were found to destabilize the protein fold, while 2MGN had a stabilizing effect. IMS drift scope analysis reveals that the tetramers and dimers of Hb adducted with DNCB, and PGE undergo gas-phase unfolding during dissociation thus causing longer drift time. However, the tetrameric state of Hb/2-MGN shows a more stable structure upon collisional activation, and no significant unfolding was observed. Our finding correlates with what we know concerning the in vivo potency of the studied contact allergens, as the murine *local lymph node assay* classifies DNCB as an *extreme*, PGE as a *strong* and 2-MGN as a *moderate* contact allergen. This is the first study to bridge the field of adductomics with native mass spectrometry showing the influence of adducts on native conformation stability and interactions. Probing biofluids with minimal sample preparation allows for facile screening for environmental exposure.

### Please explain why your abstract is innovative for mass spectrometry?

Screening environmental exposure using Native IM-MS as a tool by monitoring stability perturbations in body fluid marker proteins while their original native structure and inter/intramolecular interactions are preserved.

### Co-authors:

Nicklas Österlund, Department of Biochemistry and Biophysics, Stockholm University  
Isabella Karlsson, Department of Environmental Science, Stockholm University  
Leopold L Ilag, Department of Materials and Environmental Chemistry, Stockholm University

Poster number: LS-PB-142

## DISTINCT BINDING SELECTIVITY IN STRUCTURALLY SIMILAR BCL-2 FAMILY PROTEINS IS DRIVEN BY INNATE DYNAMIC MOTIFS

Abstract ID: 921

Presenting author: Esther Wolf, York University

### Introduction

What molecular mechanisms underly selectivity in protein-protein interactions? Our aim was to use short-timescale HDX-MS to explore anti-apoptotic proteins Bcl-2 and Mcl-1 to better understand their distinct abilities to accommodate various pro-apoptotic BH3 peptides in their binding pockets. Particularly, both Mcl-1 and Bcl-2 can bind representative BH3 peptides of Bid and Bim; however, Mcl-1 can interact with Noxa, and Bcl-2 can bind Bad, but not vice versa. These protein pathways are often implicated in various cancers by enabling cells to evade death while also providing resistance to cytotoxic chemotherapy.

### Methods

Short-timescale hydrogen-deuterium exchange mass spectrometry was performed using a time-resolved kinetic mixer. This kinetic mixer, colloquially termed "TRESI", is composed of a set of concentric capillaries which shrink down the HDX reaction into a micro-sized space and enable millisecond-second reaction monitoring. The HDX reaction is subsequently quenched and pepsin digested on-line prior to electrospray ionization and MS detection using a Waters Synapt G2. Peptide HDX analysis was done using Mass Spec Studio.

### Preliminary data (results)

With a sequence coverage of 92% and 94% for Bcl-2 and Mcl-1 respectively, short-timescale HDX was conducted at 1, 2, 4, and 18 seconds. We discovered that Bcl-2 and Mcl-1 displayed innate changes in dynamics which facilitated the binding groove accommodation regardless of the BH3 peptide bound. Mcl-1 consistently underwent a broadly distributed deuterium uptake decreases across the entire binding pocket, whereas Bcl-2 consistently showed a localized decrease around a conserved salt bridge. With homology modeling, it was revealed that Bcl-2 relies on a greater number of intermolecular bonds compared to Mcl-1. As such we propose that the molecular mechanism of binding for Mcl-1 relies heavily on conformational changes whereas Bcl-2 uses charge compensation. These data provide fundamental insight on protein function as well as additional considerations to be made in the development of anti-cancer drugs.

### Please explain why your abstract is innovative for mass spectrometry?

Protein binding is often explained using a traditional understanding of static structure; however, HDX-MS reveals that conformational dynamics are the molecular mechanism of action for the Bcl-2 proteins.

### Co-authors:

*Cristina Lento, York University*  
*Bryan Dickinson, The University of Chicago*  
*Jinyue Pu, The University of Chicago*  
*Derek J Wilson, York University*

Poster number: LS-PB-143

## INVESTIGATION OF NATIVE LESA MS AS A POTENTIAL METHOD FOR IN SITU DETECTION AND CHARACTERISATION OF ANTIBODY-DRUG CONJUGATES

Abstract ID: 939

Presenting author: Peter Macey, School of Biosciences, University of Birmingham

### Introduction

Mass spectrometry imaging (MSI) plays a key role in drug discovery, typically through the spatial profiling of small molecule drugs. In previous work, we have shown that liquid extraction surface analysis (LESA) MS may be applied for MSI of folded proteins and their complexes, i.e., native ambient MSI. We therefore hypothesised that native LESA MS may be suitable for the analysis of antibody-drug conjugates (ADCs) in tissue. Here, we investigate the native LESA MS of an ADC and its payload. Initial experiments considered the ADC dried onto a glass substrate. In subsequent experiments, the ADC was deposited onto thin tissue sections of rat liver, providing a proxy for dosed tissue.

### Methods

This work aims to determine if native LESA MS is suitable for analysis, and ultimately imaging, of both an ADC and its payload in tissue, thereby determining if the drug is delivered to target regions, e.g., tumour cells. These experiments investigated the ADC and payload deposited onto glass substrates or liver tissue. The compounds were buffer exchanged into ammonium acetate (150 mM) to yield 11  $\mu\text{M}$  solutions. Samples (1  $\mu\text{L}$ ) were dried onto glass or liver and sampled by native LESA MS by use of a Triversa Nanomate (Advion) coupled to an Orbitrap Eclipse™ Tribrid™ MS.

### Preliminary data (results)

Samples comprising 1  $\mu\text{L}$  of either analyte (11  $\mu\text{M}$ ) were dispensed onto a glass substrate and allowed to dry for 30 mins at room temperature. The dried samples were sampled by native LESA MS. Results showed that native LESA MS is suitable for the analysis of the intact ADC and its payload.

Subsequently, a solution containing both ADC (11  $\mu\text{M}$ ) and payload (11  $\mu\text{M}$ ) was prepared. The sample was directly electrosprayed into the MS. Data were acquired in four modes: high m/z range (1000-8000 m/z), low m/z range (400-2000 m/z), Single Ion Monitoring Mode (SIM), and HCD MS<sup>2</sup>. This work demonstrated that both can be detected in a single analysis.

In later work, 1  $\mu\text{L}$  each of ADC (11  $\mu\text{M}$ ) and payload (11  $\mu\text{M}$ ) was deposited onto a 10  $\mu\text{m}$  section of rat liver and allowed to dry for 30 min at room temperature. The dried sample was analysed by native LESA MS using the MS method described above. The presence of both compounds was detected in the spectra.

These results show it is possible to detect both the ADC and its small molecule payload in a single MS analysis following LESA extraction of a sample mixture deposited onto tissue.

### Please explain why your abstract is innovative for mass spectrometry?

Native LESA MS as a method for detection and characterisation of antibody-drug conjugates.

### Co-authors:

*Iain Styles, School of Computer Sciences, University of Birmingham*  
*Helen Cooper, School of Biosciences, University of Birmingham*

Poster number: **LS-PB-144**

## CHEMICAL OXIDATION OF P53 MONITORED BY MASS SPECTROMETRY

Abstract ID: **951****Presenting author: Manuel Peris Diaz, Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology**

### Introduction

The transcription factor p53 must be exquisitely sensitive and selective to a broad variety of cellular environments. Oxidative stress weakens the p53-DNA binding affinity for certain promoters, revealing the selective redox regulation of cellular functions. Ultraviolet radiation experiments demonstrated that although p53 dissociates from *Gadd45*, the binding of p53 remains intact on the *p21* promoter. Other studies revealed that in the absence of DNA, H<sub>2</sub>O<sub>2</sub>-mediated oxidation of p53 leads to Zn(II) dissociation and disulfide formation. However, the precise mechanisms by which p53 senses cellular stresses in the physiologically relevant DNA-p53 tetramer complex are still unknown. Here, we employed native MS and IM-MS coupled to chemical labelling and chemical oxidation to examine the molecular mechanism of redox regulation of p53 with *p21*.

### Methods

Recombinant wild-type human p53 DNA binding domain and the R248Q mutant were overexpressed in *E. coli* and purified by chromatography. Doubled stranded DNA was constructed by annealing DNA fragments purchased commercially. All MS and IM-MS experiments were conducted using nanoelectrospray and samples prepared in 200 mM AmAc. Ions were produced by applying a positive potential of 0.9-1.4 kV via a platinum wire. IM-MS and CIU experiments were carried out on a Synapt XS HDMS (Waters Corporation, Manchester, UK). MS experiments were performed on a Q-Exactive UHMR Orbitrap instrument (Thermo-Fisher Scientific, Bremen, Germany).

### Preliminary data (results)

Cysteine (Cys) chemical labelling by *N*-ethylmaleimide (NEM) profiled two reactive residues in p53, which upon modification did not alter p53 conformational properties or the DNA-p53 tetramer complex. Extended Cys modification first disrupted the WTp53-DNA binding, and then triggered Zn(II) dissociation and protein unfolding. Having in mind that p53 contains two reactive Cys residues, we decided to monitor H<sub>2</sub>O<sub>2</sub> redox reaction with isolated p53 and with the DNA-p53 tetramer. Incubation of Zn-p53 with H<sub>2</sub>O<sub>2</sub> initially showed a shifted charge state distribution (CSD) towards larger *z* values, which was followed by Zn(II) dissociation and protein unfolding. Interestingly, the CSD resembled that one for metal-free p53 albeit Zn(II) remained bound to p53. These results suggest a rapid formation of a sulfenic acid with a concomitant disulfide formation without involving Zn(II)-binding residues. As the reaction continues, a network of disulfides is formed. We then examined the H<sub>2</sub>O<sub>2</sub> oxidation of the (Zn-p53)<sub>4</sub>DNA complex. The first step is an oxidative dissociation where p53 dissociates from *p21* promoter. In a second step, a conformational change occurred while Zn(II) is still bound to p53. In a third step, Zn(II) dissociates with subsequent disulfide formation and protein unfolding. Here, we unveiled the molecular mechanisms which allow p53 to control the binding to its promoters under a redox cellular environment.

The research was financed by the NCN of Poland under Preludium (no. 2018/31/N/ST4/01909 to MDPD) and Opus (no. 2019/33/B/ST4/02428 to A. K).

### Please explain why your abstract is innovative for mass spectrometry?

High-resolution native MS experiments elucidated the H<sub>2</sub>O<sub>2</sub>-mediated oxidation mechanism of the DNA-p53 tetramer.

### Co-authors:

*Perdita Barran, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology*  
*Artur Krężel, Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław*

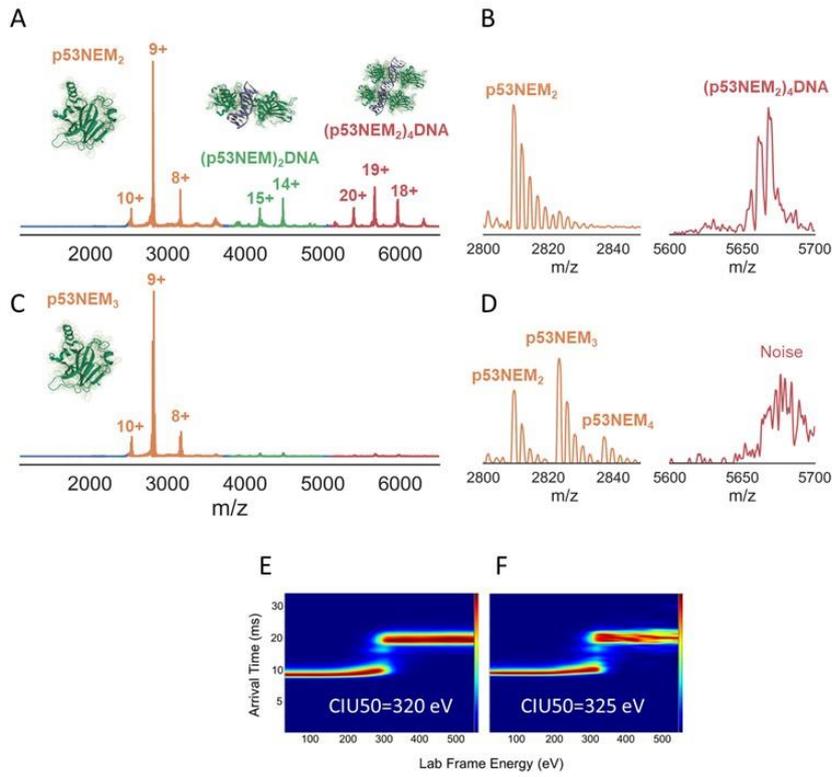


Figure 1. Chemical modification of the DNA-p53 tetramer.

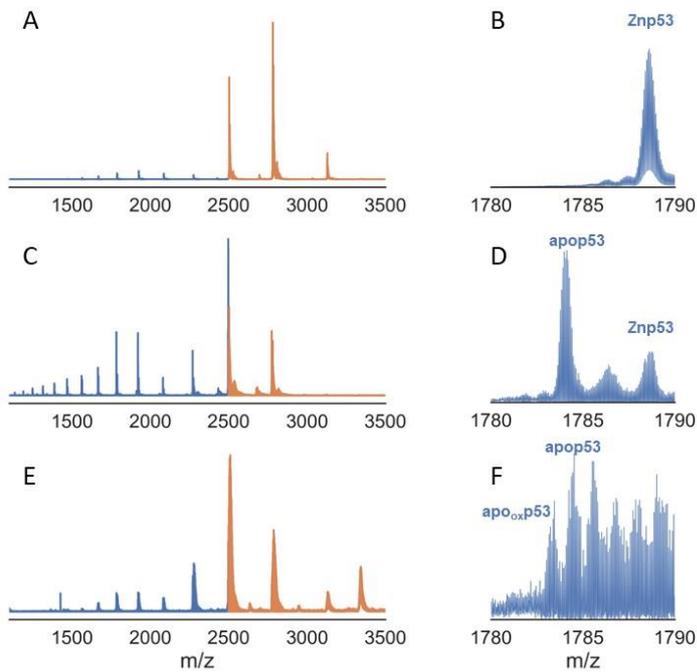


Figure 2. H<sub>2</sub>O<sub>2</sub>-mediated oxidation of DNA-p53 tetramer.

Poster number: LS-PB-145

## STEERED MD SIMULATIONS CAN MIMICK GAS-PHASE PROTEIN UNFOLDING

Abstract ID: 975

**Presenting author: Manuel Peris Diaz, Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology**

### Introduction

Collision-induced unfolding (CIU) has proved to be a powerful tool in mass spectrometry-based structural biology. In a common CIU experiment, quadrupole-selected ions are activated through a collision energy ramp increasing their internal energy which in turn causes a conformational change that can be monitored by IM-MS. On the whole, CIU IM-MS is a great method capable of detecting subtle structural changes. To further understand the structural changes at the molecular level, gas-phase computer simulations are of great interest. The IM-MS-derived CCS distributions can be readily compared with CCS values calculated for representative structures extracted from molecular dynamics trajectories. Here, we have demonstrated that steered MD provides a way to obtain partially and fully unfolded structures, and their CCS values closely agree with the experimental ones.

### Methods

Recombinant human MT2 was overexpressed in *E. coli* and purified by chromatography. IM-MS experiments were conducted using nanoelectrospray and samples prepared in 200 mM AmAc. Ions were produced by applying a 0.9-1.4 kV via a platinum wire. IM-MS and CIU experiments were carried out on a Synapt XS HDMS (Waters Corporation, Manchester, UK). Gas-phase MD simulations and SMD simulations were performed using GROMACS 2018.4 with AMBER FF19SB force field including parametrization for Zn(II) and the coordination residues. A charge placement algorithm based on the chargePlacer script was modified to include Cysteine as titratable residues and a mobile proton approach.

### Preliminary data (results)

Collision-induced unfolding experiments were performed on partially Zn(II)-loaded metallothionein-2 species, which represent one of the main cellular Zn(II) buffering systems in the cell. Metal-coupled folding effect can be observed upon Zn(II) binding: prior to CA, Zn<sub>4-7</sub>MT2 [M + 5H]<sup>5+</sup> ions populate a CCSD ~ 1000 Å<sup>2</sup> in comparison to ~ 1300 Å<sup>2</sup> for apoMT2 [M + 5H]<sup>5+</sup>. As the collision energy is increase, the CCSD shift to ~ 1150 Å<sup>2</sup>, in all of the Zn-MT2 complexes. To compare the gas-phase stabilities of Zn<sub>4-7</sub>MT2 species, the CCSD along the CE assayed were fitted to estimate the CIU<sub>50</sub> values, which indicates the energy required to activate 50% of the ions to its next conformation. A CIU<sub>50</sub> ~ 90 eV was calculated for the 5+ ions for Zn<sub>4</sub>MT2 and Zn<sub>5</sub>MT2 species. A gradual increase to CIU<sub>50</sub> ~ 110 eV was then determined for Zn<sub>6</sub>MT2 and Zn<sub>7</sub>MT2 species. Gas-phase MD simulations were performed on [Zn<sub>7</sub>MT2 + 5H]<sup>5+</sup> with a mobile proton algorithm. Neither standard MD nor a simulated annealing approach generated conformations as extended as the experimental ones. Notwithstanding steered MD simulations produced an ensemble of gas-phase MD ions with a CCS distribution that closely agrees with experimental CCSS. Moreover, force-CCS curves resemble that CIU isotherm, and then we propose that "SMD50" values can be used to relatively quantify unfolding stabilities.

MDPD acknowledge Preludium (no. 2018/31/N/ST4/01909).

### Please explain why your abstract is innovative for mass spectrometry?

We provide a framework to derive gas-phase MD simulations to CCS values that reproduce well the experimental CIU profile.

### Co-authors:

Alexey Barkhanskiy, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology  
Ellen Liggett, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology  
Artur Krężel, Department of Chemical Biology, Faculty of Biotechnology, University of Wrocław  
Perdita Barran, Michael Barber Centre for Collaborative Mass Spectrometry, Manchester Institute of Biotechnology

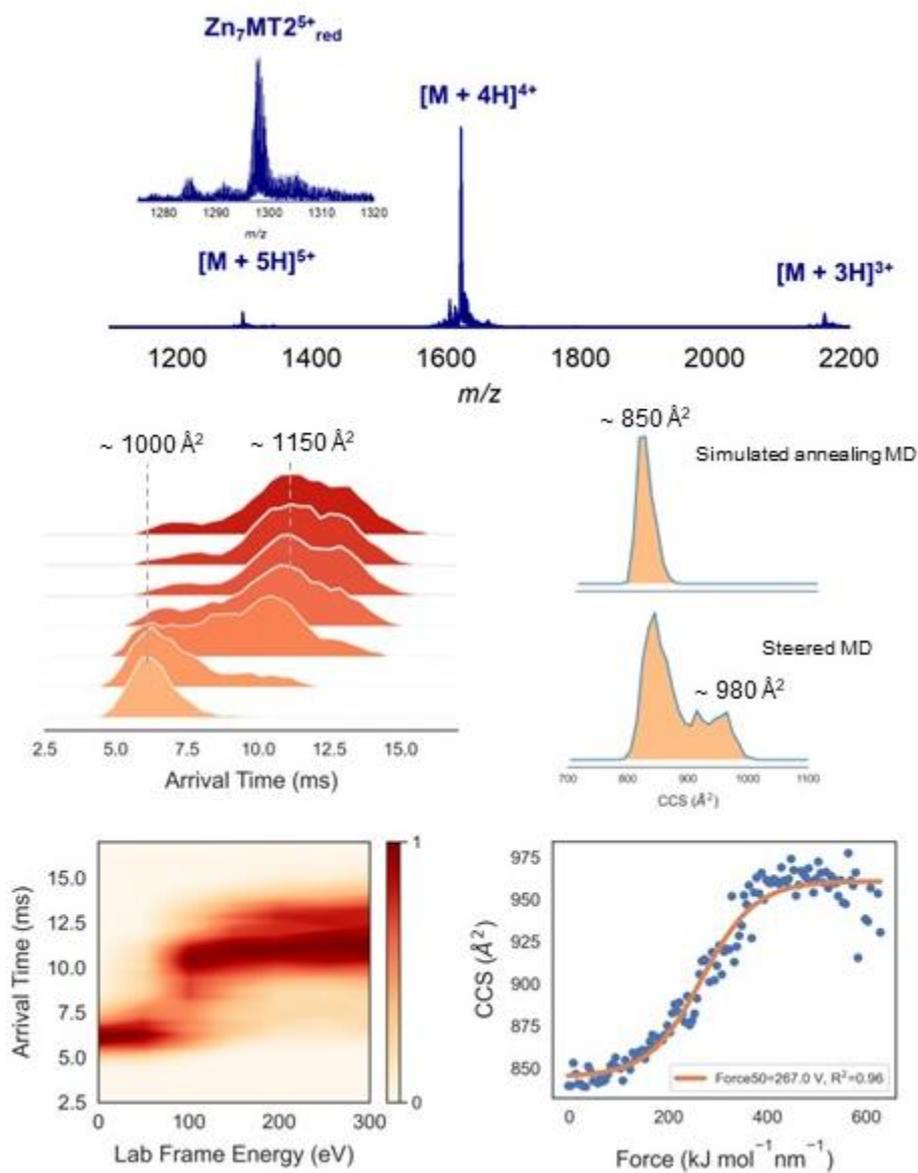


Figure 1. Steered MD simulations capture the CIU profile.

Poster number: **LS-PB-146**

## **NALIM: NATIVE LIQUID MALDI MASS SPECTROMETRY AS A NEW STRUCTURAL METHOD TO DIRECTLY CHARACTERIZE MEMBRANE PROTEIN COMPLEXES**

Abstract ID: **977**

**Presenting author: Edison Zhamungui, Centre de Biophysique Moléculaire, CNRS UPR4301, affiliated with Université d'Orléans**

### **Introduction**

Although membrane proteins (MPs) represent 2/3rds of potential therapeutic targets, so far only 10% of these proteins have been targeted, for lack of structure information. MPs are difficult to produce and analyze and most methods were designed for soluble proteins.

With carefully controlled non-denaturing conditions, native MS (nMS) methods provide complex characterization directly in the instrument. However, the detergents and salts used to stabilize MPs in aqueous buffers can suppress ions and degrade instrument performance. MALDI's tolerance to contaminants and low sample consumption makes it *de facto* attractive for native MS. Here we show how Native Liquid MALDI (**NALIM**) MS, for which proof of concept was obtained on soluble proteins [1], can be used to answer questions about the structural biology of membrane proteins.

### **Methods**

Analyses were performed on an Ultraflextreme MALDI-TOF/TOF mass spectrometer from Bruker equipped with PAN™ technology and a 2 kHz smartbeam-II laser. To eschew the transition through the solid-state and preserve noncovalent interactions, an ionic liquid matrix solution of HCCA/3-Aq/Glycerol (1:4:6 w/w) was used to prepare deposits. A 1 µL aliquot of sample-matrix solution was spotted onto a MTX stainless steel sample stage. To achieve high compatibility of NALIM with MPs at micromolar to submicromolar concentrations, we designed a strategy of optimization based on both the composition of the sample-matrix solution and instrumental parameters.

### **Preliminary data (results)**

To assess the range of potential applications for NALIM, different types of membrane proteins including transporters, receptors, and ion channels were tested. First, we focused on a multimeric ABC transporter which is an archetype for multi-drug resistance transporters. NALIM was successfully adapted and applied to address challenges related to the analysis of this dimer protein. The transporter can be stabilized through binding to its cognate ligands thanks to inorganic ion trapping. The KcsA Archaea potassium channel was the first ion channel to be crystallized and serves as the archetype of potassium channels. The Tx7335 toxin from the eastern green mamba snake (*Dendroaspis angusticeps*) is an original actitoxin, meaning that, contrary to all other known potassium channels toxins, it has an activating effect on the channel. Preliminary data from competition experiments gives us insight into the localization of binding of the actitoxin to a potassium channel. NALIM thus provides easy access to the stoichiometry of membrane protein assemblies, a monitoring method to follow complex stabilization through the binding of a specific ligand, and a tool for localization by competition.

### **Please explain why your abstract is innovative for mass spectrometry?**

NALIM is a promising alternative to native ESI, particularly for large molecular assemblies which can be used on a TOF/TOF or other instrument geometry to characterize membrane protein complexes

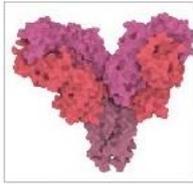
### **Co-authors:**

Cédric Orelle, Molecular Microbiology & Structural Biochemistry (MMSB) UMR 5086, CNRS/University of Lyon  
Jean-Michel Jault, Molecular Microbiology & Structural Biochemistry (MMSB) UMR 5086, CNRS/University of Lyon  
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Sébastien Poget, Department of Chemistry, College of Staten Island, City University of New York, 2800 Victory Boulevard, Staten Island

**POSTER SESSION B**

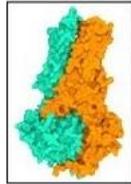
Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Martine Beaufour, Centre de Biophysique Moléculaire, CNRS UPR4301, affiliated with Université d'Orléans*  
*Martine Cadene, Centre de Biophysique Moléculaire, CNRS UPR4301, affiliated with Université d'Orléans*



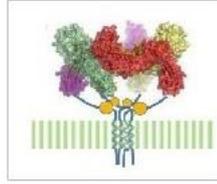
**KcsA**

K<sup>+</sup> Ion Channel



**BmrA**

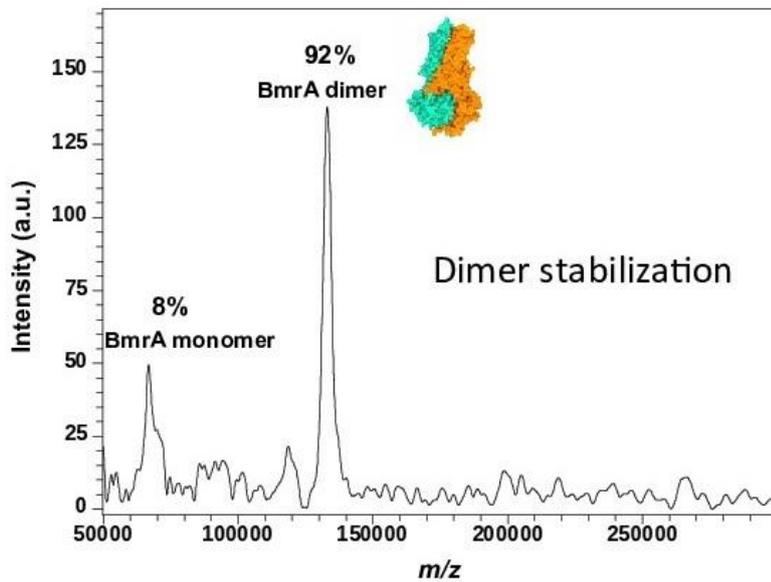
ABC Transporter



**LINGO1**

Leucine-rich coreceptor

Types of membrane proteins used for NALIM development



NALIM analysis of BmrA, an ABC transporter

## Session: Proteomics: protein -protein interaction

Poster number: **LS-PB-147**

### **DEVELOPMENT OF A NEW GENERATION OF ANTIVENOMIC APPROACH BY MASS SPECTROMETRY**

Abstract ID: **291**

**Presenting author: Damien Redureau, Mass Spectrometry Laboratory-ULiège**

#### **Introduction**

Snakebite causes the death of about 150,000 people/year. Envenomations are classically treated by injecting anti-venomous sera. However, these treatments can induce immunological reactions which can present several adverse effects to the patient. Venom compositions strongly differ from species to species, gender and habitat and providing antivenoms targeting a specific venom is then already a challenge. Quantitatively evaluating the efficacy of any antivenom is primordial to improve the production of effective sera and to determine the nature of the toxins indeed bound by the antibodies (Igs). Therefore, mass spectrometry (MS) is important to be performed, and it is then called "antivenomics". We plan to exploit the huge potential of magnetic beads and of mass spectrometry to speed up the antivenoms efficacy.

#### **Methods**

Shotgun proteomics was performed with 10µg *Echis ocellatus* and *Dendroaspis polylepis* venoms, which were reduced/alkylated/digested with a mixture of Trypsin/GluC/Chymotrypsin, then analysed using a Q-Exactive™ Plus Mass Spectrometer and the protein identification performed by Peaks Studio X+ using Uniprot/transcriptomes databases. Next step consists in grafting the antivenom antibodies to magnetic beads and incubating with the crude venoms. Comparative MS analysis of the toxins remaining in suspension (not recognized by Ig) and, more relevantly, those remaining on the beads (recognized by Ig) allows the effectiveness and selectivity of the antivenoms studied to be determined.

#### **Preliminary data (results)**

In *Echis ocellatus* venom, 82.8% were identified as toxins and 17.2% as non-toxins while for *Dendroaspis polylepis* venom we obtained 26.2% for toxins, 39.9% for non-toxins and 33.9% for cellular components. The most expressed group of toxins were metalloproteinases for *Echis ocellatus* venom and three-finger toxins for *Dendroaspis polylepis* venom. These findings will be important in the efficacy evaluation and targeting the main toxins for the next step of the development of the antivenomic approach. Snake venoms of the genus *Echis* and/or *Dendroaspis* are responsible for a large proportion of snake envenomations in sub-Saharan Africa.

#### **Please explain why your abstract is innovative for mass spectrometry?**

The optimization of the antivenomics can increase the sensibility of the antivenoms targeting the most important toxins responsible for the symptoms in the envenoming and also improve the treatment response.

#### **Co-authors:**

*Fernanda Gobbi Amorim, Mass Spectrometry Laboratory-ULiège*

*Gabriel Mazzucchelli, Mass Spectrometry Laboratory-ULiège*

*Stefanie Menzies, Centre for Snakebite Research and Interventions-Liverpool School of Tropical Medicine, Centre for Drugs and Diagnostics-Liverpool School of Tropical Medicine*

*Nicholas Casewell, Centre for Snakebite Research and Interventions-Liverpool School of Tropical Medicine, Centre for Drugs and Diagnostics-Liverpool School of Tropical Medicine*

*Loïc Quinton, Mass Spectrometry Laboratory-ULiège*

Poster number: **LS-PB-148**

## APPLICATION OF CHROMATOGRAPHIC-SPECTROMETRIC TECHNIQUES FOR THE IN VITRO EVALUATION OF POTENTIAL DRUG-DRUG INTERACTIONS IN NEWLY DEVELOPED SARMS

Abstract ID: 656

**Presenting author:** Carlotta Stacchini, Laboratorio Antidoping, Federazione Medico Sportiva Italiana, Dipartimento Chimica e Tecnologia del farmaco, "Sapienza" Università di Roma

### Introduction

Selective androgen receptor modulators are anabolic agents that modulate selectively androgen receptor activity, included in the WADA List of Prohibited Substances and Methods. The study of the metabolic profile is essential to identify optimal markers of intake: the contribution of the single recombinant isoforms allows to evaluate the alteration in the concentration of metabolites in case of co-administration of inhibitors of metabolizing enzymes; moreover, some isoforms present a wide genetic variability, so that, some subjects, defined as "poor metabolizers", may not form the metabolites identified as markers of intake. The investigations on the drug-drug interactions are therefore crucial in the anti-doping field: the alterations in the metabolites levels of prohibited substances could make more problematic the correct interpretation of the laboratory results.

### Methods

*In vitro* incubations using human liver microsomes were performed to reproduce the metabolism of five newly developed SARMS (S6, LGD2226, TFM-4AS-1, CI-4AS-1, PF-06260414). To study the contribution of the recombinant isoforms, the same procedures were carried out using single isoforms (CYP3A4, CYP2D6, CYP1A2, CYP2C9). For the studies of the drug-drug interaction, the co-administration with antifungals, antihistamines anti-H<sub>2</sub>, and progestins was evaluated. Samples were extracted with *tert*-butylmethylether and analysed by liquid chromatography (Acquity I-Class UPLC® system) coupled to mass spectrometry (Q-Trap 5500 ABSciex). Multiple reaction monitoring was used as acquisition mode.

### Preliminary data (results)

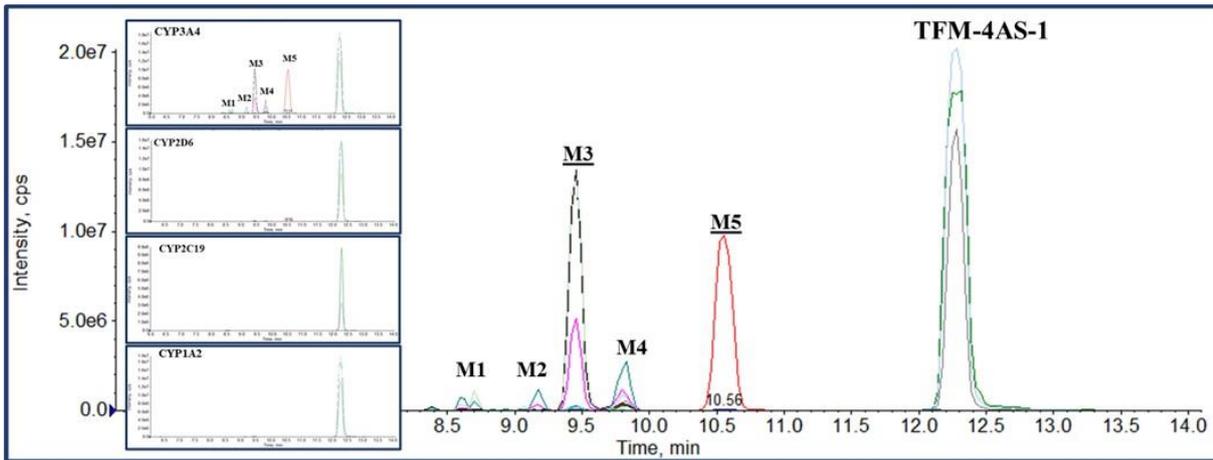
The results of the study show that the metabolizing enzyme mainly involved in phase I metabolic profile of all the SARMS under investigation is CYP3A4, which is the single isoform involved in most of the metabolic pathways of xenobiotics. For this reason, it is useful to evaluate the possible alteration of the metabolic profiles of these substances in the presence of drugs approved for clinical use, known P450 isoform inhibitors, such as antifungals, anti-H<sub>2</sub> antihistamines, and progestins. From preliminary data obtained from drug-drug interaction studies, co-administration with antifungals significantly affects the metabolic profile of SARMS under investigation, progestins, and antihistamines to a lesser extent: by significantly decreasing the concentration of metabolites that could be defined as characteristic markers of intake. As a representative example of this kind of interaction, we report the case of TFM-4AS-1, a steroidal derivative. Figure 1 shows the contribution of the single enzymatic isoform on the metabolic profile of TFM-4AS-1: CYP3A4 is the isoform involved in the formation of the most abundant metabolites, selected as possible markers of intake (M3 and M5). Being CYP3A4 the isoform most involved in the metabolism of almost 30% of the drugs on the market, the possible co-administration with known inhibitors of this enzyme was investigated: as shown in Figure 2, indeed, the co-administration with all the inhibitors, but especially with antifungals, significantly provoked a decrease of the amount of the metabolites identified as markers of intake.

### Please explain why your abstract is innovative for mass spectrometry?

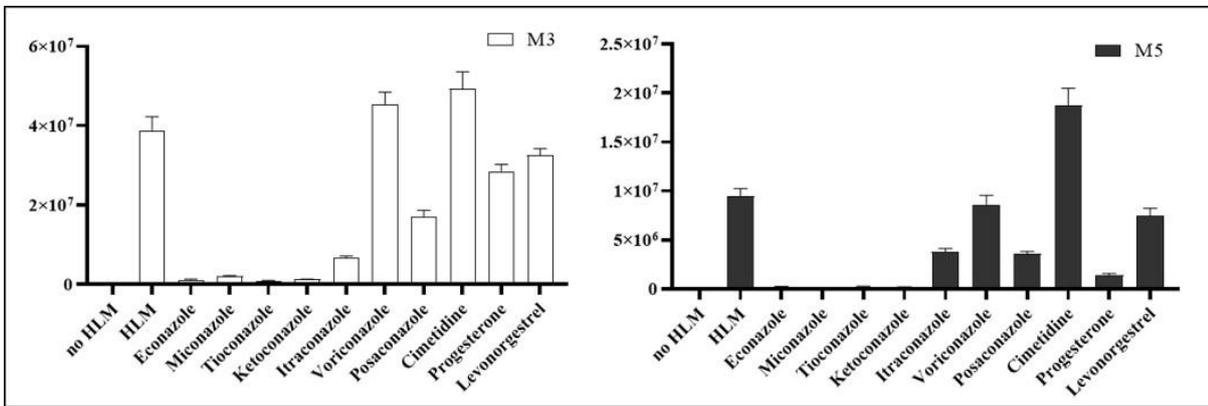
The study of drug-drug interactions by mass spectrometry for anti-doping purpose allows to refine the correct interpretation of the analytical results, especially in the case of abnormal metabolic profiles.

### Co-authors:

Francesco Botrè, Laboratorio Antidoping, Federazione Medico Sportiva Italiana, ISSUL - Institute of sport sciences, University of Lausanne, Center of Research and Expertise in Anti-Doping Sciences – REDs  
Xavier de la Torre, Laboratorio Antidoping, Federazione Medico Sportiva Italiana  
Monica Mazzarino, Laboratorio Antidoping, Federazione Medico Sportiva Italiana



TFM-4AS-1 metabolic profile: contribution of single recombinant isoforms.



Alterations of TFM-4AS-1 markers of intake with inhibitors.

Poster number: LS-PB-149

## EX VIVO CHARACTERIZATION OF THE IRF4 INTERACTOME ELUCIDATES MOLECULAR MECHANISMS OF IRF4-STEERED EFFECTOR FUNCTIONS IN TH17 AND TREG CELLS

Abstract ID: 812

**Presenting author: Anna Gabele, Institute for Immunology, University Medical Center of the Johannes-Gutenberg University, Mainz, Research Center for Immunotherapy (FZI), University Medical Center of the Johannes-Gutenberg University Mainz**

### Introduction

Interferon regulatory factor (IRF4), a key regulator in lymphoid-cell differentiation, promotes the maturation of T helper cell populations into effector cells. T helper 17 (Th17) and regulatory T cells (Treg) are important counterplayers during the development of autoimmune diseases. While Th17 cells are known to promote autoimmunity, Treg cells are important in maintaining peripheral tolerance. Despite the central role of IRF4 in T cell lineage determination, molecular mechanisms of IRF4-mediated gene expression *in vivo* are poorly understood. Also, the question how IRF4 interacting proteins steer IRF4-mediated target gene transcription in different T cell subtypes remains unsolved. Here, we present an optimized proteomic workflow enabling the *ex vivo* characterization of transcription factor interactomes combining chemical cross-linking and affinity purification of biotinylated target protein complexes.

### Methods

IRF4<sup>Bio</sup> mice, which express biotinylated IRF4 at endogenous levels were generated by crossing the ROSA26<sup>BirA</sup> strain [1] with animals expressing IRF4 fused to a BirA recognition site. Naïve CD4<sup>+</sup> T cells were isolated from spleens of IRF4<sup>Bio</sup> and control (ROSA26<sup>BirA</sup>) animals. After *ex vivo* differentiation into Th17 and Treg cells, biotinylated IRF4 along with its interactors was captured using magnetic streptavidin beads. IRF4 interactomes and full proteomes of T cells were processed using single-pot solid-phase-enhanced sample preparation (SP3) for tryptic digestion and an Orbitrap Exploris 480 instrument for LC-MS analysis. DIA data were processed using DIA-NN (version 1.8).

### Preliminary data (results)

In the present study, we focused on the characterization of the transcription factor IRF4 and its interplayers in *ex vivo* propagated CD4<sup>+</sup> T cell subsets (Th17 and Treg), for which we developed a robust and highly reproducible affinity purification protocol: Combining reversible chemical cross-linking, optimized wash and elution steps as well as SP3 (single-pot solid-phase-enhanced sample preparation) for MS sample processing, we were able to markedly reduce unspecific background as well as contamination derived from the streptavidin beads during pulldown experiments while concomitantly preserving weak and transient interactions.

We could describe a “core IRF4 interactome” which is preserved even in functionally opposed T cell subsets like Treg and Th17 cells as well as cell type specific interactors. Besides described lineage-specific interactors, like the master transcription factors RORγT (Th17) or FoxP3 (Treg), novel, yet uncharted interplayers were identified.

### Please explain why your abstract is innovative for mass spectrometry?

Chemical crosslinking combined with affinity purification mass spectrometry reveals novel players in IRF4-mediated gene regulation in CD4<sup>+</sup> T cell subsets.

### Co-authors:

Maximilian Sprang, Computational Biology and Data Mining, Johannes-Gutenberg University Mainz  
Sarah Dietzen, Institute for Immunology, University Medical Center of the Johannes-Gutenberg University, Mainz, Research Center for Immunotherapy (FZI), University Medical Center of the Johannes-Gutenberg University Mainz  
Stefan Tenzer, Institute for Immunology, University Medical Center of the Johannes-Gutenberg University, Mainz, Research Center for Immunotherapy (FZI), University Medical Center of the Johannes-Gutenberg University Mainz  
Tobias Bopp, Institute for Immunology, University Medical Center of the Johannes-Gutenberg University, Mainz, Research Center for Immunotherapy (FZI), University Medical Center of the Johannes-Gutenberg University Mainz

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Ute Distler, Institute for Immunology, University Medical Center of the Johannes-Gutenberg University, Mainz, Research Center for Immunotherapy (FZI), University Medical Center of the Johannes-Gutenberg University Mainz*

## **Session: Translational MS – Clinical and liquid biopsies**

Poster number: **LS-PB-150**

### **STEROID PROFILING FROM DRIED BLOOD SPOT SAMPLES USING QSIGHT LC-MS/MS**

Abstract ID: **34**

**Presenting author: Roberto Bozic, PerkinElmer, Milan, Italy**

#### **Introduction**

Inborn errors of metabolism include a wide variety of conditions with abnormal steroidogenesis caused by mutations affecting the enzymatic activity in the steroid biosynthesis pathways. Measurement of increased 17-hydroxyprogesterone concentration in dried blood spot (DBS) by immunoassays is associated to analytical interferences due to cross-reactivity. To allow more specific steroid marker profiling from DBS samples, we report here a gradient UHPLC-MS/MS method on the QSight mass spectrometer, which provides simultaneous specific determination of 17-hydroxyprogesterone, androstenedione, cortisol, 21-deoxycortisol and 11-deoxycortisol. Therefore, this multiple steroid profiling method enables an improved distinction between 21-hydroxylase and 11- $\beta$  hydroxylase deficiencies, and it can be also potentially used as a 2nd tier test for 17-OHP immunoassays suffering from cross-reactivity interferences.

#### **Methods**

The steroids were extracted from two 3.2 mm DBS disks using an extraction solution of water/methanol containing the internal standards. Analysis was performed on QSight LC-MS/MS system in positive mode electrospray ionization and multiple reaction monitoring acquisition. A reversed phase UHPLC gradient method was used for analytes separation.

#### **Preliminary data (results)**

All analytes produced a linear response over the measured concentration range ( $R^2 > 0.99$ ). The tested linearity ranges cover the expected normal and abnormal concentration ranges in 21-hydroxylase and 11- $\beta$  hydroxylase deficiencies. The good LLOQ results were achieved using stable isotope-labeled analogues of steroid analytes. Measured amounts of these were added to real blood from normal subjects. Good method accuracy (90 to 110%) was also obtained for all analytes. The reported method allows complete resolution of steroid isobars: 21-deoxycortisol, 11-deoxycortisol and corticosterone (another endogenous steroid). Thus, this method allows prompt confirmation and differentiation between 21-hydroxylase and 11- $\beta$  hydroxylase deficiencies. The results obtained confirm the applicability of LC-MS/MS method for profiling steroids in DBS samples with analytical sensitivity and specificity on QSight mass spectrometer. This reported method can be potentially used as a 2nd Tier test to improve the analytical interference arising from cross-reactivity associated with immunoassay measurement.

#### **Please explain why your abstract is innovative for mass spectrometry?**

Stay-clean source for complex biological matrices with less maintenance; dual ionization source (ESI and APCI).

#### **Co-authors:**

*Lorenzo Bacci, PerkinElmer, Milan, Italy*

*Andreu Fabregat, PerkinElmer, Madrid, Spain*

*Michela Perrone Donnorso, Regional Newborn Screening Laboratory, Children's Hospital V. Buzzi, Milan, Italy*

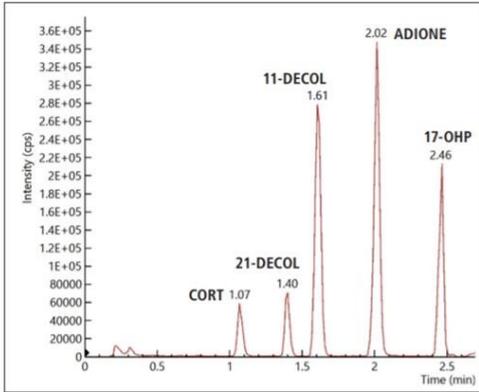


Fig.1 Steroid profiling by LC-MS/MS in DBS matrix

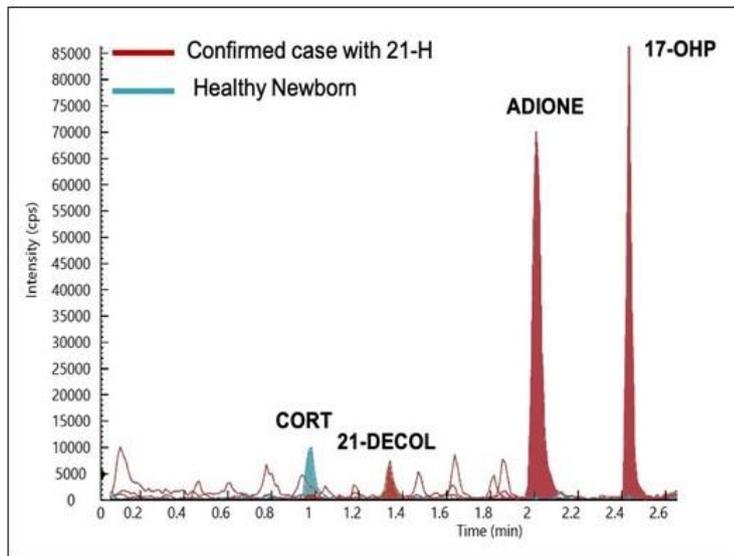


Fig.2 Healthy newborn and confirmate case with 21-hydroxylase-deficiency

Poster number: LS-PB-151

## MEASUREMENT OF ISOBARIC C5 ACYLCARNITINES IN DRIED BLOOD SPOT USING QSIGHT LC-MS/MS

Abstract ID: 46

Presenting author: Roberto Bozic, PerkinElmer, Milan, Italy

### Introduction

Inborn errors of metabolism include a wide variety of conditions caused by mutations affecting the enzymatic activity. E.g. isovaleryl-CoA dehydrogenase (IVD) deficiency results in phenotypic abnormalities due to the accumulation of isovaleric acid. Primary screening for IVD deficiency is commonly based on the measurement of increased isovalerylcarnitine (C5) concentration in blood. By direct flow injection analysis tandem mass spectrometry (FIA-MS/MS) assays without liquid chromatography (LC) separation, this specific biomarker cannot be however distinguished from other possibly existing three C5 isobars including valeryl-, pivaloyl-, and 2-methylbutyrylcarnitine. To allow more specific measurement of these isobaric C5 markers in dried blot spot (DBS) samples, we report here a gradient LC-MS/MS method on QSight mass spectrometer. It also provides potential analytical tool for the second-tier testing.

### Methods

A 3.2 mm DBS disk was extracted using a methanol:water extraction solution containing internal standard for signal normalization and quantitation. After incubation the supernatant was transferred and injected in the QSight LC-MS/MS system. Analysis was performed in positive mode electrospray ionization and multiple reaction monitoring acquisition. A reversed phase LC gradient method was used for analytes separation.

### Preliminary data (results)

This LC-MS/MS method allowed to resolve the isovaleryl-L-carnitine from pivaloyl-L-carnitine and other two C5 isobars: methylbutyryl-L-carnitine and valeryl-L-carnitine. The retention times were 4.00, 3.63, 3.75 and 4.22 min, respectively. The LC-MS/MS method characteristics were demonstrated by using non-enriched endogenous blood and several enriched concentrations of each C5 isobar alone and in combined mixture of all four C5 isobars. Quantitative determination of C5 isobars was performed using 6 level blood calibrators for each analyte. The tested linearity ranges were: 0.2-10  $\mu\text{M}$  covering the expected normal ( $\text{C5} < 0.5 \mu\text{M}$ ) and abnormal ( $\text{C5}$  typically 0.6-12  $\mu\text{M}$ ) concentration ranges in IVD deficiencies. Good overall linearity results were obtained for all four C5 isobars ( $R^2 > 0.99$ ). Acceptable precision ( $\text{CV} < 11.7\%$ ) and reasonable accuracy (96 to 115%) ranges were obtained for all analytes. Alternatively, also a direct isotope dilution quantitation without an external calibration curve was tested. For this, a compound specific relative response factor was first determined for each analyte based on six different DBS enrichment levels, and then studied with three measured control level over two test plates on two separate days. Also this approach gave reasonable accuracy (84 to 127%) and precision ( $\text{CV} < 13.5\%$ ) results for all four C5 isobars. The results obtained confirm the applicability of this LC-MS/MS method for measuring and monitoring the isobaric C5 biomarkers concentrations in DBS samples. This reported method can be potentially used as a second-tier test to reduce commonly seen false-positive rates due to the antibiotic derived C5 interference caused by pivaloylcarnitine.

### Please explain why your abstract is innovative for mass spectrometry?

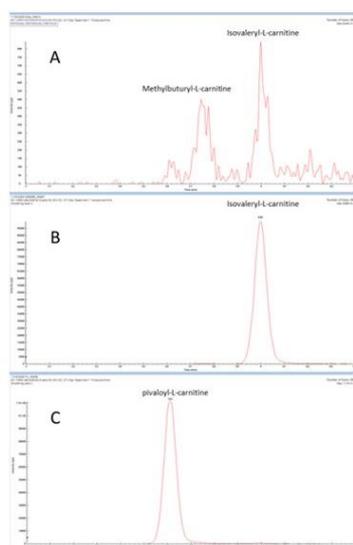
Stay-clean source for complex biological matrices with less maintenance; dual ionization source (ESI and APCI).

### Co-authors:

*Tero Lehtonen, PerkinElmer, Turku, Finland*  
*Axel Meierjohann, PerkinElmer, Turku, Finland*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



*Extracted-ion-chromatograms of healthy, positive IVD and enriched DBS samples.*

Poster number: LS-PB-153

## ABSOLUTE QUANTIFICATION OF PROTEOMES AND LIPIDOMES IN TISSUES AND BIOPSIES

Abstract ID: 397

Presenting author: Andrej Shevchenko, MPI of Molecular Cell Biology and Genetics

### Introduction

Liquid and solid biopsies is a common clinical resource extensively exploited by the *omics* sciences. These studies typically follow the fold changes of the abundances of molecules between patients cohorts, including various controls. While this is a technically mature high-throughput approach, it does not associate fold changes with the molar concentration of revealed candidates, or elucidate molar ratios within constellations and pathways encompassing molecules of different origin. We argue that accurate and consistent absolute (molar) quantification of lipids and proteins is achievable at the omics scale and may provide the molecular rationale for the multiomics interpretation of pathophysiological processes associated with perturbed lipid metabolism.

### Methods

Lipids were extracted by methyl-tert-butyl ether (Matyash et al, 2008) and quantified by high resolution shotgun lipidomics (Sales et al, 2016) (Vvedenskaya et al, 2021) using LipidXplorer software (Herzog et al, 2011). Proteome-wide absolute quantification was performed by LC-MS/MS using a generic FUGIS standard (Raghuraman et al, 2022); targeted quantification of selected protein markers by FastCAT (Rzagalinski et al, 2021) and MS Western (Kumar et al, 2018) methods.

### Preliminary data (results)

Shotgun lipidomics relies on the direct infusion of total extracts into a high resolution mass spectrometer. In this way, lipid analytes and internal standards spiked into the sample prior the lipid extraction are ionized and detected together. Molar abundance of lipid species could be quantified using a few internal standards per each lipid class. From the amount of tissue biopsies equivalent to 20 to 50 micrograms of the total proteins shotgun quantifies more than 300 consistently detectable lipid species from 22 major lipid classes, including membrane and energy storage lipids and cholesterol. Shotgun analysis corroborates lipid quantities determined by clinical chemistry (Sales et al, 2016) and is also applicable to histological zones isolated by from cryo-sections of biopsies by laser-capture microdissection (Knittelfelder et al, 2018). Independent analysis of colon cancer biopsies suggested excellent inter-laboratory concordance of lipid profiles (Wang et al, 2020) (Ecker et al, 2021).

Untargeted absolute quantification of proteins by FUGIS uses a designed chimera standard comprising peptides with "median" ionization properties that match no sequences in a database. If co-digested with the protein pellet, it allows accurate molar quantification of any protein detected with only a few peptides. Protein quantities corroborated the determinations made by independent targeted methods. In this way, a combination of shotgun lipidomics and untargeted quantification using FUGIS standard both compatible with the MTBE extraction protocol opens up novel opportunities for studying the interplay between lipid-protein interplay in metabolic disorders directly in tissue biopsies

### Please explain why your abstract is innovative for mass spectrometry?

Absolute (molar) quantification of lipids and proteins from tissues biopsies at the omics scale

Poster number: **LS-PB-154**

## TARGETED PROTEOMICS AS A TOOL TO DETECT SARS-COV-2 PROTEINS IN CLINICAL SPECIMENS

Abstract ID: **474**

**Presenting author: Jeroen AA Demmers, Erasmus MC**

### Introduction

The rapid, sensitive and specific detection of SARS-CoV-2 and yet unknown viruses is critical in responding to the current COVID-19 pandemic and future outbreaks. In this proof-of-concept study, we explore the potential of targeted mass spectrometry (MS) based proteomics for the detection of SARS-CoV-2 proteins in both research samples and clinical specimens. We show that viral proteins can be detected and quantified in patient samples such as sputum and nasopharyngeal swabs with fairly high sensitivity, clearing the way for exploration of the use of proteomics technology in clinical and diagnostics labs. In addition, we present alternative sample preparation procedures to further optimize both the sensitivity of the assay and the LC-MS throughput.

### Methods

parallel reaction monitoring (PRM) mass spectrometry on an Orbitrap Eclipse. SARS-CoV-2 was propagated on Vero E6 cells and infected cells were lysed and boiled to inactivate the virus. Proteins in patient nasopharyngeal and throat swabs or sputum samples were first precipitated with acetone-TCA to remove excessive albumin. Protein pellets were resuspended in Tris/HCl buffer and digested using the SP3 protocol. A selected set of unique SARS-CoV-2 Nucleocapsid and Spike tryptic peptides were subjected to a PRM mass spectrometry regime for targeted detection and quantitation.

### Preliminary data (results)

Using PRM MS, the limit of detection for several tryptic peptides of the most abundant SARS-CoV-2 protein, Nucleocapsid, in a complex matrix background was estimated to be in the mid-attomole range (9E-13 g). Heavy labeled AQUA peptides were used to confirm fragment ion chromatograms and retention times of target peptides. Next, targeted MS was applied to the detection of viral proteins in various COVID-19 patient clinical specimens, such as sputum and throat and nasopharyngeal swabs. In these generally heterogeneous and often heavily contaminated samples, SARS-CoV-2 proteins could be detected with high sensitivity in all specimens with reported PCR Ct values of <24 and also in several samples with higher CT values. A clear relationship was observed between summed fragment ion chromatogram areas under the curve (AUCs) for SARS-CoV-2 tryptic peptides and Ct values reflecting the abundance of viral RNA. Subsequent steps for optimization of the procedure involve the improvement of quality and speed of sample preparation and LC-MS throughput. Using offline peptide fractionation, the sensitivity could be increased several fold, albeit at the expense of analysis speed. Taken together, these results suggest that targeted MS based proteomics may have the potential to be used as an additional, orthogonal tool in COVID-19 diagnostics.

### Please explain why your abstract is innovative for mass spectrometry?

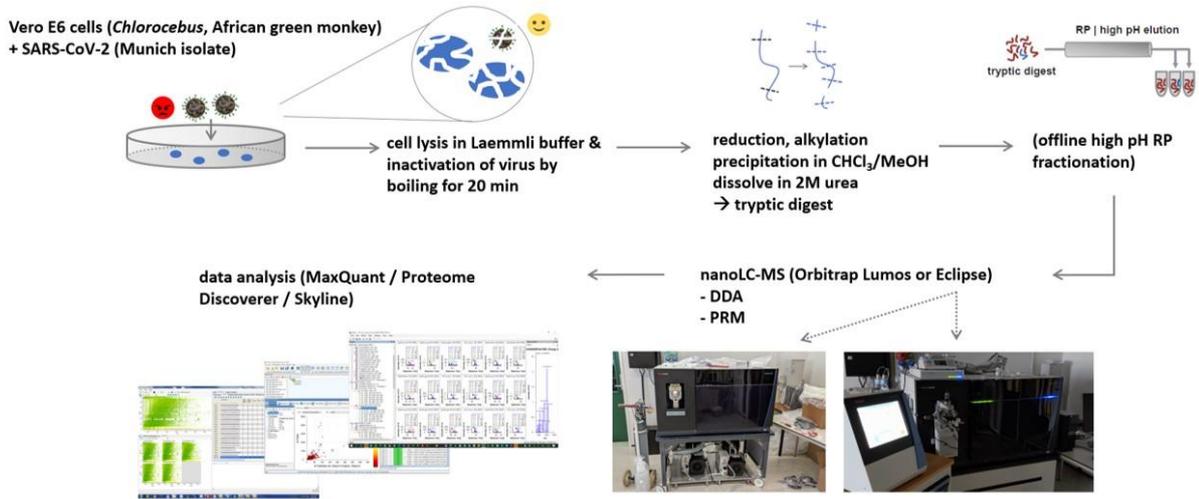
Targeted protein mass spectrometry has the potential to be used as an additional tool in COVID-19 clinical research and diagnostics.

### Co-authors:

*Mart M Lamers, Erasmus MC*  
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*Jeroen JA Van Kampen, Erasmus MC*  
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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Workflow for targeted mass spectrometry on SARS-CoV-2 proteins.

Poster number: **LS-PB-155**

## ION TRAP MASS SPECTROMETRY OF BLOOD PORPHYRINS FOR CLINICAL APPLICATION IN NEUROSURGERY

Abstract ID: **483**

**Presenting author: Anna Walke, Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster, Münster, Germany, Department of Neurosurgery, University Hospital Münster, Münster, Germany**

### Introduction

Fluorescence-guided resection allows improved tumor resection in glioma surgery. After oral administration of the prodrug 5-aminolevulinic acid (5-ALA), protoporphyrin IX (PPIX) is formed within the tumor resulting in an intraoperatively visible fluorescence area. Blood porphyrins are also diagnostic markers for, e.g., lead poisoning, and potentially for cancer progression. The majority of the analytical approaches uses spectrophotometric- or fluorescence detection taking advantage of the strong absorption of porphyrins in the region of the Soret band. However, interferences from other fluorophores and unspecific sample preparation caused inaccurate porphyrin determination from complex biological matrices. We used mass spectrometry (MS) for a more specific detection and present a robust workflow for the analysis of blood porphyrins.

### Methods

Porphyrins were isolated from EDTA-whole blood and serum by liquid-liquid extraction using water/acetonitrile (ACN) (30/70, v/v). The supernatant was purified by anionic-exchange solid phase extraction. Mesoporphyrin (MPIX) and PPIX eluted with ACN and 2% formic acid (FA), zinc-protoporphyrin (ZnPPIX) with 20% FA. Both fractions were dried and reconstituted in dimethyl sulfoxide. RP-HPLC (HP1100, Agilent) with a semi-porous, endcapped column was performed with 10 min run time. For MS detection, a time-segmented MS<sup>2</sup> method was developed on an ion trap (Esquire3000, Bruker).

### Preliminary data (results)

Choosing an MS<sup>2</sup> experiment and time-dependent screening for each single analyte improved the limit of detection. Volumes of 500 µl serum or 200 µl whole blood were required. The MS method enabled the quantification of native PPIX in serum, which so far was challenging. MPIX was used as internal standard, because it shows high structural similarity to PPIX. The singly charged [M+H]<sup>+</sup> species were fragmented; gas phase fragmentation occurred predominantly on the side chains of the tetrapyrrole core structure (losses of 59 u (-CH<sub>2</sub>COOH), 45 u (-COOH), 73 u (-CH<sub>2</sub>CH<sub>2</sub>COOH)), providing characteristic spectra. The LC-MS-based workflow is able to distinguish ZnPPIX and free PPIX, which is important for specific screening of blood samples from neurosurgical patients to test the hypothesis whether free PPIX could be a marker of the tumor burden. Time-dependent PPIX blood levels in healthy volunteers and patients with and without 5-ALA administration were detected in the range of 10 – 4,400 pmol PPIX/ml serum.

### Please explain why your abstract is innovative for mass spectrometry?

Detection of porphyrins by mass spectrometry instead of fluorescence offers greater specificity and sensitivity.

### Co-authors:

*Eric Suero-Molina, Department of Neurosurgery, University Hospital Münster, Münster, Germany*

*Walter Stummer, Department of Neurosurgery, University Hospital Münster, Münster, Germany*

*Simone König, Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster, Münster, Germany*

Poster number: **LS-PB-156**

## DEVELOPMENT AND VALIDATION OF AN LC-MS/MS METHOD FOR THE QUANTIFICATION OF CLINDAMYCIN IN HUMAN PLASMA

Abstract ID: **518****Presenting author: Laura Armengol Álvarez, Medicinal Chemistry, Rega Institute for Medical Research, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven**

### Introduction

Clindamycin (CLI) is a lincosamide antibiotic derived from lincomycin, naturally produced by *Streptomyces lincolnensis*. The semi-synthetic chlorinated derivative is the main lincosamide antibiotic applied in clinical practice. CLI is an approved drug for use in adults and children requiring systemic treatment of staphylococcal, streptococcal, and anaerobic bacterial infections. CLI directly inhibits peptide bond formation as it specifically binds to the 50S ribosomal subunit and suppresses peptidyl transferase.

Special populations have altered CYP3A4 activity together with other pharmacokinetic alterations. As CLI is metabolized by the CYP3A4/5 enzymes to bioactive N-demethyl and sulfoxide metabolites, insight on the potential relevance of drug's metabolites and disposition in special populations is of interest. In the future, a research strategy is required to address these knowledge gaps towards precision medicine.

### Methods

An LC-MS/MS method was developed and validated for the determination of CLI in human plasma. CLI and lincomycin (internal standard) were analyzed using a C18 reverse-phase column with a mobile phase consisting of a solvent mixture of 0.05% (v/v) formic acid in water (eluent A) and acetonitrile: water (1:1 v/v) (eluent B). Multiple reaction monitoring (MRM) on a triple quadrupole with ESI in positive mode was used with an MRM setting of 407 → 359 and 425 → 377 to quantify lincomycin and CLI, respectively. Human plasma sample preparation was undertaken by protein precipitation with acetonitrile.

### Preliminary data (results)

Different experiments were set up to examine the performance characteristics of the method in terms of linearity, LLOQ, precision, peak symmetry, sensitivity (LOD and LOQ), matrix effect and selectivity. A limit of detection and quantification of 0.04 and 0.12  $\mu\text{g mL}^{-1}$  were found, respectively. The assay exhibited a linear range of 0.05 – 5  $\mu\text{g mL}^{-1}$  and gave a good coefficient of determination ( $r^2$ ) of 0.9997. The chromatographic run time was approximately 17 min. Precision and accuracy corresponded with the followed guidelines (FDA and EMEA), showing RSD% values that did not exceed 15%. The method was proven to be selective and with satisfactory peak symmetry CLI was separated from spiked human plasma proteins by protein precipitation with acetonitrile and no interferences of the biological matrix were observed during the analysis. However, the method was not applied to real patient plasma samples, as these were not yet available. The procedure was linear, accurate, precise, and selective.

In course of time, the method is expected to be successfully applied to real patient plasma samples, but since these were not yet available, upcoming experiments for CLI will include this analysis. Furthermore, the method will also be validated against CLI's active metabolites: CLI sulfoxide and N-desmethyl CLI.

### Please explain why your abstract is innovative for mass spectrometry?

A novel and sensitive LC-MS/MS method was developed and validated for the quantification of clindamycin in human plasma.

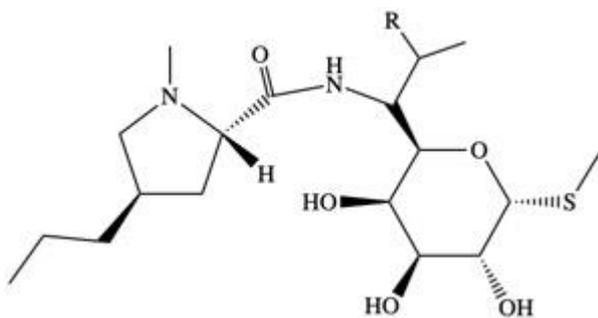
### Co-authors:

Isabel Spriet, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven  
Karel Allegaert, Organ Systems Unit, Department of Development and Regeneration, KU Leuven & Department of Pediatrics, UZ Leuven

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Jef Rozenski, Medicinal Chemistry, Rega Institute for Medical Research, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven*



Chemical structure of clindamycin (R= -Cl) and lincomycin (R= -OH).

Poster number: LS-PB-157

## DISTINGUISHING PATIENTS WITH BENIGN BREAST LESIONS, DCIS, AND INVASIVE DUCTAL CARCINOMA BY MALDI IMAGING MASS SPECTROMETRY SERUM N-GLYCAN ANALYSIS

Abstract ID: 547

Presenting author: Calvin Blaschke, Medical University of South Carolina

### Introduction

Breast cancer (BC) remains the most common type of cancer in females. Early detection drastically increases the survival of BC patients and provides multiple treatment options. While mammography is the standard tool for BC screening, it often results in unnecessary biopsies of benign lesions. Incorporating additional assays could improve the performance of BC screening. Changes in the glycan composition of blood glycoproteins are dynamic metabolic indicators of health and immune status. Prior serum N-glycan studies have evaluated healthy versus cancer conditions, but clinical decisions are made between benign disease versus cancer. Here, we investigate the serum N-glycan profiles of patients with benign breast lesions, ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC) and identify N-glycan classes that can classify these patient groups.

### Methods

Serum samples from patients with benign lesions ( $n = 200$ ), DCIS ( $n=30$ ), or IDC ( $n=70$ ) were used. Amine-reactive slides were used to immobilize serum glycoproteins from 1 microliter of samples spotted on to the slides. The samples were delipidated and desalted with washes of Carnoy's solution and water. Peptide N-glycosidase F was sprayed on the samples to cleave the N-glycans, and a layer of matrix was applied. The released N-glycans were detected across the samples by MALDI-QTOF imaging mass spectrometry. Significant differences in N-glycan and N-glycan class intensities were determined by linear regression modelling at a 10% false discovery rate.

### Preliminary data (results)

A recently developed high-throughput and rapid method for biofluid N-glycan analysis by MALDI IMS was used, and displayed high sensitivity (over 125 N-glycans detected across all samples) and reproducibility (7% average coefficient of variation for the 20 most abundant N-glycans) with peptide N-glycosidase F treatment. Peptide N-glycosidase F is an enzyme that cleaves all N-glycans at the core of their structure, retaining the full composition of the N-glycan. When comparing the benign samples to the cancer samples (DCIS and IDC), two fucosylated, sulphated, bisecting N-glycans ( $m/z$  1952.6047 and  $m/z$  2265.6820) had lower intensities in the cancer samples.

The benign samples and DCIS samples had 13 N-glycans with significantly different intensities, while the benign and IDC samples had no N-glycans with significantly different intensities. This indicates that serum N-glycan analysis may be a valid approach for distinguishing patients with DCIS, but not IDC. When comparing the DCIS samples to the benign samples, a fucosylated di-sialylated bi-antennary N-glycan with and without a bisecting N-acetylglucosamine ( $m/z$  2594.9095 and  $m/z$  2391.8301, respectively) had a higher intensity. A series of fucosylated mono-galactosylated bisecting N-glycans ( $m/z$  2287.8192,  $m/z$  2265.6820, and  $m/z$  1952.6047) had a lower intensity in the DCIS samples compared to the benign. A high-mannose N-glycan ( $m/z$  1257.4226) had a lower intensity in DCIS samples as well. A receiver operating characteristic curve utilizing these six N-glycans, race, and BMI had an area under the curve of 0.790, displaying moderate discriminative power between the benign and DCIS samples.

### Please explain why your abstract is innovative for mass spectrometry?

Utilizing the speed and sensitivity of MALDI-IMS, serum N-glycans from clinically relevant patient groups were analyzed for intensity differences and breast lesion classification.

### Co-authors:

*Christine Laronga, Moffitt Cancer Center*  
*Peggi Angel, Medical University of South Carolina*  
*Anand Mehta, Medical University of South Carolina*  
*Richard Drake, Medical University of South Carolina*

Poster number: **LS-PB-158**

## **UTILIZING MALDI IMAGING MASS SPECTROMETRY TO ANALYZE PATIENT-MATCHED HEPATOCELLULAR CARCINOMA TISSUE AND SERUM N-GLYCOSYLATION**

Abstract ID: **550**

**Presenting author: Andrew Delacourt, Medical University of South Carolina, Department of Pharmacology**

### **Introduction**

The development of more robust and sensitive biomarkers is a focal point of cancer research to detect tumors at an earlier stage. Hepatocellular carcinoma (HCC) in particular has much better survival outcomes when detected earlier. The application of cancer-correlated N-glycosylation has shown significant promise in HCC biomarker development, and MALDI-IMS techniques have been developed to analyze N-glycosylation of both serum and tissue samples. In order to further apply N-glycosylation to HCC biomarker development, this work analyzes 26 patient-matched serum/tissue samples through MALDI-IMS. Previous studies have shown HCC tumor-associated N-glycosylation, although in a heterogenous manner related to genetic/molecular heterogeneity. Therefore, the tissue/serum samples are categorized into the Hoshida tumor subtyping system for analysis, which classifies HCC tumors based on genetic/molecular features.

### **Methods**

To analyze the N-glycosylation of HCC tissue and serum samples, established MALDI-IMS protocols were utilized on matched tissue/serum samples classified by the Hoshida subtyping system. Formalin-fixed paraffin embedded tissues were prepared through applying N-glycan cleaving enzymes PNGase F Prime™ and Sialidase Prime™, and application of alpha-cyano-4-hydroxyxinnamic acid (CHCA). Serum samples were prepared for MALDI-IMS total serum analysis or specific glycoprotein-capturing antibody arrays. Each sample was analyzed via a timsTOF Flex QTOF mass spectrometer (Bruker Daltonics) operating in positive ion mode. Data is analyzed utilizing SCiLS Lab 2022 (Bruker), and m/z peaks are assigned using GlycoWorkBench databases.

### **Preliminary data (results)**

This work applied recently published discoveries regarding subtype-dependent N-glycosylation of HCC tissues to patient-matched, genetically subtyped serum and tissue samples. Previous data has demonstrated that while fucosylation and branching of N-glycans are increased in HCC as a whole, it is in a heterogeneous manner that makes biomarker development difficult. Additional work attributed some of this heterogeneity, particularly regarding fucosylation, to differing genetic/molecular/histological features of the tumor. This analysis utilized the Hoshida HCC subtyping system, which classified tumors as subtype 1 (aggressive stromal), subtype 2 (aggressive stemness), or subtype 3 (well-differentiated). Through on-tissue MALDI N-glycan imaging, fucosylation was shown to be increased in S1 tumors and not increased in S2 tumors, demonstrating a difference between aggressive, clinically similar tumors.

To continue this work, we analyzed fucosylation of 78 subtyped HCC serum samples, 26 of which including matching tissue samples. Serum was analyzed through two distinct MALDI-IMS techniques developed to analyze serum N-glycosylation in a high-throughput manner appropriate for biomarker discovery, with focus on either total serum glycosylation or glycoprotein-specific N-glycosylation. This work validated what was seen on-tissue, that overall fucose expression on N-glycans is significantly different between S1 and S2 tumors. In a further step, patient-matching serum/tissue samples allowed for comparison of MALDI-IMS N-glycan data between serum and tissue of the same patient, which is novel. These samples demonstrated consistency in the fucosylation of glycoproteins between tumor and serum, with S1 tumors demonstrating higher expression of fucosylated N-glycans both on tissue and in serum compared to S2 tumors.

### **Please explain why your abstract is innovative for mass spectrometry?**

It is novel to utilize MALDI imaging mass spectrometry to analyze N-glycosylation of patient-matched serum and tissue samples for cancer biomarker development.

### **Co-authors:**

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours

Thursday 1 September 2022 from 14:00 to 15:30 hours



*Peggi Angel, Medical University of South Carolina, Department of Pharmacology*  
*Richard Drake, Medical University of South Carolina, Department of Pharmacology*  
*Yujin Hoshida, University of Texas Southwestern Medical Center, Department of Internal Medicine*  
*Amit Singal, University of Texas Southwestern Medical Center, Department of Internal Medicine*  
*Anand Mehta, Medical University of South Carolina, Department of Pharmacology*

Poster number: LS-PB-159

## ANALYSIS OF KIDNEY TRANSPLANT BIOPSY TISSUE: AIDING HISTOPATHOLOGY BY PROTEOMICS AND DEEP LEARNING TO IMPROVE PATIENT OUTCOME

Abstract ID: 705

**Presenting author: Rianne Hofstra, Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Amsterdam, The Netherlands, Department of Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands**

### Introduction

Transplantation is the preferred treatment for end-stage kidney disease, requiring life-long medication to suppress rejection. These medicaments have serious side-effects, in some patients they are less effective at treating rejection than anticipated. The cornerstone of current transplant diagnostics is pathological analysis of a biopsy. New methods have emerged to improve the challenging classification of patients. The most important is Deep Learning: machine learning for automated pattern recognition in digital pathology images. Deep Learning is often considered a blackbox, a powerful approach, but difficult to understand. An alternative approach is analysis of molecular data obtained by mass spectrometry. Molecular features can help understand differences among patients to clarify why some are more prone to rejection, while others have a greater risk of toxicity or viral infection.

### Methods

The molecular analysis of these biopsies tissues, which are preserved using formalin fixed paraffin embedding (FFPE) or fresh frozen (FF), needs to be consistent and quantitatively accurate. A concern is the impact formalin fixation has on the molecular composition of the sample. We have investigated and developed a suite of methods suitable for tissue (FF&FFPE) MS analysis. These methods consistently perform well with DDA and SWATH-MS analysis for high protein coverage and quantitative accuracy. DIA-NN in library-free mode was used for data acquisition.

### Preliminary data (results)

This research is part of the multidisciplinary DEEPGRAFT project (Kers et al. 2021 Lancet Digital Health). The overarching goal of DEEPGRAFT is to develop quantitative machine learning tools (deep learning in digital biopsy images coupled to molecular tissue analysis) that aid in clinical decision making in clinical practice. Here we present our approach to acquire quantitative and reproducible data from fresh frozen (FF) and especially difficult to handle formalin-fixed paraffin-embedded (FFPE) biopsy tissues (typically 1x10 mm tissue dimensions). Due to the crosslinks that are created in FFPE material, influencing the molecular composition, it was presumed that the analysis of FF would provide us with more information. However, the FFPE material is still able to provide us with a large amount of relevant protein identifications. Methods for direct analysis of tissues have also been reported (Dapic et al. 2022 Molecules) and additional changes to the methods will be reported at IMSC, concerning optimal tissues dimensions, size and area, with respect to column configuration and MS operation. Figure 1 shows the reproducibility once these optimal parameters were applied to our complex samples.

### Please explain why your abstract is innovative for mass spectrometry?

SWATH-MS on kidney biopsy samples is challenging, but feasible with potential for clinical translation. Our approach can help other histopathology fields suffering from low sample input and archived FFPE tissues

### Co-authors:

*Aleksandra Chojnacka, Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Amsterdam, The Netherlands*

*Irena Dapic, International Centre for Cancer Vaccine Science, University of Gdansk, 80-309 Gdansk, Poland*

*Jesper Kers, Department of Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands,*

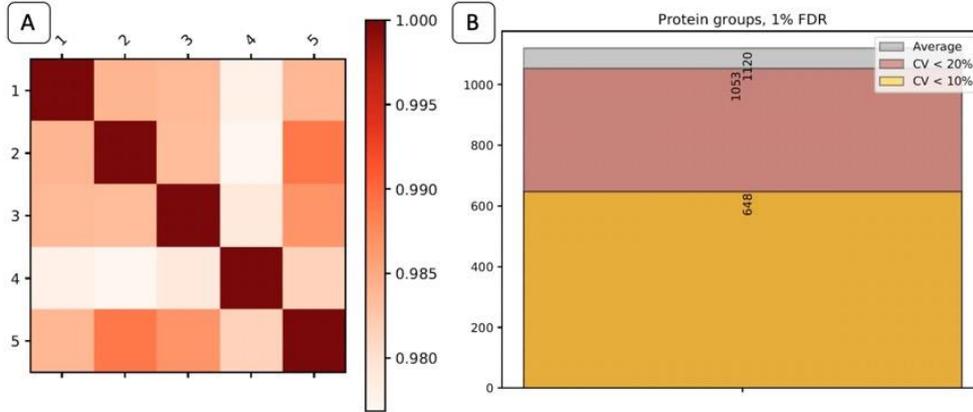
*Department of Pathology, Leiden University Medical Centre, Leiden, The Netherlands, Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Amsterdam, The Netherlands*

*Garry Corthals, Van 't Hoff Institute for Molecular Sciences (HIMS), University of Amsterdam, Amsterdam, The Netherlands*

**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Netherlands , Department of Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands,  
Maastricht MultiModal Molecular Imaging Institute, Maastricht University, 6229 ER Maastricht, The Netherlands*



Reproducibility of 5 injected tissues samples using our optimized parameters.

Poster number: **LS-PB-160**

## **MASS SPECTROMETRY HISTOCHEMISTRY FOR SINGLE CELL NEUROPEPTIDE IMAGING OF THE FFPE HUMAN BRAIN. COMPLEMENTING THE HUMAN BRAIN ATLAS**

Abstract ID: **707****Presenting author: Peter Verhaert, ProteoFormiX, M4i Maastricht University**

### **Introduction**

We previously developed a workflow for label-free top-down MS neuropeptide analysis in sections of formalin-fixed paraffin-embedded (FFPE) tissue. In analogy with immunohistochemistry (IHC), the method was designated mass spectrometry histochemistry (MSHC). MSHC paves the way for innovative (neuropeptide) biomarker research in pathology, as biopsy material from human clinical samples is rendered accessible for in situ (neuro)peptide studies. In addition, the vast collections of well-conserved human brain post mortem samples documented in biobanks such as at the renowned Netherlands Institute for Neuroscience, can now be incorporated in this type of biomolecular discovery investigations. The first step is to map all 'proteoforms' (or 'peptidoforms') of FFPE detectable neuropeptides throughout the human brain, complementing the existing human brain atlases with this biomolecular information.

### **Methods**

Sections (5µm) from selected human FFPE brain regions, including hypothalamus and pituitary, were mounted on regular (non-ITO) microscope glass slides. After deparaffinization through xylene and ethanol, and MALDI matrix spray-coating (DHB), MSHC was performed on a platform consisting of an AP MALDI UHR source fitted to an LTQ Orbitrap Velos. HRMS data were acquired at lateral resolution between 10 and 20µm. Data files (\*.raw and \*.xml) were processed by Mozaic software (Spectroswiss). Metabolite image data were annotated within METASPACE after conversion to \*.imzML. Adjacent sections were histochemically stained with H&E and with IHC using neuropeptide antibodies (Fig. 1).

### **Preliminary data (results)**

MSHC of a tissue section of 5x12.5 mm<sup>2</sup> at 20µm pixel size (scanning at m/z 150-2000) generates >4GB \*.raw and 11 MB \*.xml files (>68 h of continuous MS acquisition time). In human pituitary sections comprising neurohypophysis tissue processed through standard hospital FFP embedding protocols, vasopressin (VP) and oxytocin (OT), two disulfide-bridged neuropeptides, are unambiguously detected (<2ppm) by high resolution (HR) MSHC in neurosecretory nerve fibers (Fig. 1).

To find out whether MSHC allows for the detection of single neuronal cell bodies, we also focused on two regions in the hypothalamus where the perikarya are localized which produce these nonapeptides, i.e., nucleus paraventricularis and nucleus supraopticus. Averaging the complete (multi-GB) MS data does not allow for the neuropeptide MS peaks to be readily observed. Yet, mining the data for monoisotopic peaks (with the survey mass accuracy window of 20 ppm, and with post-acquisition re-calibration) suggests that neurosecretory nonapeptide ions were recorded at various x,y-coordinates along the extensive MS imaging runs. In addition, the MSHC distribution of the monoisotopic peptide peak corresponding to m/z [VP+H]<sup>+</sup> agrees with the IHC distribution of VP immunoreactive cell bodies as demonstrated on adjacent hypothalamic tissue sections. We will discuss the various modes of neuropeptide MS acquisition which can be optimized for more efficient (low abundant) neuropeptide signals detection, as well as different soft- and hardware features of our platform which can be exploited to increase the performance of MSHC (neuro)peptide workflows.

### **Please explain why your abstract is innovative for mass spectrometry?**

Single cell neuropeptide imaging of the human brain is achieved on histological sections of well-documented FFPE tissues from hospital biobanks.

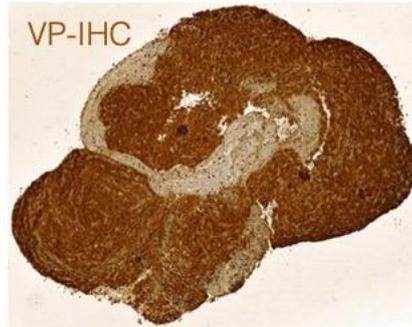
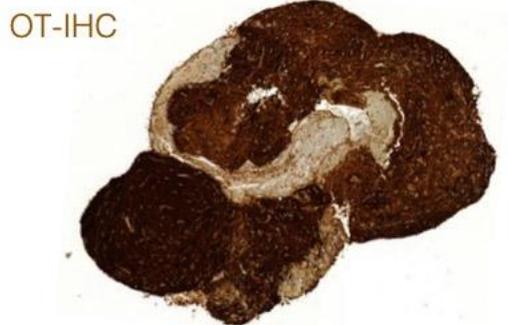
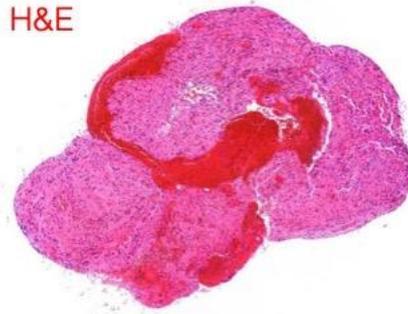
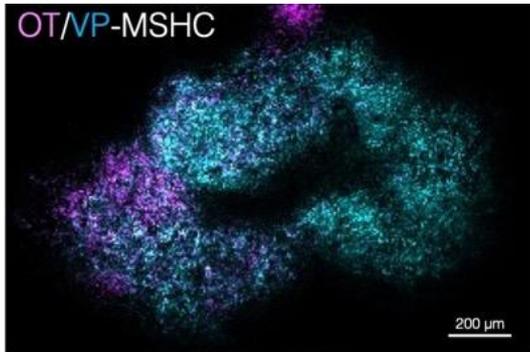
### **Co-authors:**

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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours

*Marthe Verhaert, University Hospital VUB, ProteoFormix*  
*Ling Shan, Netherlands Institute for Neuroscience*  
*Lin Zhang, Netherlands Institute for Neuroscience*  
*Olya Vvedenskaya, Spectroswiss*  
*Konstantin Nagornov, Spectroswiss*  
*Yury Tsybin, Spectroswiss*  
*Raf Sciot, University Hospital KUL*



Adjacent histological sections through FFPE-processed surgically resected human pituitary adenoma.

Poster number: LS-PB-161

## GANGLIOSIDE PROFILING IN CEREBRAL ORGANOID: EXPLORING AGING AND ALZHEIMER'S DISEASE RELATED CHANGES

Abstract ID: 732

**Presenting author: Durga Jha, RECETOX, Faculty of Science, Masaryk University**

### Introduction

The prevalence of neurodegenerative diseases is rising with the aging human population. However, currently used in-vitro and animal models have not succeeded in translating these diseases from the bench to the bedside. Development in three-dimensional cerebral organoid (CO) generation could revolutionize our understanding by recapitulating the critical aspects of brain development and marked signatures of neurodegenerative diseases such as Alzheimer's disease (AD).

In AD, progressive accumulation of amyloid-beta plaques leads to cellular degeneration, thus, dysregulating membrane lipid homeostasis. Since gangliosides are abundant in the neuronal membrane, the current study aims to analyze these lipids in wild-type (WT) and AD pathology, presenting COs to assess their utility as a functional 3D human neural tissue.

### Methods

We analyzed longitudinal changes in ganglioside levels in wild-type (WT) COs harvested at four time-points – 50, 85, 110, and 130 days. We also profiled the gangliosides in WT and AD COs with different ApoE genotypes at similar time points. There were four biological replicates per time point. Lipids were extracted from a lyophilized CO using 80% isopropanol and analyzed using ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) in positive and negative ion detection modes. The selective reaction monitoring (SRM) method was developed to quantify 42 GS molecular species.

### Preliminary data (results)

Profiling of the GS levels revealed an abundance of simple GSs such as GD3 and GM3 compared to the complex GSs in the COs. We observed a subsequent increase in all neuron-specific GS – GM1, GD1a, GD1b, and GT1b levels till D110, followed by a decline at later time points. Simple GSs such as GM3 and GD3 did not undergo massive changes with time. GSs such as GD2 and GM2 kept increasing till D160, resulting from the degradation of complex neuronal GSs.

Wild-type COs with ApoE3/4 genotype had higher levels of GSs compared to ApoE3/3. In the ApoE3/3 line, there was a higher GS level of GSs in AD COs than WT COs. This result contrasted with the ApoE3/4 line, where the AD COs had lower levels of GSs compared to WT. GSs of the same sub-class portrayed similar trends reported above, irrespective of their acyl chain lengths.

Correlation of the lipids with proteins revealed a strong correlation between longer acyl chains of GM3 and ApoE, probably due to their co-localization in astrocytes.

### Please explain why your abstract is innovative for mass spectrometry?

We profiled multiple ganglioside molecular species using the UHPLC-MS/MS (SRM) method in a single cerebral organoid with WT, AD pathology, along with different ApoE variants.

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*Tereza Váňová, Dept. of Histology and Embryology, Faculty of Medicine, Masaryk University, International Clinical Research Center (ICRC), St. Anne's University Hospital*

*Dáša Boháčiková, Dept. of Histology and Embryology, Faculty of Medicine, Masaryk University, International Clinical Research Center (ICRC), St. Anne's University Hospital*

Poster number: LS-PB-162

## BIOMARKER DISCOVERY OF MAJOR DEPRESSIVE DISORDER WITH SERUM OF USING METABOLOMICS ANALYSIS

Abstract ID: 769

**Presenting author: Seungyeon Lee, Department of Senior Healthcare, Graduate School, Eulji University - Uijeongbu Campus, Uijeongbu, Republic of Korea**

### Introduction

Major depressive disorder (MDD) is the most common mood disorder with symptoms of depression, and anxiety. Diagnosing MDD is based on subjective evaluations of clinicians and patients, such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) and Beck Depression Inventory (BDI). However, there is still no specific diagnostic method for MDD. Because MDD exhibits clinical heterogeneity between individuals, it is important to find biomarkers specific for MDD based on biological understanding. Metabolite analysis is a tool that can identify different biological systems for each individual and explore evidence of heterogeneous clinical features based on measuring the participants, intermediates and products of biological pathways. In this study, we aim to discover potential biomarkers of MDD through metabolite analysis.

### Methods

The discovery set consisted of 32 MDD patients and 26 controls, and the validation set consisted of 45 MDD patients and 35 controls. After extracting metabolites from the serum of participants belonging to the discovery set, filtering to remove impurities, and then profiling the metabolites through liquid chromatography-tandem mass spectrometry (LC-MS/MS). Thereafter, statistical analysis was performed to identify metabolites that showed a significant difference between the MDD patient and the control groups. and biomarker candidates were selected through the process of identification using a database. Afterwards, multiple reaction monitoring (MRM) was conducted to validate the selected candidates.

### Preliminary data (results)

The total number of molecular features (MFs) identified in the discovery set was 62901, and the number of MFs present in the MDD or control group was filtered to 1331. The volcano plot analysis was performed with the filtered list of MFs, and 61 MFs passed the fold change > 2.0 and p-value < 0.05 conditions, among which 53 showed an increase in MDD and 8 showed a decrease in MDD. In the subsequent sparse partial least squares discriminant analysis (sPLS-DA), MFs constituting the first component that contributed the most to the distinction between MDD and control groups were listed. A total of 168 candidates were listed by integrating the Volcano plot analysis and the list of MFs culled from sPLS-DA. Then, the metabolites were identified in an online database. Finally, acetylcarnitine was selected as a potential biomarker candidate and validated using MRM. As a result, it was confirmed that the distinction between MDD patients and controls through acetylcarnitine was statistically significant even in a larger sample set.

### Please explain why your abstract is innovative for mass spectrometry?

Profiling and quantifying metabolites using mass spectrometry to discover potential biomarkers for specific diagnosis of MDD. The discovered biomarkers will be available for specific diagnosis of MDD.

### Co-authors:

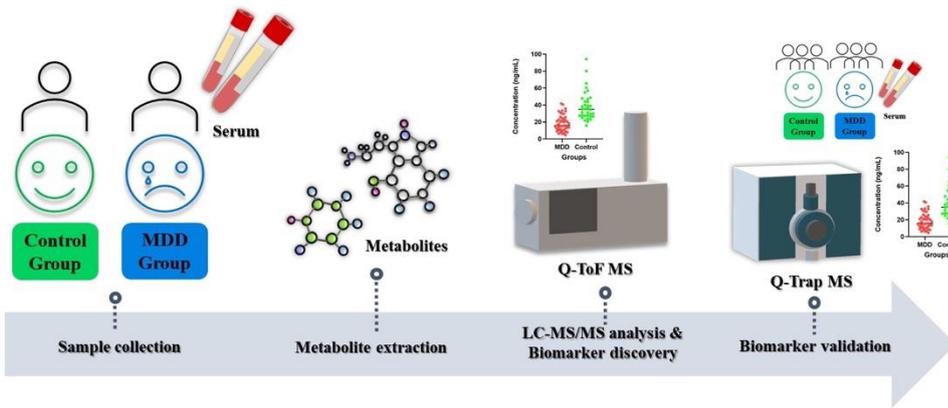
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**POSTER SESSION B**

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Poster number: **LS-PB-163**

## **IOHEXOL QUANTITATION AND POSSIBLE DEGRADATION KINETICS IN HUMAN URINE USING MASS SPECTROMETRY COUPLED TO LIQUID CHROMATOGRAPHY (LC)**

Abstract ID: **866****Presenting author: Philippe Massonnet, Department of Clinical Chemistry - CHU de Liège**

### **Introduction**

Iohexol is a well-known marker used to evaluate glomerular filtration rate (GFR) which is one indicator of kidney function. This GFR is often calculated using Iohexol intensity decay calculated using LC-MS/MS approaches on human plasma and urines. In these approaches, urines or plasma are taken from patients who were administered Iohexol at different timepoints and Iohexol is quantified at each time using one MRM approach. Once those values obtained, kinetics can be performed and GFR is calculated. However, some discrepancies can occur between urine and plasma results from the same patient and no study clearly explained this. Based on this, this work aims at assessing molecule profile variations occurring over time with patients that took Iohexol using LC coupled with high resolution mass spectrometry.

### **Methods**

In this project, urine samples are taken at given timepoints from patients who received Iohexol. The samples are first centrifuged, and the supernatant is diluted 100 times with water before injection in a NanoACQUITY UPLC system coupled with a SYNAPT XS instrument operating in positive ion mode. The mobile phases are composed of water (+0.1% formic acid) and of acetonitrile (+0.1% formic acid). Standard samples (commercial Iohexol drug) are also analyzed as quality control.

### **Preliminary data (results)**

The Iohexol LC-MS/MS method on a triple quadrupole instrument has successfully been implemented on the SYNAPT XS – NanoACQUITY UPLC system. The first results on different patient urines show the presence of other peaks than Iohexol in patient samples. Interestingly, those peaks are not present in standards and in urines of patients who did not receive the drug. Investigation of all mass spectra is in progress and these results open the possibility for a large screening over time. Comparison with data obtained using LC-MS/MS is also in progress and the next step is the comparison with data obtained on plasma samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

Use of high-resolution mass spectrometry coupled to liquid chromatography for drug kinetic assessment in human fluids.

### **Co-authors:**

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*Jordi Farre Segura, Department of Clinical Chemistry - CHU de Liège*  
*Pierre Delanaye, Department of Nephrology-Dialysis-Transplantation - CHU de Liège*  
*Caroline Le Goff, Department of Clinical Chemistry - CHU de Liège*  
*Etienne Cavalier, Department of Clinical Chemistry - CHU de Liège*

Poster number: **LS-PB-164**

## CONTRIBUTION OF MASS SPECTROMETRY IN THE FIELD OF CARDIOVASCULAR RISK IN CHRONIC KIDNEY DISEASE: FOCUS ON HDL PROTEIN CARGO

Abstract ID: **876****Presenting author: Frédéric Delolme, Protein Science Facility, SFR BioSciences CNRS UMS3444, Inserm US8, Université Lyon 1, ENS de Lyon**

### Introduction

Chronic kidney disease (CKD) is associated with an increased cardiovascular risk as it exposed patients to an accelerated onset of atherosclerotic and atherothrombotic lesions. Altered biological properties of high-density lipoproteins (HDL) may play a role in these pathological phenotype. Indeed, several evidences of the impaired biological functions of HDL were highlighted so far. Modification of HDL protein cargo and post-translational modifications of the latter were highlighted as potential mediators of HDL dysfunction. This study aimed to i) describe the HDL proteome from non-diabetic haemodialysis patients (HD) and identify potential pathways affected by the dysregulated expression of HDL protein and ii) describe the adductome of HDL from non-diabetic hemodialysis patients with a focus on glycation and the nature of the modified proteins.

### Methods

HDL were sampled from the plasma of 9 non-diabetic hemodialysis and 8 potential kidney-donors patients with a sequential potassium bromide stepwise density gradient ultracentrifugation. After reduction/alkylation/digestion steps, the samples were analysed on a nano-RSLC coupled with a hybrid quadrupole-Orbitrap Q-Exactive HF. Data were processed by database searching using SequestHT against a human Swissprot database and quantified with a label-free quantification approach. Oxidation, acetylation, carbonylation (by 4-HNE), carbamylation, glycation (carboxy-methyl-lysine, carboxy-ethyl-lysine, Glyoxal-H1, Methyl-Glyoxal-H1, Fructosyl-Lysine), guanydination, chlorination, nitration and nitrosylation were investigated. Protein quantitation was based on pairwise ratios and ANOVA hypothesis test.

### Preliminary data (results)

522 proteins were identified in the proteomic study of HDL. Among these proteins, 151 proteins were found in every sample and 326 were present in at least five of the eight control and six of the nine HD patients (60% of the samples). Among these 326 proteins, 10 were significantly upregulated and 9 downregulated in HD patients compared to the control patients ( $p < 0.05$ ). Up and downregulated proteins were involved in lipid metabolism, haemostasis, wound healing, oxidative stress, and apoptosis pathways.

We found as well, among the 522 proteins, 40 (i.e. 11%) that presented adduction sites. The main post-translational modifications were glycation (26%), guanidinylation (17%), carbamylation (15%), nitration (14%), carbonylation by 4-HNE (11%), nitrosilation (9%) and chlorination (8%). Those proteins were involved in lipid metabolism, acute phase response, haemostasis, wound healing and muscular metabolism. Regarding the amount of amino-acids in the protein sequence, apolipoprotein A2 and 1 were the proteins the more prone to adduction (28 and 27% respectively) followed by serum albumin (15%), apolipoprotein C3 (9%) and serum amyloid A4 (8%). Most of the key-proteins of HDL metabolism were found to be adductable.

### Please explain why your abstract is innovative for mass spectrometry?

Confirmation that HDL protein cargo is deeply modified in CKD and is involved in CV burden in this population with a focus on the relevance of glycation modification.

### Co-authors:

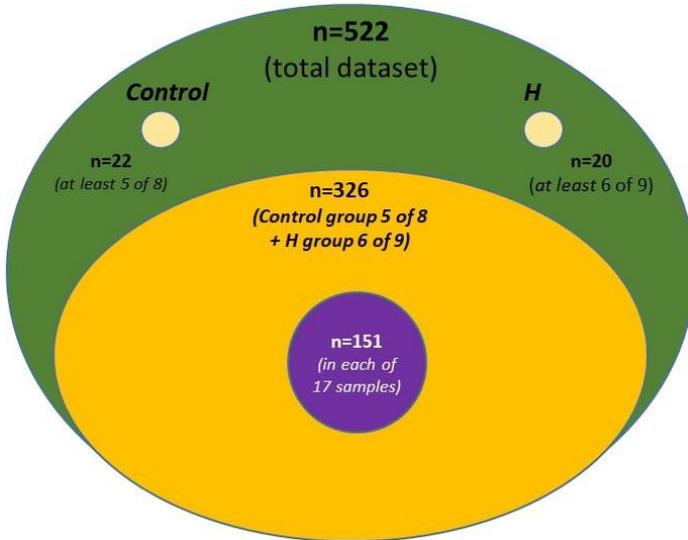
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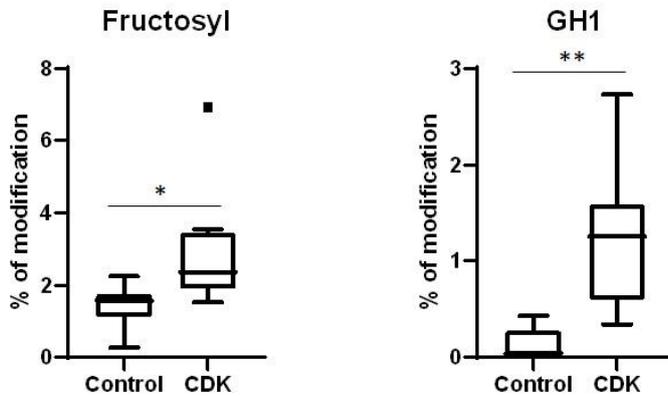
Catherine CALZADA, Univ. Lyon, CarMeN, INSERM U1060, INSA de Lyon, Université Claude Bernard Lyon 1, INRA U1397

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HDL proteome composition



Increase of glycation modification in CDK patients vs kidney-donors patients

Poster number: **LS-PB-165**

## PULMONARY AND RENAL LONG COVID AT TWO-YEAR REVISIT

Abstract ID: **880****Presenting author: Xiao Liang, Westlake University**

### Introduction

More than 460 million people have been affected by COVID-19 as of 19 March 2022, with more than 98.5% of these cases cured. Long COVID symptoms emerged in over 70% of the recovered patients and have become a global concern. These symptoms usually persisted for several months. Within them, long-lasting lung lesions had been reported up to 12 months after discharge, with 24% of the enrolled patients exhibiting unresolved radiological changes. Renal long COVID were reported up to 6 months after discharge, with 22% of the cases exhibiting persistent renal dysfunctions since the disease onset. Nevertheless, due to the limitations of monitoring timespan, longer-term consequences of these long COVID symptoms are unclear. Also, the molecular mechanisms underlying long COVID remain elusive.

### Methods

In this study, we applied cutting-edge mass spectrometry (MS)-based multi-omics technologies (proteomics and metabolomics) to the analysis of 991 serum and urine specimens from 144 COVID-19 patients who had been monitored for 763 days. These multi-omics data were integrated with clinical manifestation, including 182 clinical indices and 435 chest CT scan images.

### Preliminary data (results)

Abnormal clinical indices were detected in over 30% of the patients, involving lung, kidney, and liver. Pulmonary and renal long COVID of one-year revisit can be predicted by a machine learning model based on clinical and multi-omics data collected during the first month from the disease onset with an ACC of 87.5%. Proteomics revealed that lung fibrous stripes was related to continuous down-regulation of SFTPB in the sera, which might be a potential therapeutic target for pulmonary long COVID. Notably, our data show that all the patients with persistent pulmonary ground glass opacity or patchy opacity lesions developed into pulmonary fibrous stripes at two-year revisit. Together, this study depicts the longitudinal clinical and molecular landscape of COVID-19 with up to two-year follow-up and presents a method to predict pulmonary and renal long COVID.

### Please explain why your abstract is innovative for mass spectrometry?

We integrated a mass spectrometry-based proteomic and metabolomic data resource to enable multi-dimensional interpretation of long COVID.

### Co-authors:

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## Pulmonary and Renal Long COVID at Two-year Revisit

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Poster number: **LS-PB-166**

## **FAIMS-PRM IMPROVES PROTEIN LIMITS OF DETECTION AND QUANTIFICATION IN NATIVE PLASMA**

Abstract ID: **901**

**Presenting author: Simonas Savickas, Biognosys AG**

### **Introduction**

The PQ500™ Assay Panel was designed for comprehensive proteomics approaches. Data can be acquired in a targeted fashion, i.e. via Multiple Reaction Monitoring (MRM, also called SRM) and Parallel Reaction Monitoring (PRM) – which focus on quantifying predefined sets of plasma proteins with high sensitivity and reproducibility. PQ500™ data extraction can be improved by ion mobility enabled devices, i.e. FAIMS (Field Asymmetric Ion Mobility Spectrometry). Which uniquely increases the sensitivity of the targeted assays by reducing noise. Here, we showcase an application of FAIMS-PRM to our PQ500™ kit in native plasma.

### **Methods**

PQ500™ peptides are spiked into a trypsinized native human plasma of 5-fold dilutions, 3 replicates. Each plasma dilution is invertedly matched with peptides from chicken plasma. 1ug of peptide matrix is loaded onto the column, eluted at 250nl/min over 40min, and analyzed using Thermo Fisher Exploris 480 mass spectrometer. We identify the perfect Compensation Voltage (CV) of PQ500™ synthetic peptides using Single Ion Monitoring FAIMS CV ramp method. It follows a comparison of two high resolution mass spectrometry-based methods w/ and w/o FAIMS device. Collected raw data is analyzed using FAIMS supported SpectroDive 11.

### **Preliminary data (results)**

SIM scan indicated a gaussian distribution of targets raw intensity across CV ramp from -100 to 0. The average target FWHM is 14.4 (+/-2) CV values of selected peptides using "Standard resolution" option. The optimized targeted assay with perfect CV values shows an increase in sensitivity of FAIMS assisted PRM method. In very low concentrations of native human plasma (<0.02%) unproductive ion intensity coming from co-isolated human and chicken plasma peptides is very efficiently removed, when usually they are co-isolated in a classical PRM. Among quantified targets in plasma were low abundance proteins as IL6 & ANT3. They were confirmed according to SIS peptides, spectrum library, and response to their native plasma dilution. FAIMS device has improved the LOD & LOQ of some targets up to 5 fold. In one example, we were able to confidently quantify down to 0.14amol of endogenous ANT3 eluted from the column, which amounts to approximately 84000 copies of the protein. Therefore, the improved sensitivity allows us to observe down to 589pg of a single protein in 1ml of native plasma. However, some peptides limit of detection does not improve nor the noise level decreases after the addition of FAIMS device to PRM analysis. With the results at hand we highlight the merit of the method to endogenously quantify low plasma proteins using PQ500 kit. Taken together, FAIMS technology allows lower limits of detection for targets masked by co-eluted peptides. Which ultimately gives access to lower abundant proteome in native plasma samples.

### **Please explain why your abstract is innovative for mass spectrometry?**

FAIMS device connected to Exploris 480 reduces the level of ionization noise entering the Orbitrap analyzer, which correspondingly improves raw intensity of the product ions.

### **Co-authors:**

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Poster number: LS-PB-167

## LONG-LIVED PROTEINS AND DNA IN TISSUES AND THE PERIPHERY AS PREDICTIVE DISEASE BIOMARKERS

Abstract ID: 918

**Presenting author: Christoph W. Turck, Max Planck Institute of Psychiatry, Munich, Germany, Silantes GmbH, Munich, Germany**

### Introduction

Protein turnover is an important mechanism to preserve cellular integrity. The majority of cellular proteins are broken down over a period of days to weeks to prevent a buildup of proteins that lose their function due to damage caused by misfolding, modifications or degradation. Only a small fraction of the proteome is long-lived. In the current study we have subjected mice to *in vivo* stable isotope labeling. Ten tissues and plasma were then analyzed by mass spectrometry and interrogated for long-lived proteins and DNA. The combined data reveal tissue proteins that may be involved in pathology due to their low degree of renewal and the resulting increased risk for incurring damage.

### Methods

For the determination of long-lived proteins and long-lived DNA, mouse pups, immediately after birth, were initially exposed to stable isotope labeling through the breast milk of the mother that was fed a  $^{15}\text{N}$ -diet. Following weaning, the pups were then fed the same diet until postnatal day 89. Individual tissues and plasma were isolated and proteins and DNA processed for mass spectrometry analyses. Protein tryptic digests were subjected to deep profiling shotgun mass spectrometry. DNA from the same tissues was hydrolyzed and the isolated deoxynucleosides served as proxy for DNA  $^{15}\text{N}$  incorporation levels determined by targeted mass spectrometry.

### Preliminary data (results)

The number of long-lived proteins (LLPs) differed between tissues, with some shared by almost all tissues. Their functional analysis and protein-protein interaction networks suggest that LLPs that are found in almost all tissues are mainly involved in 'nucleosome organization', 'ubiquitin mediated proteolysis' and 'proteasome'. This is of interest due to the reported involvement in aging. Interestingly, a significant percentage of LLPs was detected in plasma. The majority of the LLPs identified in plasma are also present in most of the tissues and belong to the cytoskeleton and desmosome. These proteins are made very early in development during organ formation and apparently a significant fraction is not turned over. The third significant group of plasma LLPs are involved in 'protein homeostasis', a central cellular mechanism essential for many physiological processes and critical for preventing an accumulation of misfolded, damaged or aggregated proteins. Dysfunctional protein homeostasis is presumed to be a major cause for aging and disease. The relative quantification of non-labeled and labeled DNA-derived deoxynucleosides from the same tissues by mass spectrometry provided information about cellular renewal and showed good correlation with long-lived proteins in the brain. After 89 days, by far the most non-labeled DNA is detected in the brain which is consistent with its low cellular renewal.

### Please explain why your abstract is innovative for mass spectrometry?

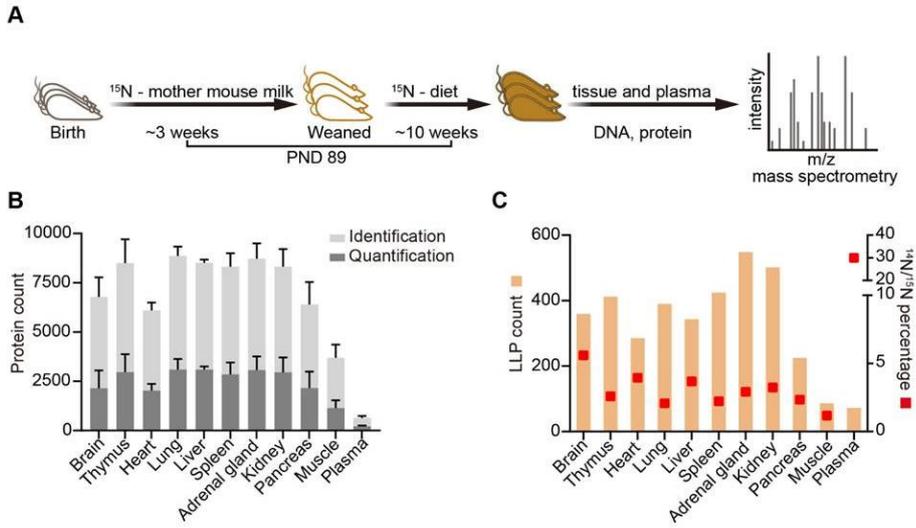
$^{15}\text{N}$  labeling of live animals allows the delineation of not only protein but also DNA renewal in tissues and body fluids by assessing  $^{14}\text{N}/^{15}\text{N}$  peptide and deoxynucleoside ratios, respectively.

### Co-authors:

Xiaosong Liu, *Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China*  
Bozidar Novak, *Max Planck Institute of Psychiatry, Munich, Germany*  
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POSTER SESSION B

Wednesday 31 August 2022 from 14:00 to 15:30 hours  
Thursday 1 September 2022 from 14:00 to 15:30 hours



Identification of long-lived proteins in mouse tissues and plasma.

Poster number: **LS-PB-168**

## **MASS SPECTROMETRY EVALUATION OF TYPE I COLLAGEN IN-SITU DIGESTION IN BIOLOGICAL MATRICES**

Abstract ID: **925**

**Presenting author: Justine Demeuse, CHU Liège, Department of Clinical Chemistry , ULiege, CIRM, Laboratory of Clinical Chemistry**

### **Introduction**

Type I collagen crosslinked telopeptide (b-CTX) is a biomarker commonly used to monitor compliance of osteoporotic patients to antiresorptive therapy. It is currently used by an algorithm recommended by the IOF and the IFCC. Three immunoassays are currently commercially available for the quantitation of b-CTX. However, plasma and serum samples collected from the same patients do not provide the same concentration and do not degrade at the same rate. As b-CTX is an uncharacterized fragment of the C-terminal telopeptide of type I collagen, it was decided to study the in-situ digestion of type I collagen in plasma and serum in order to explain the differences between both matrices.

### **Methods**

One pool of EDTA plasma and one pool of serum were prepared with remnant samples from hemodialyzed patients in order to obtain high concentrations of peptides. Pools were precipitated with zinc sulfate and acetonitrile, evaporated to dryness and reconstituted with 1 mL of injection solvent. Each reconstituted pools was then analysed using a LC-HDMSe (SYNAPT XS, Waters) workflow. PLGS software was then used to identify the fragments belonging to type I collagen.

### **Preliminary data (results)**

Fragments from all the different regions of the type I collagen (namely the telopeptides and the helix) were identified in both matrices. Fourteen peptides were found in EDTA plasma while twenty-nine were identified in serum. This difference could be explained by the fact that divalent ions such as  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  are chelated in EDTA plasma thus preventing proteolytic enzymes present in blood to cleave the type I collagen. Further purification steps will be considered in order to identify more peptides from type I collagen in both matrices by decreasing ion suppression triggered by highly concentrated peptides. Purification by preparative liquid chromatography and immunocapture will be planned.

### **Please explain why your abstract is innovative for mass spectrometry?**

Use of LC-HDMSe approach for the assessment of in-situ digestion of type I collagen in human matrices.

### **Co-authors:**

*Elodie Grifnée, CHU Liège, Department of Clinical Chemistry*

*Philippe Massonnet, CHU Liège, Department of Clinical Chemistry*

*Loreen Huyghebeart, CHU Liège, Department of Clinical Chemistry*

*Caroline Le Goff, CHU Liège, Department of Clinical Chemistry*

*Etienne Cavalier, CHU Liège, Department of Clinical Chemistry, ULiege, CIRM, Laboratory of Clinical Chemistry*

Poster number: **LS-PB-169**

## **SINGLE SHOT QUANTIFICATION OF 4000 PROTEINS IN BLOOD PLASMA USING DATA INDEPENDENT ACQUISITION MASS SPECTROMETRY**

Abstract ID: **942**

**Presenting author: Lukas Reiter, Biognosys**

### **Introduction**

Human blood is a readily available, highly relevant sample type carrying information from virtually all parts of the human body. Until recently, single shot proteome coverage in human blood using mass spectrometry was rather limited due to the large dynamic range of protein abundance, spanning more than ten orders of magnitude. Improvements at every step of the workflow have led to huge increases in proteome coverage in the last year. We show evidence that this is due to the particular shape of the protein abundance distribution of blood. Finally, we show some data how modern workflows can quantify 4000 plasma proteins using single shot data independent acquisition mass spectrometry.

### **Methods**

Human plasma samples were measured using Biognosys' TrueDiscovery platform. In short, samples were depleted and downstream sample prep was performed in 96-well format. Samples were analyzed on a Thermo Scientific EASY-nLC 1200 coupled to a Thermo Scientific Orbitrap Exploris 480. A FAIMS Pro was connected to the Exploris 480. LC-MS DIA methods were optimized for the respective gradient lengths. DIA data was either directly searched using directDIA or analyzed with a library using Spectronaut (Biognosys).

### **Preliminary data (results)**

We optimized every step of a single shot data independent acquisition mass spectrometry workflow including sample preparation, liquid chromatography separation, ion mobility separation (FAIMS), mass spectrometry acquisition and data analysis.

We show that with this optimized workflow we can quantify 4000 plasma proteins in a human cancer plasma study. This corresponds to a 800% improvement compared to the roughly 500 proteins which were achieved few years ago using single shot acquisition workflows.

We show evidence that the massive gains in proteome coverage with this optimized workflow are caused by the inherent protein abundance distribution of plasma. The data indicate that current workflows reached a dynamic range such that the lower end is at the point of highest protein density in plasma and hence any improvements in dynamic range lead to significant increases in proteome coverage.

Going forward we see possibilities for improvements on every step in the workflow cumulatively pushing the boundaries of unbiased, ultra-deep plasma profiling in the coming years. Proteomics in plasma will become an indispensable tool for precision medicine and will deliver biomarkers used as surrogate endpoints in clinical trials.

### **Please explain why your abstract is innovative for mass spectrometry?**

A high throughput sample preparation and LC-MS setup is estimated to profile up to 4000 proteins in human plasma studies

### **Co-authors:**

*Marco Tognetti, Biognosys*  
*Christopher Below, Biognosys*  
*Sebastian Müller, Biognosys*  
*Roland Bruderer, Biognosys*

Poster number: **LS-PB-170**

## PROTEIN DEPOSIT TYPING IN CARDIAC AMYLOIDOSIS BY S-TRAP-BASED MICROPROTEOMICS WORKFLOW

Abstract ID: **993****Presenting author: Francesco Greco, Sant'Anna School of Advanced Studies, Pisa, Fondazione Pisana per la Scienza ONLUS**

### Introduction

Cardiac amyloidosis is a disease characterized by the deposition of proteinaceous material in the extracellular space of cardiomyocytes, which cause heart muscle function disruption and cardiac dysfunction. Transthyretin (ATTR) and immunoglobulin light chain (AL- $\lambda$  or AL- $\kappa$ ) amyloidosis are the most common amyloidosis types presenting cardiac involvement. Amyloid typing is crucial for patient management since ATTR and AL are characterized by different treatments and prognosis. Endomyocardial or subcutaneous fat biopsies are needed to identify the constituent proteins of the amyloid deposit, for which proteomics has quickly become the gold standard for amyloid typing. Here we present a laser-capture microdissection/microproteomics workflow based on S-Trap to characterize protein deposits in cardiac amyloidosis.

### Methods

Endomyocardial biopsies were collected from 13 cardiac amyloidosis patients, fixed in formalin, and embedded in paraffin. 8  $\mu$ m thick sections were mounted on glass slides and stained with Congo Red to highlight amyloid deposits. Congo Red positive areas were isolated by laser-capture microdissection and processed with a modified S-Trap protocol. Immunohistochemistry (IHC) was performed on consecutive sections.

### Preliminary data (results)

A microproteomics workflow for the typing of cardiac amyloidosis was developed. The protocol included FFPE endomyocardial biopsies sectioning, Congo Red staining, and the isolation of protein deposit by Congo Red autofluorescence-guided laser-capture microdissection. A modified S-Trap bottom-up microproteomics workflow was applied to the samples. Peptides were analyzed by nLC coupled with an Orbitrap Fusion mass spectrometer. The protein forming the amyloid deposit was defined by the most abundant amyloidogenic protein in the sample. 10 out of 13 samples showed a concordance between proteomics and immunohistochemical validation, with 8 ATTR cases, 1 AL- $\lambda$  case and 1 negative case. Only 1 of the remaining 3 cases were discordant (ATTR according to IHC and AL- $\lambda$  according to proteomics); for the remaining 2 cases either IHC or proteomics were inconclusive. ApoE, ApoA-IV and SAP, known to co-precipitate in the deposit and described as a characteristic amyloid protein signature, were detected in all samples. The principal advantage of the S-trap microproteomics workflow is that it is fast, sensitive, robust to co-factors that may be introduced during routines clinical sample handling, and does not require highly trained personnel.

### Please explain why your abstract is innovative for mass spectrometry?

We used for the first time a microproteomics workflow to characterize amyloid deposit in cardiac amyloidosis, demonstrating its full compatibility with typing of amyloidosis patients.

### Co-authors:

Angela Pucci, Dpt. of Pathology, Pisa University Hospital

Veronica Musetti, Fondazione Toscana Gabriele Monasterio

Asia Botto, Dpt. of Chemistry, University of Pisa, Fondazione Pisana per la Scienza ONLUS

Michele Emdin, Fondazione Toscana Gabriele Monasterio, Sant'Anna School of Advanced Studies, Pisa

Liam A. McDonnell, Fondazione Pisana per la Scienza, ONLUS

Poster number: LS-PB-172

## QUANTIFYING PERFORMANCE DIFFERENCES IN DECONVOLUTION FOR TOP-DOWN PROTEOFORM IDENTIFICATION

Abstract ID: 666

Presenting author: Kyowon Jeong, Tübingen University

### Introduction

Deconvolution plays a key role in the identification of top-down proteoforms. At both MS and MS/MS levels, software must infer charges (for example from isotope spacing), shift intensity from higher isotopes into the monoisotope, and decharge ions for representation at neutral or singly-charged mass. Because both FT- and TOF-based instruments are now used for top-down identification, deconvolution algorithms need to manage resolution and mass accuracy differences as well as artifacts and signal-to-noise variation. Comparison of spectra to appropriate sequences requires that precursor mass and charge are accurately inferred. Proteoform-spectrum matching can succeed only when fragments are not eliminated as noise. Because each proteoform identifier is coupled with its own deconvolution engine, researchers may be unaware that alternative deconvolution routes could improve their identification yield.

### Methods

Tested deconvolution engines included two by instrument vendors (Thermo Xtract and Bruker SNAP2/MaxEnt) and two from the open source community (FLASHDeconv and TopFD). These were paired with the TopPIC proteoform identifier. Support tools were created to convert from MGF to MSAlign format, to extract MSAlign data from Xtract Proteome Discoverer, and to combine duplicate MS2s prior to deconvolution. Metrics for comparison included scan and peak counts, identification overlap, and mass/charge distributions.

### Preliminary data (results)

We evaluated two datasets: PXD019247 (*E. coli*, Thermo Orbitrap Fusion Lumos) and PXD010825 (*S. scrofa*, Bruker maXis2). For the bacterial dataset, TopFD, FLASHDeconv, and Xtract handled deconvolution, but Bruker DataAnalysis replaced Xtract for *S. scrofa*.

The numbers of MS2 spectra and deconvolved mass distribution varied highly per tool per dataset (image 1 and 2). FLASHDeconv and Bruker reported 60-80% fewer MS2 spectra than the others due to summing duplicate MS2 scans, but they reported larger precursor masses. The Q-TOF mass distributions were higher than seen in the Orbitrap.

The precursor mass overlap between tools was very low. The number of universally observed precursor nominal masses for *S. scrofa* was only 157 while the total distinct mass count was 5465 (3% overlap). For *E. coli*, these values were 1726 and 16181 (10%), respectively. For only universally deconvolved MS2 spectra, the overlap increased to 8% for *S. scrofa* and 18% for *E. coli*. 10% and 20% of PrSM masses overlapped for *S. scrofa* and for *E. coli*, respectively. The unique proteoform count was not highly dependent on MS2 count. FLASHDeconv reported 123 proteoforms from 5328 deconvolved MS2s while TopFD reported 63 proteoforms from 15276 MS2s for *S. scrofa*. For *E. coli*, TopFD reported the largest number of proteoforms. The results suggest that deconvolution algorithms results are rather inconsistent and quality and error control should follow.

We also found that taking the moving average of MS2 spectra along retention time significantly improves signal-to-noise ratio and in turn deconvolution in Q-TOF datasets.

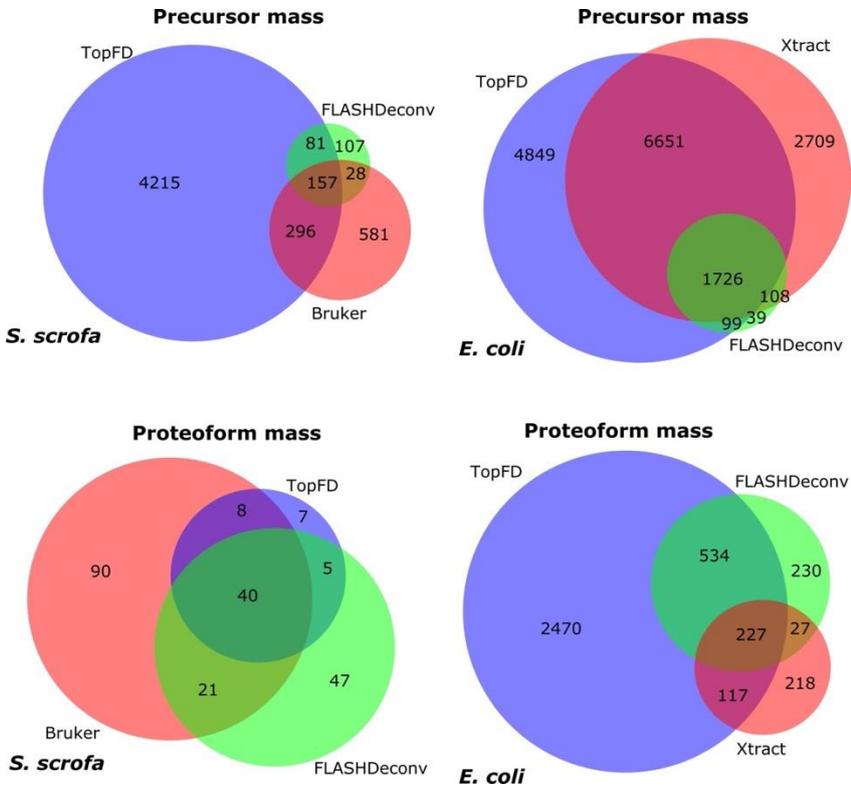
### Please explain why your abstract is innovative for mass spectrometry?

Top-down proteomics may fragment massive proteoforms but fail to deconvolute them correctly. We quantify retrieval of large ions via four different deconvolution engines in Q-TOF and FT experiments.

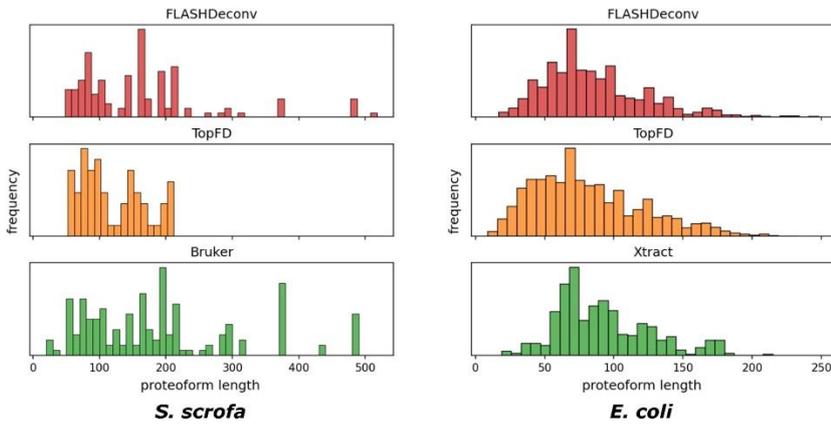
### Co-authors:

Jihyung Kim, Tübingen University  
David L Tabb, Institut Pasteur

Julia Chamot-Rooke, Institut Pasteur  
 Oliver Kohlbacher, Tübingen University



Venn diagrams for precursor and proteoform nominal masses



Proteoform sequence length distributions



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