27th meeting of the Belgian Society for Mass Spectrometry

Book of abstracts

Program of the day

9h00	Coffee & welcome
9h30	Opening
9h35	*Mass Spectrometry for Mechanistic Studies in Chemical Reactions*
	Prof Marianne Engeser – University of Bonn
10h20	*A case study of aptamer-target complexes characterization by kinetic capillary electrophoresis and ion mobility mass spectrometry*
	Hajjar Zeina – University of Liège
10h40	*Optimization of a liquid chromatography tandem mass spectrometry method for the enantioselective analysis of amino acids using a chiral derivatization reagent*
	Cinzia Lella – Vrije Universiteit Brussel
11h00	Coffee & networking
11h25	Flash presentations (2 min/speaker – *best poster award candidates*)
	Jelle Verdonck – KU Leuven
	Emma Piplart – University of Mons
	Linus Donvil – Vrije Universiteit Brussel
	Lisa Mussoi – University of Mons
	Eva Seyssens – University of Liège
	Pauline Blanckaert – University of Mons
	Ruth Kamguem Kamga – University of Mons
	Saral Bilal – Vrije Universiteit Brussel
	Louis Groignet – University of Mons
	Lena De Hondt – Vrije Universiteit Brussel
	Thomas Robert – University of Mons
11h55	Sponsor presentations (5min/speaker)
	Waters; Bruker; Agilent; MsVision; ThermoFisher Scientific; Sciex; Leco
12h30	Lunch, networking & posters

13h40	*Hydrogen/ Deuterium exchange coupled to Mass Spectrometry - A multifaceted tool to study membrane proteins* **Prof Chloé Martens** – Université Libre de Bruxelles
14h25	*Phenylazothiazole photoswitches on macromolecules for solar energy storage : photoisomerization and thermal back isomerization kinetics by MS* **Gwendal Henrard** – University of Mons
14h45	*HILIC-MS/MS-Based Strategy for Quantitative Amino Acid Profiling in Multiple Biological Matrices and Its Application in Kidney Disease Biomarker Discovery* **Xiongwei Yin** – KU Leuven
15h05	Coffee & networking
15h35	*Analyzing Protein Folding Dynamics Using Multi-Dimensional Varying Coefficient Models* **Jürgen Claesen** – Amsterdam UMC
15h55	*Structural Characterization of Dimeric Perfluoroalkyl Carboxylic Acid Using Experimental and Theoretical Ion Mobility Spectrometry Analyses* **Aurore L. Schneiders** – University of Liège
16h15	Poster award & closing ceremony
16h25	Farewell

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Keynote lectures

Prof Marianne Engeser – University of Bonn

Mass Spectrometry for Mechanistic Studies in Chemical Reactions

Prof Chloé Martens – Université Libre de Bruxelles

Hydrogen/ Deuterium exchange coupled to Mass Spectrometry - A multifaceted tool to study membrane proteins

Oral Communications

Oral 1 - Hajjar Zeina – University of Liège

A case study of aptamer-target complexes characterization by kinetic capillary electrophoresis and ion mobility mass spectrometry

Oral 2 - Cinzia Lella – Vrije Universiteit Brussel

Optimization of a liquid chromatography tandem mass spectrometry method for the enantioselective analysis of amino acids using a chiral derivatization reagent

Oral 3 - Gwendal Henrard – University of Mons

Phenylazothiazole photoswitches on macromolecules for solar energy storage photoisomerization and thermal back isomerization kinetics by MS

Oral 4 - Xiongwei Yin – KU Leuven

HILIC-MS/MS-Based Strategy for Quantitative Amino Acid Profiling in Multiple Biological Matrices and Its Application in Kidney Disease Biomarker Discovery

Oral 5 - Jürgen Claesen – Amsterdam UMC

Analyzing Protein Folding Dynamics Using Multi-Dimensional Varying Coefficient Models

Oral 6 - Aurore L. Schneiders – University of Liège

Structural Characterization of Dimeric Perfluoroalkyl Carboxylic Acid Using Experimental and Theoretical Ion Mobility Spectrometry Analyses

Posters + Flash presentation

Poster 1 – Jelle Verdonck – KULeuven

Coupling ICP-MS and LC-MS for Analysis of Cr Species in Parenteral Nutrition Solution

Poster 2 – Emma Piplart – University of Mons

Self-assembled monolayers based on Azobenzene derivatives as MOST systems

Poster 3 – Linus Donvil – Vrije Universiteit Brussel

The development of a targeted UHPLC-MS/MS method for the quantification of myelin basic protein size isoforms as a biochemical marker for differential diagnosis, disease progression and therapeutic monitoring in neurological disorders

Poster 4 – Lisa Mussoi – University of Mons

Discovering carotenoids in symbiotic crustaceans: qualitative and quantitative analysis by LC-MS

Poster 5 – Eva Seyssens – University of Liège

Characterization of peptides using bidimensional separation by preparative Centrifugal Partition Chromatography (CPC) and Capillary Zone Electrophoresis coupled with Mass Spectrometry (CZE-MS) produced during the revalorization of byproducts and leftovers from the agrifood industry

Poster 6 – Pauline Blanckaert – University of Mons

Design of a peptoid-based Artificial Catalyst inspired by Carbonic Anhydrase for CO_2 Capture

Poster 7 – Ruth Stella Kamguem Kamga – University of Mons

Design and Evaluation of Azobenzene-Functionalized Macrocyclic Peptoids as Tunable Molecular Photoswitches

Poster 8 – Sarah Billal – Vrije Universiteit Brussel

Development of an untargeted brain metabolomics method using supercritical fluid chromatography-mass spectrometry: screening and evaluation of potential chromatographic systems

Poster 9 – Louis Groignet – University of Mons

On the Impact of Post-Translational Succination on SUMO1 Structure: A Dual Approach Combining Gas-Phase and Solution Analysis

Poster 10 – Lena De Hondt – Vrije Universiteit Brussel

On the Impact of Post-Translational Succination on SUMO1 Structure: A Dual Approach Combining Gas-Phase and Solution Analysis

Poster 11 – Thomas Robert – University of Mons

On the determination of the activation energies for the thermal relaxation of photoisomers by state-of-the-art mass spectrometry methods

Posters

Poster 12 – Quentin Bastiaens – University of Liège

Mass Spectrometry-Based Imaging and Molecular Profiling of Snake Venom Effects on Murine Tissues

Poster 13 – Sarajit Naskar – University of Mons

Three-dimensional Structural Investigation of Polystyrene-based Gold Containing Single Chain Nanoparticles using a Multidisciplinary IMS-MS/MD Approach

Poster 14 – G. Scholl – University of Liège

Validation of a GC-MS/MS Method for the Quantification of Six Banned Smoke Flavoring Compounds in Traditionally Smoked Fish, Meat and Cheese

Poster 15 – Thomas Crasset – University of Liège

Development of an Analytical Framework to Evaluate Antivenom Efficacy through Magnetic Beads and BioLayer Interferometry

Poster 16 – Kristian Serafimov – UCLouvain

UCL-MetIsoLib: A Public High-Resolution Tandem Mass Spectrometry Library for HILIC-Based Isomer-Resolved Profiling of Glycolysis, Central Carbon Metabolism, and Beyond in Urine, Plasma, Tissues, Cells, and Patient-Derived Organoids

Poster 17 – Laetitia Ghiande – University of Mons

Development of a LC-MS/MS analysis method to quantify bufalin and marinobufagenin in plasma

Poster 18 – Maxime Benonit – University of Liège

Unfolding the Truth: α-Synuclein and A-Syn 1 Aptamer as a Model System to Explore Binding and Unfolding Dynamics in the gas phase

Poster 19 – Jan Claereboudt – Waters NV/SA

Novel semi-targeted mass spectrometry imaging approach using the SELECT SERIES MRT mass spectrometer with DESI XS source

Poster 20 – Lucia Cocorullo – University of Liège

MICROBoost: HILIC-FLD-MS/MS characterization of prebiotic oligosaccharides

Poster 21 – Steven Daly – MSVision

Introducing the first prototype of preparative native ion mobility mass spectrometer for mass and shape selected pseudomolecular ions for ion soft landing built from a substantially modified Waters Synapt G2s ("SoftSynapt")

Poster 22 – Blandine Chazarin – University of Mons

Plasma proteome characterization: each step is to be optimized

Poster 23 – Jan Claereboudt – Waters NV/SA

Parallel targeted and untargeted metabolite analysis of mouse plasma samples using a benchtop multi-reflecting time of flight mass spectrometer

Poster 24 – Jason Fauquet – University of Mons

From Folk Remedy to Discovery: An HPLC-HRMS Untargeted Metabolomics Workflow Unveil Species-Specific Metabolites in Sideritis herba

Poster 25 – Lou Freuville – University of Liège

Affinity-Capture Mass Spectrometry for the Discovery of Receptor-Targeting Peptides in Animal Venoms

Poster 26 – Jan Claereboudt – Waters NV/SA

Analysis of Per-and-polyfluoroalkyl substances in soil and wastewater/groundwater using a benchtop multi-reflecting Time-of-flight Xevo MRT mass spectrometer

Poster 27 – Hugo B. Muller – University of Liège

Gas Chromatography–Trapped Ion Mobility Mass Spectrometry: A Highly Specific and Ultra-Sensitive Platform for Quantifying Sub-ppt Levels of Dioxins and PCBs in Food

Poster 28 – Bastien Cabrera-Tejera – University of Liège

Development of a novel non-targeted approach in mass spectrometry for PFAS imaging

Poster 29 – Charles Delvaux – University of Liège

Thin layer chromatography coupled to MALDI in source decay imaging (TLC/MALDI-MSI-ISD) for whole sequence coverage of oligonucleotides

Poster 30 – Anisha Haris – Waters NV/SA

Novel heated inlet for in-source activation of native protein complexes

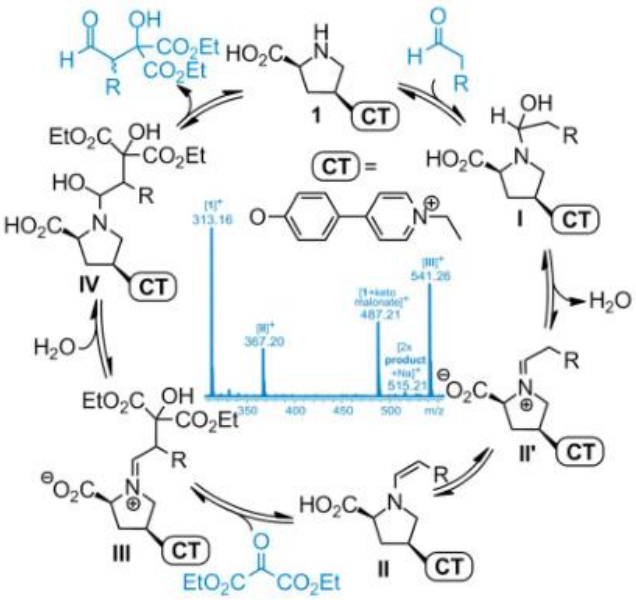
Keynote 1 - Mass Spectrometry for Mechanistic Studies in Chemical Reactions

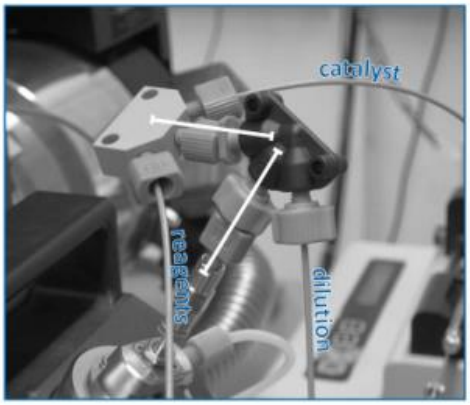
M. Engeser, J.A. Willms, R. Beel, A. Schnell, K. Schuppener, M.T.S Krause

Kekulé-Institute for Organic Chemistry and Biochemistry, University of Bonn, Germany

E-mail: marianne.engeser@uni-bonn.de

The development and optimization of new reactions often is a highly challenging task. Undirected screening of a multitude of different reaction parameters typically blocks a lot of resources without guarantee for satisfying improvements. In this context, mechanistic insight in kinetics, relevant intermediates, as well as side products or unproductive resting states can be of great help. Modern mass spectrometry offers powerful tools for mechanistic studies, particularly when different (complimentary) techniques are combined. The high sensitivity of electrospray mass spectrometry allows the detection of low-concentrated ionic species present in the reacting solution. Online microreactor techniques give access to short reaction times and reproducible detection of reactive intermediates. We have developed a charge-tagging strategy for proline organocatalysis and present experimental results on proline-catalyzed aldol- and Diels-Alder reactions. Further, a complex dual activation coupling and cyclization reaction catalyzed by gold and a proline-derived organocatalyst is addressed with a particular emphasis on the different roles the organocatalyst plays therein. For the copper-catalyzed oxidative derivatization of tetrahydroisoquinolines, mechanistic investigation by mass spectrometry combined with electrochemical techniques led us to an increased mechanistic knowledge based on which we could achieve a widely broadened and synthetically highly useful reaction scope.





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Keynote 2 - H/D exchange coupled to Mass Spectrometry: a multifaceted tool to study transport proteins.

Martens Chloé

Université Libre de Bruxelles

Transport proteins participate in a myriad of vital biological processes, including nutrient and drug import, cell-cell signaling and toxin export. This central role makes them an attractive class of drug targets and uncovering their molecular mechanism is an intense area of research. They are, however, notoriously difficult to work with, mainly due to their localization within the heterogeneous of environment of the biological membrane and instability once extracted from the lipid bilayer.

Hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) has recently emerged as a powerful method to investigate aspects eluding traditional biophysical tools, such as the conformational dynamics of membrane proteins and their interactions with substrates, ions and lipids. This technique measures the rate of H/D exchange of labile protons from backbone amides[1]. This exchange rate is directly correlated to solvent accessibility and local structural dynamics. Specifically, we have demonstrated that HDX-MS is an ideal tool to decipher the mechanism of membrane transporters[2].

They undergo important structural rearrangements, alternating between inward-facing (IF) and outward-facing (OF) states to actively shuttle their substrate across the biological membrane. Changes in the conformational landscape caused by different lipid environments lead to changes in global and local dynamics measurable by HDX-MS. In combination with predictions from molecular dynamics simulations, specific lipid-modulated changes in the conformational dynamics can be identified at the molecular level[3]. Similarly, the coupling between a substrate and an ion in a secondary transporter leads to a structural fingerprint that can be observed with this approach[4]. A summary of the insights that HDX-MS applied to membrane transporters can reveal, as well as the practical means to obtain those will be presented.

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Oral 1 - A case study of aptamer-target complexes characterization by kinetic capillary electrophoresis and ion mobility mass spectrometry

Hajjar Zeina[a], Davoine Clara[b], Kune Christopher[a], De Pauw Edwin[a], Far Johann[a], Fillet Marianne[b], Eppe Gauthier[a]

[a] *Mass Spectrometry Laboratory, MolSys Research Unit, Quartier Agora, University of Liège, Allée du Six Août 11, B-4000 Liège, Belgium.*

[b] *Laboratory for the Analysis of Medicines (LAM), Department of Pharmacy, CIRM, University of Liege, Belgium*

In recent years, oligonucleotide-based therapeutics—particularly aptamers—have gained growing interest due to their versatility, high affinity, and selectivity. However, designing and fully characterizing aptamers remain challenging, as their key properties depend on their higher-order structures (HOS) and thermodynamic behavior. As a result, the development of advanced analytical techniques capable of probing both the structure and binding dynamics of aptamers is essential. In this study, we investigated the aptamer-target complex formed between OTC5 and oxytetracycline (OXY), with a magnesium ion (Mg^{2+}) stabilizing the interaction. Our goal was to characterize the complex in both solution and gas phases using a complementary strategy combining kinetic capillary electrophoresis (KCE), MS-based titration and ion mobility mass spectrometry (IM-MS).

KCE analysis provided an electropherogram displaying the dissociation pattern of the complex during migration in solution. The profile exhibited three key zones: a peak corresponding to unbound OXY, a peak representing the intact complex, and a region indicative of OXY dissociating from the complex during migration. From the area under these zones, we applied two equations to determine the dissociation constant (Kd) and the dissociation rate constant (koff), yielding insight into the complex's thermodynamic and kinetic behavior in solution.

We carried out an MS-based titration to determine the gas-phase Kd. A series of equimolar mixtures of OTC5 and OXY were prepared at increasing concentrations with a fixed $MgCl_2$ concentration. For each spectrum, the intensities of free OTC5 and the formed complex were extracted, and the ratio between them was plotted against the total equilibrium concentration. Upon fitting the data, the binding pattern suggested a ternary 1:1:1 complex, involving one aptamer, one target molecule, and one Mg^{2+} ion. Simulations confirmed the formation of a stable three-component complex. These findings indicate that magnesium is an essential part of the complex.

To further investigate the role of Mg^{2+}, ion mobility spectrometry will be employed to assess potential conformational changes in OTC5 upon binding to the magnesium ion, and how these changes may affect its binding to the target and the global kinetic model.

Furthermore CE-UV titration will be performed taking into account the fact that the system forms a ternary complex, in order to compare the kinetic and thermodynamic parameters with those obtained from MS titration.

Oral 2 - Optimization of a liquid chromatography tandem mass spectrometry method for the enantioselective analysis of amino acids using a chiral derivatization reagent

Lella, Cinzia; [a] Nestor, Liam; [a] Hennecke, Ulrich; [b] Vander Heyden, Yvan; [c] Van Eeckhaut, Ann. [a]

[a] *Vrije Universiteit Brussel (VUB), Experimental Pharmacology Research Group (EFAR), Center for Neurosciences (C4N), Laarbeeklaan 103, Brussels, Belgium;*
[b] *Vrije Universiteit Brussel (VUB), Organic Chemistry Research Group (ORGC), Pleinlaan 2, 1050 Brussels, Belgium;*
[c] *Vrije Universiteit Brussel (VUB), Department of Analytical Chemistry,Applied Chemometrics and Molecular Modelling (FABI), Laarbeeklaan 103, 1090 Brussels,Belgium.*

Studying the complex neurochemical pathways in both healthy and diseased brains is crucial for improving our understanding of the mechanisms behind neurological disorders. The quantification of metabolites that act as neurotransmitters, gliotransmitters, orneuromodulators could help to identify novel drug targets and to develop new therapeutic strategies. Among these metabolites, D-amino acids have emerged as potential biomarkers, not only in metabolic disorders, but also in cognitive and neurodegenerative diseases. Therefore, enantioselective analysis of these compounds is essential to expand our knowledge of their biological roles under physiological and pathological conditions.

This study aims to develop an ultra-high-performance liquid chromatography - electrospray ionization - tandem mass spectrometry (UHPLC-ESI-MS/MS) method for the sensitive and enantioselective quantification of amino acids in volume-limited microdialysis samples. The preliminary results obtained during method development will be presented.

The detection and quantification of chiral amino acids entails a pre-column derivatization reaction with diacetyl-L-tartaric anhydride (DATAN) to form diastereomers and achieve their separation on an achiral stationary phase, enhancing both sensitivity and metabolite stability. Additionally, an isotope-coded DATAN derivative is synthesized to generate isotopically labelled internal standards for each amino acid, facilitating the correction for matrix effects.

Derivatization was carried out using (+)-DATAN on dried standard solutions of amino acids, incubated at 75°C for two hours. Analysis was performed in positive ionization mode using a UHPLC system coupled to a Xevo TQ-MS (Waters) with an Acquity BEH C18 column (2.1 mm × 100 mm, 1.7 µm, 130 Å) at a flow rate of 0.3 mL/min and column temperatures of 60°C and 30°C. Water and acetonitrile, both with 0.1% of formic acid, were chosen as mobile phases A and B, respectively. Instrumental parameters, including capillary voltage, cone voltage, collision energy, and MRM transitions, were optimized for each analyte. Preliminary results show successful enantioseparation of proline, phenylalanine, tryptophan and leucine, with the following elution gradient: 0-1 min, 0.2%B; 1-3 min, 0.2% to 5%B; 3-6 min, 5% to 10%B; 6-8 min, 10% to 15%B; 8-10 min, 15% to 20%B; 10-11 min, 20% to 95%B; 11-11.10 min, 95% to 99.8%B, 11.10-13 min, held at 99.8%B; 13-13.10 min, return to 0.2%B and re-equilibration. Moreover, partial separation was achieved for glutamic acid, cysteine, valine and methionine. Further optimization is ongoing to achieve complete enantioseparation of all proteinogenic amino acids.

Oral 3 - Phenylazothiazole photoswitches on macromolecules for solar energy storage : photoisomerization and thermal back isomerization kinetics by MS

[a,b]Henrard, Gwendal; [a]Robert, Thomas; [a,b]Tassignon, Benjamin; [a]De Winter, Julien; [b]Cornil, Jérôme; [a]Gerbaux Pascal

[a] *Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium;*

[b] *Laboratory for Chemistry of Novels Materials, Center of Innovation and Research in Materials and Polymers, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium*

Molecules that undergo light-induced isomerization to metastable isomers can be used to store solar energy. Such systems are known as MOlecular Solar Thermal systems (MOST). Exposing photoswitching molecules to sunlight generates high energy photoisomers whose lifetime is considered as a key criterion for storage purpose. When energy is needed, the photoisomer is converted back to the stable form, releasing the excess energy under the form of heat. Azobenzenes (AB) with their *E* → *Z* photoisomerization are among the most widely studied molecular photoswitches although properties such as the storage enthalpy, half-life time and optical properties need to be improved. Two strategies are considered in our group to optimize azobenzene-based chromophores for MOST applications; *i.e.* (i) the substitution of one phenyl group by a thiazole moiety to red shift the absorption of the chromophores in the visible region, and (ii) the grafting of several azobenzene residues at selected positions along a polymer backbone to enhance the storage enthalpy and the metastable isomer half-life time via cooperating effects. 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. Two different residues are incorporated in our photoactive peptoids; *i.e.*, (*S*)-phenylethylamine (N*spe*) and (E)-4-(thiazol-2-yldiazenyl) aniline (N2tz). Three peptoids have been successfully synthesized, namely N2*tz*N*spe*N*spe* (N-ter position), N*spe*N2*tz*N*spe* (center position) and N*spe*N*spe*N2*tz* (C-ter position). Their sequences were confirmed via tandem mass spectrometry (MS/MS) experiments, based on the B/Y, A/Y, and Side Chain Loss (SCL) fragmentation patterns. All peptoids were subjected to photo-illumination experiments. LC-MS experiments were carried out before and after irradiation to separate/identify/quantify the stereoisomers. The kinetics of thermal back-isomerization were studied by performing repeated LC- MS measurements while maintaining the peptoid methanolic solutions in the HPLC autosampler, kept in the dark and at a controlled temperature. E and Z isomers were mostly detected as protonated species $[MH]^+$. Extracted Ion Current (EIC) chromatograms were utilized to detect the stable and metastable isomer ions and determine the overtime evolution of their relative proportions. Repeating the kinetic measurements at different temperatures allows determining the kinetic parameters, including the activation enthalpy and entropy, of our MOST candidates, further affording evidences on the relaxation mechanisms.

Oral 4 - HILIC-MS/MS-Based Strategy for Quantitative Amino Acid Profiling in Multiple Biological Matrices and Its Application in Kidney Disease Biomarker Discovery

Xiongwei Yin, Erwin Adams, and Ann Van Schepdael

KU Leuven/ Pharmaceutical Analysis, Department of Pharmaceutical and Pharmacological Sciences Leuven, Belgium.

Amino acid metabolism disorders have strong links to various kidney diseases, and the kidney is essential for maintaining systemic amino acid homeostasis. Given the lack of obvious clinical symptoms in the early stages of kidney disease, searching for reliable metabolic biomarkers is essential for early diagnosis. Endogenous amino acids are widely present in human biological matrices, making it difficult to obtain analyte-free blank matrices for method validation. This poses challenges in the preparation of calibration standards, leading to uncertainty and reduced consistency in the results. This study aimed to develop a rapid hydrophilic interaction liquid chromatographic method coupled with tandem mass spectrometry (HILIC-MS/MS). It could analyze approximately 50 AAs and related compounds, present in biological matrices (e.g., human plasma, urine, and cyst fluids). The Stripped Matrix method was utilized to prepare blank matrices for constructing calibration curves. The slopes of the different calibration curves were then systematically compared, in comparison with the standard addition method, fully demonstrating the accuracy and reliability of the method. Method validation was conducted for the target analytes and comprised the following items: linearity (r^2 at least 0.99), limit of quantification, precision, accuracy, recovery, and sample stability. To further investigate the applicability of this method to large-scale sample analysis, the analysis of plasma samples, urine samples, and cyst fluid samples obtained from patients with two types of kidney disease were performed. Orthogonal partial least squares discriminant analysis (OPLS-DA) identified 11 potential differential metabolites with VIP scores > 1 from plasma samples, among which 6 showed statistically significant differences (p<0.05) upon further analysis. Kidney diseases may be influenced by multiple amino acid metabolic pathways. Besides validating the practicality of this approach, these exploratory results demonstrate that specific amino acids could be used to identify kidney disease biomarkers, providing a valuable tool for future metabolic research and quantitative analysis of amino acids.

Oral 5 - Analyzing Protein Folding Dynamics Using Multi-Dimensional Varying Coefficient Models

Claesen, Jürgen[a,b]

[a] *Amsterdam UMC, The Netherlands*
[b] *Hasselt University, Belgium*

Proteins are initially synthesized as unstructured polymers on ribosomes and fold into functional forms. The folding process is influenced by both intrinsic protein properties and interactions with external factors, leading to a range of behaviors from rapid folding to stable unfolding. Protein folding mechanisms can be investigated using pulsed hydrogen-deuterium exchange mass spectrometry (HDX-MS), which tracks both local and global exchanges. In a pulsed HDX experiment, proteins are exposed to deuterium for a set period, causing hydrogen atoms to exchange with deuterium and resulting in a measurable mass increase. This change in mass is captured by a mass spectrometer coupled with liquid chromatography (LC). By monitoring shifts in retention time and mass at various stages of the folding process, the mechanism and rate of folding can be determined. To examine folding differences across multiple proteins, we developed a multi-dimensional varying coefficient model. In this model, protein mass and retention time are treated as covariates within one-dimensional smooth functions. The product of these one-dimensional smooths creates a smoothed surface. We also incorporated interactions between categorical variables (protein identity and folding time) and the two smooths (mass and retention time), yielding multiple smoothed surfaces. The model, structured with main effects and interactions, allows for the estimation and testing of smoothed differences between the reference surface and other surfaces, with results evaluated using simultaneous confidence bands.

Oral 6 - Structural Characterization of Dimeric Perfluoroalkyl Carboxylic Acid Using Experimental and Theoretical Ion Mobility Spectrometry Analyses

[a] Aurore L. Schneiders, [a] Johann Far, [b] Lidia Belova, [c] Allison Fry, [b] Adrian Covaci, [c] Erin S. Bake, [a] Edwin De Pauw, [a] Gauthier Eppe

[a] *Mass Spectrometry Laboratory, MolSys Research Unit, Chemistry Department, University of Liège, Liège (4000);*
[b] *Belgium; Toxicological Centre, University of Antwerp, 2610 Wilrijk, Belgium;*
[c] *Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States*

Per- and polyfluoroalkyl substances (PFAS) are contaminants of increasing concern, with over seven million compounds inventoried in the PubChem PFAS Tree, according to the OECD definition. Ion mobility spectrometry (IMS) combined with liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) has recently advanced PFAS analysis. Interestingly, under negative electrospray ionization, perfluoroalkyl carboxylic acids (PFCAs) form dimeric ions ($[2M-H]^-$). These dimers were detected using traveling wave, drift-tube, and trapped ion mobility. It is still unclear how the formation of dimers affects the analytical performance of analytical methods aiming to qualitatively and/or quantitatively analyze this compounds class. Additionally, as PFCA dimer conformations are unknown, this study aimed at proposing potential gas-phase structures for these ions. Preliminary data revealed that the CCS versus mass-to-charge ratio (m/z) for PFCA dimers suggested that proton-bound PFCA homodimer ($[2M-H]^-$) likely adopt a V-shaped structure, as the dimer trend deviated from the linearity observed for cylindrical shapes (Haler et al., JASMS, 2022) and was best described by a power regression model. This hypothesis was supported by using DFT modeling and CCS calculations tools. The workflow used was the following: generating of large set of conformers, selecting a subset for CCS calculations, and the lowest-energy conformer with CCS values within 2% of experimental measurements was considered plausible. Two general structural trends were identified for PFCA dimers: either both fluorinated chains align closely, or one chain bends near its carboxyl group toward the other. These findings raise questions about whether such conformations are maintained when dimers are complexed with larger cations like Na^+ or K^+. Preliminary CCS data for such cation-bound dimers have been acquired, and further modeling is ongoing. Additionally, perfluoroether carboxylic acids (PFECAs) were observed predominantly as dimers, suggesting a role for oxygen atoms in influencing dimer geometry. Finally, MS/MS breakdown curve experiments are currently being conducted for these different dimers to obtain additional structural information and insights into their relative stability.

Poster 1 - Coupling ICP-MS and LC-MS for Analysis of Cr Species in Parenteral Nutrition Solution

Verdonck, Jelle; [a] Poels, Katrien; [a] Vanoirbeek, Jeroen; [a] Godderis, Lode; [a,b] Smolders, Erik; [c]

[a] *Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium;*
[b] *IDEWE, External Service for Prevention and Protection at Work, Heverlee, Belgium;*
[c] *Department of Earth and Environmental Sciences, KU Leuven (University of Leuven), Leuven, Belgium*

Background

Chromium (Cr), a transition metal, exhibits a wide range of oxidation states from -2 to +6, with trivalent Cr(III) and hexavalent Cr(VI) being the most stable and environmentally relevant forms. Cr(III) is an essential micronutrient for human physiology, while Cr(VI) is recognized for its pronounced toxicity and carcinogenicity. Accurate differentiation between these two species is critical due to their contrasting toxicological profiles. However, reliable speciation remains challenging, as chromium species can undergo interconversion under varying pH and temperature conditions, particularly within complex matrices such as biological fluids. Additionally, both species are often present at trace levels, further complicating their quantification.

Aim

To address these challenges, this study presents a sensitive and robust method for the simultaneous quantification of Cr(III) and Cr(VI) in biological matrices, including exhaled breath condensate, urine, wastewater, and nutritional solutions.

Methods

The developed analytical strategy integrates micro-liquid chromatography (µLC) with inductively coupled plasma mass spectrometry (ICP-MS). To prevent interconversion and stabilize both Cr species, a pH-adjusted EDTA complexation step was employed prior to analysis. Separation of Cr(III) and Cr(VI) was achieved using a micro-scale anion exchange column, yielding distinct retention times of 170 seconds for Cr(III) and 230 seconds for Cr(VI). Method performance was evaluated by spiking known concentrations of Cr(III) and Cr(VI) into diverse biological matrices, with additional validation performed using a certified drinking water proficiency testing sample.

Results and Conclusion

The optimized method demonstrated effective separation and reliable quantification of Cr species, with high precision and accuracy, even in complex biological matrices. This approach provides a rapid and dependable tool for the routine monitoring of chromium speciation, contributing to improved assessment of chromium exposure and toxicological risk in clinical and environmental studies.

Poster 2 - Self-assembled Monolayers Based on Azobenzene Derivatives as MOST Systems

[a,b] Piplart, Emma; [a,b] Serez, Ari; [a,b] Henrard, Gwendal; [a] De Winter, Julien; [c] Frère, Marc; [b] Cornil, Jérôme; [a] Gerbaux, Pascal

[a] *Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium ;*
[b] *Laboratory for Chemistry of Novels Materials, Center of Innovation and Research in Materials and Polymers, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium ;*
[c] *Thermodynamics and Mathematical Physics Unit, University of Mons – UMONS, 31 Boulevard Dolez, B-7000 Mons, Belgium*

Azobenzene (AZO) derivatives exhibit a reversible photoisomerization between ground-state trans (E) and metastable cis (Z) forms[1]. Pristine AZO and many of its derivatives absorb in the UV range to promote E-to-Z isomerization while the reverse isomerization is generally triggered by visible light for releasing the energy. Densely packed self-assembled monolayers (SAM) of azobenzene derivatives anchored on rigid metal substrates exhibit a high yield of photoisomerization (>96%) due to cooperative switching effects[1]. For grafting on metallicsurfaces such as gold or silver, the azobenzene core is typically end-substituted by a thiol group to generate a covalent Au/Ag-S bond upon chemisorption[2]. Interestingly, switches can also be grafted on glass substrates using silanes as anchoring unit[3]. Glass is a strongly appealing substrate for our project due to its transparency, further allowing for spectroscopic investigations, and its high commercial availability. In the present study, the strategy for anchoring azobenzene derivatives onto glass surfaces is to perform a click reaction between azobenzenes with an alkyne group and grafted silanes end-substituted by an azide group.

In the present communication, we will present our preliminary results related to the preparation of azobenzenes and fluorine-substituted azobenzenes chromophores. The determination of the MOST properties of the isolated chromophores are performed before the SAM preparation to assess the impact of alkyne groups on the chromophore photoswitching properties and also to further evaluate the role of the intermolecular interactions within the close-packed assemblies on the MOST properties. Thus, we evaluated the absorption properties by UV-Vis spectroscopy and the half-life time by mass spectrometry. LC-MS (Liquid Chromatography- Mass Spectrometry) is here originally used to separate the isomers and to measure the proportion of both as a function of time to obtain the azobenzene half-life time.

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Poster 3 - The Development Of A Targeted UHPLC-MS/MS Method For The Quantification Of Myelin Basic Protein Size Isoforms As A Biochemical Marker For Differential Diagnosis, Disease Progression And Therapeutic Monitoring In Neurological Disorders

Donvil, Linus [a,b] ; Van Eeckhaut, Ann[a]; Bjerke, Maria [b,c]

[a] *Vrije Universiteit Brussel, Research Group Experimental Pharmacology (EFAR), Center for Neurosciences (C4N), Jette, Belgium ;*

[b] *Universitair Ziekenhuis Brussel, Department of Clinical Biology, Laboratory of Clinical Neurochemistry, Jette, Belgium;*

[c] *Neuroprotection and Neuromodulation (NEUR) Research Group, Center for Neurosciences (C4N), Vrije Universiteit Brussel (VUB), Brussels, Belgium*

Myelin Basic Protein (MBP) is one of the major constituents in the myelin sheath around nerve axons. This myelin sheath is essential to ensure proper signal transduction in the nervous system. Damage to the myelin sheath is characteristic for many neurological diseases, such as multiple sclerosis. Classical antibody-based assays have shown that cerebrospinal fluid MBP is a marker for myelin damage and of disease progression in multiple sclerosis[1], and in stroke patients[2] . It has also been shown to be a marker that allows for differential diagnosis between different neurodegenerative diseases[2,3] . Human MBP exists in six different size isoforms, originating from alternative splicing. Of these six isoforms, four are found in central nervous system (CNS) myelin (isoforms 3,4,5 & 6). These isoforms are expressed during different stages of myelin sheath formation; isoforms 3 & 4 are mostly found during early myelination and remyelination, isoforms 5 & 6 are the predominant forms in adult myelin. The goal of this project is to develop a ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) method for the quantification of these different MBP isoforms in CSF, to allow for differential diagnosis and the monitoring of disease progression of neurodegenerative diseases. Characteristic peptides for each MBP isoform, obtained after trypsination of the protein were determined in silico using Skyline, and five peptides were selected for further method optimization. All experiments were performed on a AQUITY UPLC M-Class system with iKey separation device, coupled to a Xevo TQ-XS (all from Waters) run in multiple reaction monitoring (MRM) mode. First, optimal MRM transitions were selected based on a combination of automatic MS tuning and in silico predictions. Furthermore, the cone voltage, collision energy, the dissolution solvent, dilution solvent and injection solvent of the lyophilized standards, the LC gradient, column temperature, flow rate and mobile phase composition were optimized. For four of the five analyzed peptides a limit of detection (LOD) was found in the 10 pM range, for the fifth peptide the LOD was approximately 100 pM. Further optimization of the method, including also the sample preparation, is necessary before the method could be used to analyze real cerebral spinal fluid samples.

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Poster 4 - **Discovering carotenoids in symbiotic crustaceans: qualitative and quantitative analysis by LC-MS.**

Mussoi, Lisa;[a,b] Groignet, Louis; [b] Lourtie, Alexia[a,c] ; Hédouin, Laetitia[d] ; Maire, Julie[a] ; Caulier, Guillaume[a]* ; Gerbaux, Pascal[b]*

* These authors contributed equally to this project

[a] *Biology of Marine Organisms and Biomimetics Unit, University of Mons, 6 Avenue du Champs de Mars, Mons, Belgium ;*
[b] *Organic Synthesis and Mass Spectrometry Laboratory, Université de Mons, 24 Place du Parc, 7000 Mons, Belgium;*
[c] *Culture and Patrimony unit, University of Mons, 24 Place du Parc, 7000, Mons, Belgium,*
[d] *PSL Research University, USR 3278 CRIOBE, BP 1013, 98729, Papetoai, Mo'orea, French Polynesia.*

Carotenoids are fat-soluble pigments with hues ranging from yellow-orange to red, sometimes even mauve. Present in a wide variety of organisms, they are responsible for the characteristic orange-red coloration of crustaceans. In crustaceans, this coloration plays a number of biological roles, including communication, aposematism and various types of mimicry, such as camouflage. This mimicry is particularly important in symbiotic crustaceans, which are able to adapt their colors and patterns to those of their host. However, certain environmental conditions can lead to the separation of symbionts from their host. This rupture causes a phenomenon known as "separation syndrome", resulting in a weakening of the symbiont's general condition and a loss of pigmentation. Two symbiotic species of the coral Pocillopora acuta, the crab Trapezia serenei and the shrimp Alpheus lottini, are particularly affected by this phenomenon. Marked discoloration has been observed in individuals separated from their coral host. This study proposes a chemical approach to this phenomenon, starting by identifying the carotenoids present in these two species using LC-MS (liquid chromatography coupled with mass spectrometry). The results show that T. serenei and A. lottini share several carotenoids, including astaxanthin. Preliminary quantitative analyses suggest a significant decrease in carotenoid concentration in isolated individuals, which would explain their discoloration. One hypothesis is that these crustaceans obtain part of their carotenoids by feeding on their host. Separation would then result in a pigment deficit, leading to a loss of coloration.

Poster 5 - Characterization of peptides using bidimensional separation by preparative Centrifugal Partition Chromatography (CPC) and Capillary Zone Electrophoresis coupled with Mass Spectrometry (CZE-MS) produced during the revalorization of byproducts and leftovers from the agrifood industry.

Seyssens, Evan ;[a] Léonard, Kévin ;[c] Fernandez Salina, Ana ;[b] Hamzaoui, Mahmoud ;[c] Hilali, Soukaina ;[c] Gillard, Nathalie ;[b] Far, Johann ;[a] Gauthier Eppe [a]

[a] *Laboratory of Mass spectrometry, University of Liège;*
[b] *CER Group ;*
[c] *CELABOR SRL*

The production of bioactive peptides for food and feed produced from protein hydrolysates without chemical modifications from leftovers of the Wallon agrifood industries is assessed in the PeptiBoost project (FoodWal, Wallonia region). The (poly)peptides released during the process still retains chemical cross-links (disulfide bridges) and the chemical modifications (e.g. oxidation, glycation…) of the residues induced by the food processing steps. There is a need for green analytical methods exhibiting enough theoretical plates (peak capacity) for the characterization of such peptides.

Centrifugal Partition Chromatography (CPC) is basically a biphasic liquid-liquid separation method queuing cells where centrifugal force is used to keep the equivalent of the stationary phase immobilized, allowing the fractionation of grams of compounds. Capillary zone electrophoresis–mass spectrometry (CZE–MS) use high voltage to separate analytes by charge and shape. It usually offers excellent separation efficiency, low sample consumption and green chemistry compliance. In this work, we present an analytical pipeline that combines sheath-liquid interface fitted for CZE–MS with high-resolution Orbitrap detection and open-source informatics to characterize peptides derived from protein standards, food proteins, and hydrolysates of biological interest.

The developed protocol was applied to protein hydrolysates obtained from enzymatically digested bovine colostrum and lentil flour extracts or from CPC fractions. FragPipe and custom FASTA databases informed by LC–MS proteomic data achieved the identification of hundreds of peptides per sample. Five distinct hydrolysis conditions compatible with food and feed purposes involving the same set of proteases applied to bovine colostrum yielded peptide mixtures showcasing different peptides size distribution and compositions.

33 CPC fractions produced from colostrum hydrolysates were characterized by CZE-MS. >500 unique peptides were identified, with significant overlap across adjacent fractions.

The usefulness of CPC fractionation prior CZE-MS characterization of the peptides is under investigation in term of orthogonality between the methods.

Poster 6 - Design of a peptoid-based Artificial Catalyst inspired by Carbonic Anhydrase for CO_2 Capture

P. Blanckaert[1,2], D. Dellemme[2], L. Groignet[1,2], M. Surin[2] and P. Gerbaux[1]

[1] *Organic Synthesis & Mass Spectrometry Lab (S2MOs), Center of Innovation and Research in Materials and Polymers (CIRMAP), University of Mons - UMONS, Place du Parc 23, B-7000 Mons, Belgium*

[2] *Laboratory for Chemistry of Novel Materials (CMN), Center of Innovation and Research in Materials and Polymers (CIRMAP), University of Mons - UMONS, Place du Parc 23, B-7000 Mons, Belgium*

The capture and conversion of CO_2 are critical challenges in mitigating the impact of anthropogenic emissions on climate change. In living organisms, carbonic anhydrase, a metalloenzyme containing a zinc ion at its active site, efficiently catalyzes the reversible hydration of CO_2 into bicarbonate [1]. However, its application at a large-scale is hindered by stability and cost limitations.

In order to develop an efficient alternative, an artificial carbonic anhydrase is being designed using peptoids. These peptide-like oligomers possess an N-substituted glycine backbone that offers enhanced stability and structural tunability to mimic the enzyme active site [2].

In the present communication, we report on the investigation by mass spectrometry of the complexation of various metal ions (Zn^{2+}, Cu^{2+}, Ni^{2+}), known as modulators of the enzymatic activity [3], with tailor-made peptoid sequences bearing from zero to three pending imidazole metal ligands. The designed original peptoids have been prepared using an optimized solid-phase protocol based on the method developed by Zuckermann et al.,[2] and have been fully characterized by LC-MS and LC- MSMS experiments. Electrospray ionization of peptoid/metal salt solutions revealed the formation of different peptoid ions, depending on the nature of the metal ion and the sequence of the peptoids. Notably, we found that at least two imidazole ligands are required for the detection of potentially catalytically active $[M+Zn]^{2+}$ ions. To gain deeper insights into the conformations of these complexes in the gas phase, we conducted ion mobility spectrometry (IMS) experiments using a Waters Synapt G2-Si mass spectrometer. The experimental collision cross sections of all detected singly and doubly charged peptoid ions were measured and compared with theoretical CCS values derived from the 3D atomistic structures generated via molecular dynamics (MD) simulations. This structural analysis provides crucial information on the stability and conformational changes of $[M+Met]^{2+}$ complexes in the gas phase, offering key insights for optimizing metal coordination and enhancing catalytic efficiency.

By combining synthetic chemistry, ion mobility spectrometry, and molecular modelling, our approach provides a framework for a rational design of efficient artificial enzymes for CO_2 capture.

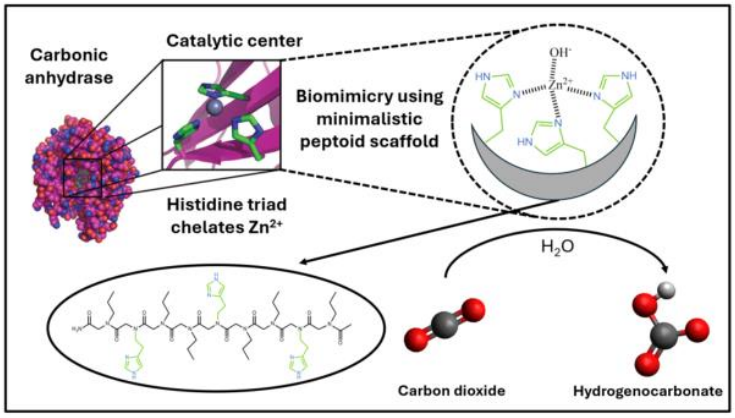

Figure 1. Minimalist design of a peptoid-based artificial enzyme inspired by carbonic anhydrase.

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Poster 7 - Design and Evaluation of Azobenzene-Functionalized Macrocyclic Peptoids as Tunable Molecular Photoswitches

Kamguem Kamga, Ruth Stella; Duez, Quentin; Robert, Thomas; Gerbaux, Pascal; De Winter, Julien

Organic Synthesis and Mass Spectrometry laboratory (S²MOs) University of Mons,
23 Place du Parc, B-7000 Mons Belgium

Azobenzene (**AB**) derivatives are a particularly interesting class of molecular photoswitches due to their capacity to reversible photoisomerize between their trans and cis configurations. This property is exploited in many fields, including solar energy storage, smart materials and pharmacology. By dynamically modifying their molecular interactions and physico-chemical properties, **AB**s may be associated to innovative applications such as light-activable sensors, organic transistors and controlled-release drugs [1], [2], [3]. However, the absorption of pristine AB is limited to the UV range, restricting therefore their use in many applications, such as drug release, due to the harmful nature and the low tissue penetration of UV light [2]. Furthermore, the relatively short half-life time ($t_{1/2}$) of the cis isomer (4.2 days at 20°C in toluene) limits its use in various applications [1]. Hence, there is a need to develop strategies to improve the **AB** photoswitching properties. In particular, several methods have already been explored to improve the t1/2 of **AB**, including grafting several **AB**s onto the backbone of polymers, including peptoids [4]. Promising results have been obtained reaching a $t_{1/2}$ of 17 days at 20°C in methanol [4]. To push further the molecular design of **AB**-containg peptoids, we are now developing cyclic peptoids [5] functionalized with **AB** derivatives as novel, tunable photoswitches with selected wavelength of excitation (from UV to NIR) and extended metastable stereoisomer half-life times. Key results reveal that the interplay between the conformational rigidity imposed by the peptoid backbone (through amide bonds) and the nature of the side-chains combined with the additional constraints from macrocyclization has a profound effect on the back-isomerization kinetics of the **AB** stereoisomers We present here the initial results regarding the synthesis of cyclic peptoids, as well as their characterization by MS, MS/MS, LC-MS, and IMS-MS. The back-isomerization kinetics of the linear and cyclic peptoids were also determined and compared with their precursor linear counterparts. As a starting point, we synthesized a six-unit peptoid containing a single **AB** chromophore and further decorated with propyl side chains (Figure 1).

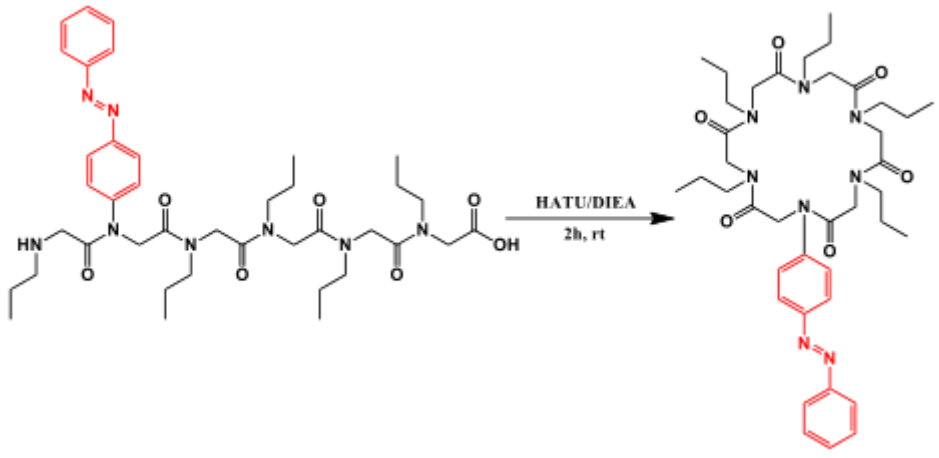

Figure 1. Structures of the synthesized linear and cyclic peptoids NpropNazo(Nprop)4OH.

The results of the back-isomerization kinetics studied at 40°C by LC-MS (Figure 2) reveal a clear improvement of the half-life time of the metastable isomer after cyclization. Specifically, while the linear peptoid back-isomerizes within 3.2 hours in methanol at 40 °C, its cyclic analog exhibits a significantly extended half-life time of 39.2 hours under identical experimental conditions.

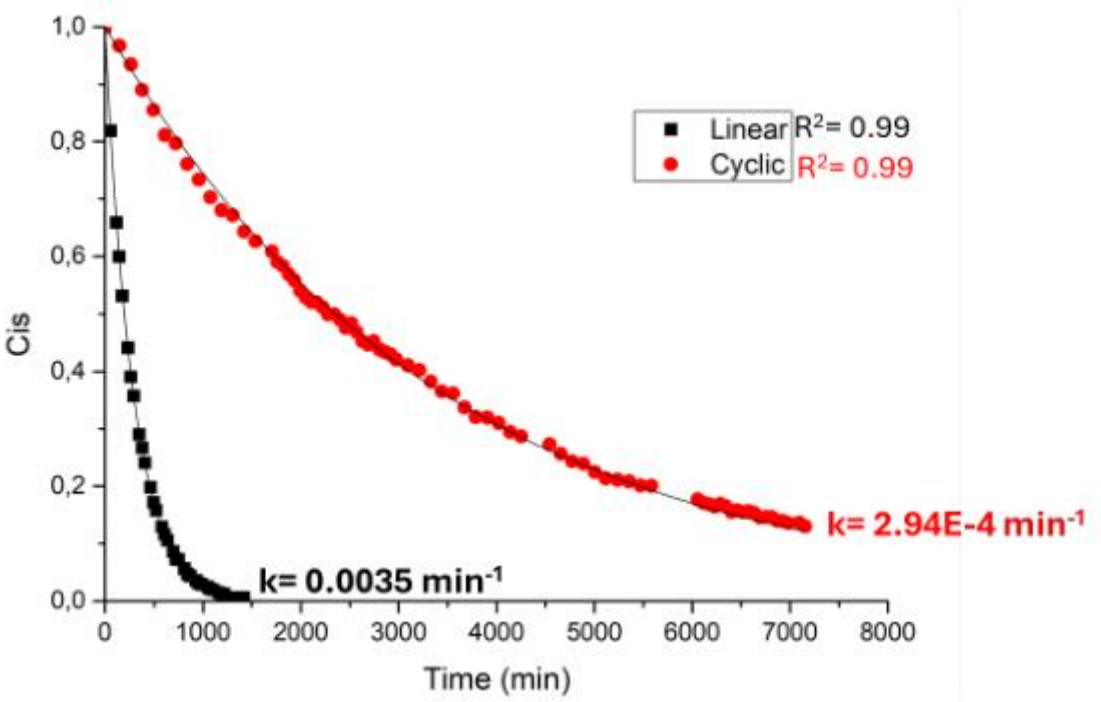

Figure 2. Evolution of the cis isomer proportion over time for linear and cyclic peptoids at 40°C. Analysis performed by HPLC- MS (C18 Agilent Eclipse plus column, gradient H2O (0.01% HCOOH)/ACN, 95/5 to 0/100).

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Poster 8 - Development of an untargeted brain metabolomics method using supercritical fluid chromatography-mass spectrometry: screening and evaluation of potential chromatographic systems

Sarah Bilal[a], Ann Van Eeckhaut[b], Yvan Vander Heyden[a], Debby Mangelings[a]

[a] *Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Research group of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Laarbeeklaan 103, 1090 Jette, Belgium*

[b] *Vrije Universiteit Brussel (VUB), Faculty of Medicine and Pharmacy, Research group of Experimental Pharmacology (EFAR), Center for Neurosciences (C4N), Laarbeeklaan 103, 1090 Jette, Belgium*

Metabolomics refers to the measurement of small molecules (<1500 Da) in complex biological matrices. In untargeted metabolomics, it is aimed to analyze as many metabolites as possible in a sample without a prior hypothesis. This approach is valuable in the context of neurological diseases, where it may provide insight into underlying biological processes and support the identification of potential biomarkers.

Chromatographic separation techniques are commonly employed to develop comprehensive metabolic profiles that cover a wide range of metabolite polarities, concentrations, and chemical classes. Supercritical fluid chromatography (SFC) is an efficient separation technique that uses a supercritical fluid, with low viscosity and high diffusivity enabling fast and efficient separations. Carbon dioxide is the preferred supercritical fluid in SFC due to its low critical pressure and temperature, as well as its more sustainable characteristics. To extend the range of analyzed compounds, a unified chromatography approach is sometimes applied, where a gradual transition from a supercritical to liquid mobile phase is made during the analysis. Hyphenating SFC with mass spectrometry (MS) enhances detection capabilities, and provides better sensitivity and selectivity.

This work aims to establish a robust untargeted SFC-MS method for metabolic profiling of biological samples (e.g., plasma, urine, cerebrospinal fluid) to identify alterations associated with neurological disorders.

Neurologically relevant metabolites, such as amino acids, neurotransmitters, sugars, nucleosides, vitamins, organic acids, and hormones, with a wide range of physicochemical properties (e.g. log P values from –5 to 10), were used to create a representative analyte mixture. This mixture was used for the screening of different potential chromatographic systems. Seven dissimilar stationary phases (bare silica, aminopropyl, diol, ether-linked phenyl, 2-ethylpyrrolidone, 1-aminoanthracene, and ethylene-bridged hybrid silica) were tested. Each stationary phase was evaluated using a 65 min gradient ranging from 2% to 100% co-solvent, consisting of methanol with 3% water and different additives, such as 10 mM ammonium formate, 10 mM ammonium acetate, 10 mM ammonium hydroxide, and a combination of 0.8 mM ammonium fluoride and 10 mM ammonium formate. The selection of the best chromatographic system(s) was based on the number of separated peaks as the main criterion. Peak shape, retention behavior, and mass spectrometric response are also taken into account.

Poster 9 - On the Impact of Post-Translational Succination on SUMO1 Structure: A Dual Approach Combining Gas-Phase and Solution Analysis

Louis Groignet[a,b], David Dellemme[b], Quentin Duez[a], Afaf Fizazi[c], Jean-Marie Colet[c], Patrick Brocorens[b], Mathieu Surin[b], Pascal Gerbaux[a], Julien De Winter[a]*

[a] *Organic Synthesis and Mass Spectrometry Laboratory (S²MOs), UMONS, Belgium*
[b] *Laboratory for Chemistry of Novel Materials (CMN), UMONS, Belgium*
[c] *Human Biology and Toxicology Laboratory, UMONS, Belgium University of Mons, Place du Parc 23, B-7000 Mons, Belgium*

*E-mail: julien.dewinter@umons.ac.be

In certain metabolic diseases, such as Hereditary Leiomyomatosis and Renal Cell Carcinoma, mutations lead to intracellular accumulation of fumarate[1]. This excess promotes a non- enzymatic post-translational modification (PTM) known as succination[1], where fumarate covalently binds to cysteine thiols via a Michael addition (Figure 1). In this study, we investigated if Small Ubiquitin-like Modifier 1 (SUMO1) may be succinated in vitro, and whether this PTM would affect its conformation and activity. SUMO1 is a protein involved in SUMOylation, a process regulating essential cellular functions, including protein localization, activity, and complex assembly[2].

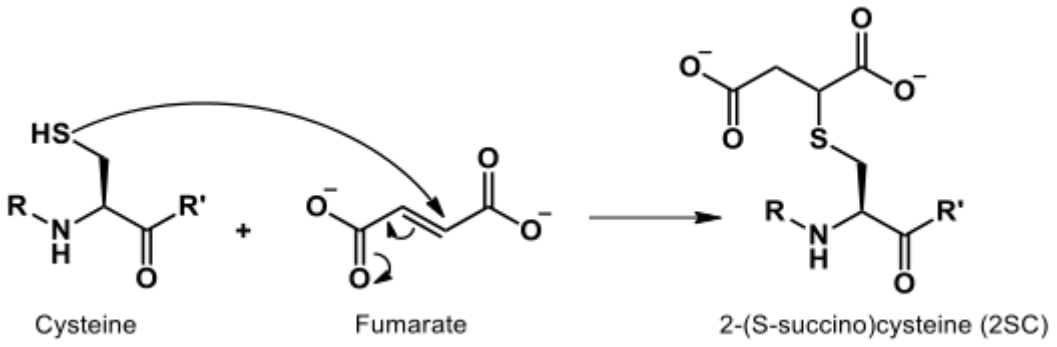

Figure 1: Irreversible succination reaction between cysteine residue and fumarate through a Michael addition leading to 2-(S-succino)cysteine (2SC).

SUMO1 contains a single cysteine residue (Cys52), known for its high reactivity and crucial role in maintaining the protein structural stability3. Previous studies have demonstrated that structural alterations of Cys52 significantly impact the three-dimensional conformation of SUMO1 and impair its biological function[3].

In this study, we demonstrate that SUMO1 undergoes succination in vitro, leading to important conformational changes. These findings provide insights into the susceptibility of SUMO1 to metabolic alterations. In this study, we employ a multidisciplinary approach, combining advanced gas-phase and solution-phase techniques: Native mass spectrometry (MS) confirmed the formation of covalent adducts, while Ion Mobility Spectrometry-MS (IMS-MS) was employed to assess structural changes via Collisional Cross-Section (CCS) measurements and stability differences through Collision-Induced Unfolding (CIU). Complementary biophysical experiments in solution, circular dichroism (CD) and Hydrogen-Deuterium Exchange MS (HDX-MS), offered insights into alterations in secondary structure and solvent accessibility. These experimental results were supported by molecular dynamics (MD) simulations performed in both gas and solution phases.

Our integrative analysis revealed that succination induces structural rearrangements without complete unfolding. IMS-MS showed similar CIU50 values for unmodified and modified SUMO1 at low collision energy but diverging unfolding trajectories at higher energy levels. Notably, native MS indicated a shift in charge state distribution favoring higher charge states in the succinated form, consistent with protein unfolding. HDX-MS further

confirmed enhanced backbone solvent accessibility upon modification, while CD indicated that secondary structures are preserved. MD simulations highlighted local unfolding and disruption of hydrogen bonding networks in the vicinity of the modification site.

Altogether, these results demonstrate that succination alters SUMO1 tertiary structure and conformational dynamics, potentially impairing its interaction with partners. This study highlights the power of integrating gas-phase MS, solution-phase techniques, and atomistic modeling to decipher the structural consequences of metabolite-induced PTMs.

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Poster 10 - Quantification of ADHD Medication in Breast milk: An LC-MS/MS Analysis

Lena De Hondt[1], Joke Muyldermans, Eline Tommelein[1], Ann Van Eeckhaut[1]

[1] *Experimental Pharmacology Research Group, Department of Pharmaceutical and Pharmacological Sciences, Center for Neurosciences, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Jette, Belgium*

Introduction: Attention-Deficit/Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder of which the prevalence and medical treatment in adults is increasing over the last years. Methylphenidate and (lis)dexamphetamine are the first-line treatment, followed by atomoxetine. Guanfacine and bupropion are also used. The question has been raised whether ADHD medication can be used safely during the breastfeeding period. Information about ADHD medication milk levels during breastfeeding is however very limited. A validated and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify these active substances simultaneously in breast milk has not yet been described.

Methods: LC-MS/MS analysis was carried out on an Acquity UPLC® system, coupled with a Xevo TQ MS triple quadrupole system, equipped with a Z-spray™ electrospray ionization source (all from Waters). We used an Acquity UPLC® BEH C18 column (2.1 x 100 mm, 1.7 µm), a mobile phase A/B being ultrapure water/acetonitrile with 0.1% formic acid, an injection volume of 5 µL, and a column temperature of 60°C. MS detection parameters for methylphenidate, dexamphetamine, atomoxetine, guanfacine, bupropion and hydroxybupropion were determined with the Waters Intellistart® program or via manually tuning. The desolvation temperature was set at 500°C, desolvation gas flow at 1000 L/hr, and cone gas flow at 200 L/hr. Blank breast milk samples have been collected, spiked with ADHD medication, and pretreated before injected.

Results and discussion: First, the MS parameters were optimized for every compound, i.e. precursor and product ion, collision energy and cone voltage. Second, the LC parameters were optimized. Several gradients were tested to obtain baseline separation between the compounds within a 18 min run. Third, a protein precipitation method for breast milk analysis was optimized. Several precipitation agents (methanol, acetonitrile, ethanol), volumes, and temperatures were tested. Centrifugation speed, temperature and time was optimized and an incubation step was included to increase sensitivity. The supernatant was dried in a rotary vacuum concentrator RVC 2-18 CDplus (Christ) and the residue was resolubilized in mobile phase before injection. At the moment, the method is validated by investigating limit of detection, limit of quantification, linearity, precision, accuracy, matrix effects and stability.

Conclusion: An LC-MS/MS method for quantifying ADHD medication in breast milk was developed and is being validated. In a further study, this method will also be evaluated for the quantification of these compounds in plasma samples in order to correlate maternal plasma and breast milk concentrations for clinical lactation studies for ADHD medication.

Poster 11 - On the determination of the activation energies for the thermal relaxation of photoisomers by state-of-the-art mass spectrometry methods.

Thomas Robert[a]; Gwendal Henrard[a]; Benjamin Tassignon[a]; Ari Serez[a]; Julien De Winter[a]; Philippe Dugourd[b];Jérôme Cornil[a]; Fabien Chirot[b]; Pascal Gerbaux[a]

[a] *Université de Mons, MONS, Belgium ;*
[b] *Université Claude Bernard Lyon 1, LYON, France*

Photoswitchable molecules are gaining prominence in scientific research due to their ability to switch structures and properties upon UV-Visible light absorption.[1] Azobenzenes are a highly promising family of photoswitches thanks to their versatile synthesis which allows the modification of its properties such as the half-life time of the metastable photoisomers. This property is crucial, as it determines the applications targeted for the systems, ranging from the scale of the milliseconds to the year.[2] Liquid chromatography-mass spectrometry (LC-MS) is used to study thermal back-isomerization kinetics in solution by monitoring the photoisomer population over time.[3] Since back-isomerization follows first-order kinetics, the decay of metastable photoisomers is fitted with a mono-exponential to determine the rate constant at various temperatures and calculate activation energy. To extend the temperature range beyond solvent limits, we use a tandem ion mobility instrument to study gas-phase kinetics. Metastable ions are selected, trapped, and analyzed after relaxation at a given temperature to determine activation energy. Additionally, collisional activation is proposed to induce back- isomerization, monitored via ion mobility spectrometry on a Synapt G2-Si.

Several systems based on azobenzene (pristine and chemically modified) have been synthesized with peptoid backbones to tune properties such as the half-life of metastable photoisomers. Activation parameters (enthalpic and entropic contributions of the free Gibbs activation energy) were measured in methanol using our original LC/MS method. While enthalpy varies minimally, entropy emerged as a critical factor, with $\Delta\Delta S^*$ reaching 50 J mol^{-1} K^{-1}, consistent with insights by Reiman et al. on azobenzene's thermal back-isomerization mechanism and addressing the so-called "entropy puzzle".[4] Tandem IMS was used to study thermal relaxation kinetics in the gas phase, revealing a complex interplay between structural features and desolvation effects. While some systems displayed similar parameters in solution and gas phases, others indicated a shift in the back- isomerization mechanism, reflected in entropy differences.[5] This technique enables fast screening of activation energies and mechanisms but is limited by the specialized equipment required. To address scalability, we propose to use a widespread activation method in mass spectrometry, the collisional activation CA, to trigger thermal back-isomerization in the gas phase via inelastic collisions. This process converts kinetic energy into internal energy and requires temperature calibration to determine kinetic parameters. These parameters are then derived by combining tandem ion mobility spectrometry (IMS) data with collision-induced isomerization, based on previous work by the Prell group on protein ions.[6,7]

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Poster 12 - Mass Spectrometry-Based Imaging and Molecular Profiling of Snake Venom Effects on Murine Tissues

Bastiaens Quentin, Axel de Monts de Savasse, Virginie Bertrand, Loïc Quinton

Mass Spectrometry Laboratory, University of Liège

Snake envenomation is classified as a Neglected Tropical Disease and is responsible for approximately 100,000 fatalities worldwide each year. In addition to its high mortality, snake venom is a complex biochemical mixture composed predominantly of proteins and peptides collectively referred to as toxins. These toxins exhibit a broad spectrum of biological activities, including neurotoxic, hemotoxic, and cytotoxic effects, which can severely damage various tissues and organ systems.

This thesis aims to develop and optimize a Mass Spectrometry-based workflow to investigate and visualize the molecular effects of snake venom on murine tissues. The overarching objective is to elucidate the biochemical and spatial alterations induced by venom exposure in tissues commonly affected during envenomation, such as skeletal muscle, liver, heart, and brain. Experimental models will involve the controlled application of snake venom to these tissues, followed by analysis to characterize the resulting molecular perturbations. Moreover, to assess potential therapeutic interventions, selected venom inhibitors and commercially available antivenoms will be co-incubated with venom-treated tissues to evaluate their efficacy in neutralizing toxin activity and mitigating tissue degradation.

The project comprises two complementary components: Mass Spectrometry Imaging (MSI) and Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS). The imaging component will employ Matrix-Assisted Laser Desorption/Ionization MSI (MALDI-MSI), enabling both the acquisition of high-resolution mass spectra and the spatial localization of ionized species across tissue sections. This approach provides critical insight into the distribution of venom-derived and host-response molecules within the affected tissues.

The second component involves LC-MS/MS, which will be used for detailed molecular profiling and identification. This analytical strategy will facilitate the detection and structural characterization of specific toxins, degradation products, and host-derived biomolecules implicated in venom-induced pathophysiology.

Together, these methodologies aim to provide a comprehensive understanding of snake venom action at the molecular level as well as evaluate the mechanistic basis of antivenom and inhibitor efficacy.

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Poster 13 - Three-dimensional Structural Investigation of Polystyrene-based Gold Containing Single Chain Nanoparticles using a Multidisciplinary IMS-MS/MD Approach

Sarajit Naskar,[a,b,c] Aidan Izuagbe,[b] Vincent Lemaur,[c] Quentin Duez,[a] Andrea Minoia,[c] Julien De Winter,[a] Stephen J. Blanksby,[b] Jérôme Cornil,[c] Christopher Barner-Kowollik,[b] and Pascal Gerbaux[a]

[a] *Organic Synthesis and Mass Spectrometry Laboratory, Center of Innovation and Research in Materials and Polymers (CIRMAP), University of Mons, B-7000 Mons, Belgium*

[b] *Center for Materials Science, School of Chemistry and Physics, Queensland University of Technology,Queensland 4000, Australia*

[c] *Laboratory for Chemistry of Novel Materials, Center of Innovation and Research in Materials andPolymers (CIRMAP), University of Mons, B-7000 Mons, Belgium*

The most important contributing factors to the catalytic activity and specificity of single chain nanoparticles (SCNPs) are their size and shape, as these can be linked to the location and the accessibility of the catalytic center. SNCPs are promising candidates for catalysis applications and biomimetic materials, yet predicting their three-dimensional structure, position and accessibility of the catalytic center using conventional methods is highly challenging. The mass, dispersity, and nature of the monomeric units are the only information provided by most analytical methods. Herein, we combine ion mobility spectrometry mass spectrometry with molecular dynamics simulations to characterize the folding and the three-dimensional structure of Polystyrene-based, gold containing, single chain nanoparticles. We find that the folding process of the copolymer is initiated by π-π stacking interactions between adjacent styrene units with the possible formation of H-bond helping stabilising the final 3D structure of the folded SNCP. The chloride atom of the catalytically active Au- Cl unit can form a strong hydrogen bonds with the protonated 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) end group, while a weaker H-bond can also be formed between the protonated TEMPO end group and the hydroxyl group of styrene-CH2-OH monomers. By allowing a detailed characterization of size, shape and position of the catalytic centers of a SCNP, our joint experimental/theoretical approach allows establishing a precise structure-activity relationship for SNCPs.

Poster 14 - Validation of a GC-MS/MS Method for the Quantification of Six Banned Smoke Flavoring Compounds in Traditionally Smoked Fish, Meat and Cheese

Scholl, G.[a,b] ; Dubois E. [a] ; Eppe, G. [a]

[a] *Mass Spectrometry Laboratory, MolSys Research Unit, Chemistry Department, University of Liège, Liège (4000) Belgium*

[b] *Center for Analytical Research and Technology (CART), MolSys Research Unit, Chemistry Department, University of Liège, Liège (4000) Belgium*

In August 2024, the European Commission announced the non-renewal of authorizations for eight liquid smoke flavoring agents used in food products, following the November 2023 EFSA re-evaluation that highlighted their genotoxic and carcinogenic potential. Among the concerning compounds are six small, volatile molecules—2(5H)-furanone, 3-methyl-2(5H)- furanone, 4-methyl-2(5H)-furanone, 4H-pyran-4-one, 2,3-benzofuran, and styrene, found both in industrial smoke flavorings and formed endogenously during traditional smoking processes via Maillard reactions. To support future regulatory enforcement and risk assessments, sensitive and validated analytical methodologies are essential. In this study, we developed and validated a quantitative analytical protocol for the determination of these six compounds in smoked cheese, fish, and meat. The method combines a QuEChERS-based extraction and cleanup with GC-MS/MS analysis (EVOQ- Speed, Bruker). Key innovations include the use of an optimized programmable temperature vaporization (PTV) injection to mitigate acetonitrile peak broadening and a 15 m column under helium constant flow to resolve isomeric compounds. The EVOQ-Speed system's capability to detect ions down to 10 Da allowed the selection of two specific CID transitions under argon for each analyte, ensuring high selectivity and sensitivity. Full validation in all three matrices demonstrated quantification limits as low as 10 µg/kg for all compounds, with expanded uncertainties ranging from 16% to 26%, except for 2,3-benzofuran (42%). This robust method enables accurate monitoring of genotoxic contaminants in traditionally smoked foods and provides a foundation for improved safety control and regulatory measures ahead of the 2029 phase-out deadline.

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Poster 15 - Development of an Analytical Framework to Evaluate Antivenom Efficacy through Magnetic Beads and BioLayer Interferometry.

Crasset, Thomas;[a] Besonhé, Germain;[a] Redureau, Damien;[a] Gobbi Amorim, Fernanda;[a] Casewell, Nicholas R;[b] Menzies, Stefanie K;[c] Berger-Schaffitzel, Christiane;[d] Vandevenne, Marylène;[e] Quinton, Loïc[a]

[a] *Laboratory of Mass Spectrometry, MolSys Research Unit, University of Liège, 4000 Liège, Belgium;*
[b] *Centre for Snakebite Research and Interventions, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK;*
[c] *Department of Biomedical and Life Sciences, Lancaster University, Lancaster LA1 4YG, UK;*
[d] *School of Biochemistry, University of Bristol, 1 Tankard's Close, Bristol BS8 1TD, UK;*
[e] *Laboratory of Enzymology and Protein Folding, Centre for Protein Engineering, InBioS, University of Liège, Liège, Belgium*

Snake envenomation remains a critical yet neglected public health issue across Africa, the Middle East, Asia, and subtropical regions, affecting mostly rural populations. Despite the availability of antivenoms, their limitations, such as poor thermal stability, risk of adverse reactions, and ethical-concerned production relying on hyperimmunized animals, underscore the urgent need for innovative therapeutic approaches. The European ADDovenom project (2021–2025, FET-Open H2020) addresses these challenges by developing a new generation of antivenoms based on ADDomers: virus-like particles that are thermally stable, cost- effective, and capable of displaying 60 high-affinity binding sites. A major bottleneck in antivenom development and quality control lies in the limited information on the specific venom toxins targeted by antivenom antibodies. To overcome this, we present integrated, automatable workflows for captured toxin identification, quantification, and affinity assessment. Using tosylactivated or Protein G magnetic beads coupled to EchiTab G, a monospecific antivenom prepared to neutralize Echis ocellatus toxins, we probed its cross-reactivity with Echis romani. Toxins captured and uncaptured were analyzed via LC-MS/MS following tryptic digestion, enabling detailed mapping of the antivenom's immunorecognition profile. Additionally, biolayer interferometry (BLI) was employed to determine the apparent dissociation constants of whole antivenoms against crude venoms, facilitating direct comparison with emerging alternatives like nanobodies, monoclonal antibodies, and Addobody constructs. These streamlined methods require minimal sample quantities, are fully automatable, and hold strong potential for routine application in antivenom quality control—paving the way toward more rational, targeted, and effective snakebite therapies.

Poster 16 - UCL-MetIsoLib: A Public High-Resolution Tandem Mass Spectrometry Library for HILIC-Based Isomer-Resolved Profiling of Glycolysis, Central Carbon Metabolism, and Beyond in Urine, Plasma, Tissues, Cells, and Patient-Derived Organoids

Kristian Serafimov[a,b], Maria Virginia Giolito[a], Sébastien Ibanez[a], Ophélie Renoult [a], Sarah Srhir[a], Paula Varon Rugeles[a], Florine Laloux-Morris[a], Cyril Corbet[a], Julien Pierrard[c,d], Marc Van den Eyndec,[e], Olivier Feron[a]

[a] *UCLouvain, Institut de Recherche Expérimentale et Clinique (IREC), Pole of Pharmacology and Therapeutics, Brussels, Belgium*

[b] *UCLouvain, IREC Metabolomics Core, Institut de Recherche Expérimentale et Clinique, Brussels, Belgium*

[c] *UCLouvain, Institut de Recherche Expérimentale et Clinique (IREC), Center of Molecular Imaging, Radiotherapy and Oncology (MIRO), Brussels, Belgium*

[d] *Radiation Oncology Department, Cliniques Universitaires Saint-Luc, Brussels, Belgium*

[e] *Medical Oncology Department, Cliniques Universitaires Saint-Luc, Brussels, Belgium*

We introduce **UCL-MetIsoLib**, a publicly accessible high-resolution tandem mass spectrometry (HRMS/MS) library developed to fill a critical gap in ion-pair free isomer-resolved metabolomics. Optimized for HILIC-based workflows using a bioinert UHPLC system and the Acquity Premier BEH Amide column, the library ensures broad applicability and ease of integration for users with varying instrumentation and workflows. The platform integrates two complementary methods operating under distinct chromatographic conditions (pH 3.5, ESI+; pH 11.0, ESI−), enabling broad metabolic coverage. A total of 334 metabolites are annotated in the library structure, with thiol derivatization incorporated into the extraction protocol to mitigate redox-driven artifacts. Metabolite identification is supported by 245 authentic reference standards and curated according to MSI Level 1 and Level 2 criteria. Validation followed FDA guidelines for bioanalytical method validation and was performed across five biological matrices—urine, plasma, tissues, cultured cells, and patient-derived colorectal organoids—with a U-^{13}C, U-^{15}N-labeled Amino Acid Mixture used as an isotope labeled internal standard. The method demonstrated high precision (<15% RSD intra-/inter-day) and recovery (85–115% across all QC levels). To demonstrate biological applicability, UCL-MetIsoLib was applied to a case study comparing healthy and cancer-derived colorectal organoids. The method provided a robust solution for resolving metabolite isomers, enabling confident and unambiguous annotation of closely related species including key glycolytic intermediates such as DHAP and GA3P, as well as sugar phosphates from both glycolysis and the pentose phosphate pathway. Metabolic alterations were further observed in tumor organoids, including accumulation of nucleotide derivatives and shifts in central carbon metabolism. In conclusion, these findings highlight the value of isomer- resolved spectral libraries in uncovering biologically significant differences often overlooked by traditional untargeted metabolomics workflows. UCL-MetIsoLib offers a robust, reproducible, and versatile resource that enhances high-confidence metabolite annotation, making it an invaluable tool for both translational and systems-level metabolomics research.

Poster 17 - Development of a LC-MS/MS analysis method to quantify bufalin and marinobufagenin in plasma

Ghiande Laëtitia[a], Wells Mathilde[a], Genbauffe Aline[b], Journé Fabrice[b], Lefebvre Victor[a], Beukens Delphine[a], Karasiewicz Tania[c], Leroy Baptiste[c], Wattiez Ruddy[c], Blankert Bertrand[a]

[a] *Laboratory of Pharmaceutical Analysis, Faculty of Medicine, Pharmacy and Biomedical Sciences (FMPB), Health Research Institute, University of Mons, Avenue Victor Maistriau 15, 7000 Mons, Belgium.*

[b] *Laboratory of Human Biology and Toxicology, Faculty of Medicine, Pharmacy and Biomedical Sciences (FMPB), University of Mons, Avenue du Champ de Mars 6, 7000 Mons, Belgium.*

[c] *Laboratory of Proteomics and Microbiology, Faculty of Science, University of Mons, Avenue du Champ de Mars 6, 7000 Mons, Belgium.*

Bufalin (BFL) and marinobufagenin (MBG) are bufadienolides that belong to the cardiotonic steroid family. They inhibit Na^+/K^+ ATPase pump activity by binding to the α-subunit. Many therapeutic properties have been associated with these compounds, such as anti- inflammatory, anaesthetic, antimicrobial, and anticancer effects. Additionally, BFL and MBG have been shown to be endogenously present in human plasma, suggesting their potential as biomarkers in pathological contexts. A study highlighted BFL as a potential marker of hepatocarcinoma, with lower levels observed in cancerous patients. This project applies the theory to melanoma patients. Although melanoma represents about 5% of skin cancer cases, it accounts for 80% of related deaths. MBG, at high concentrations, induces hypertension in preeclamptic patients, making it a promising early-onset preeclampsia biomarker, a condition affecting roughly 5% of pregnancies. Efficient quantification of these compounds could help clinicians personalize monitoring and follow-up for affected patients.

This study used a microLC-MS/MS method for quantification at very low plasma levels. A major challenge in quantifying endogenous steroids is their binding to carrier proteins. This work focused on pre-treatment protocols to reduce protein-binding and improve sensitivity.

Two matrices were prepared: (i) an authentic matrix combining plasma from five healthy individuals for standard addition, and (ii) a “steroid-free” surrogate matrix produced via charcoal-stripping. These were aliquoted and spiked with increasing BFL and MBG concentrations. Plasma, being protein-rich, required pre-treatment to precipitate and eliminate proteins before extraction. This step enhances yield and cleanliness before the final evaporation to concentrate BFL and MBG.

Several pre-treatment protocols were tested using methanol, trichloroacetic acid and proteinase K. This was followed by supported liquid extraction (SLE). Samples were concentrated before injection. Cinobufagin-d3 was used as internal standard. Samples were analyzed by µLC-QTRAP in MRM mode. Preliminary results showed that methanol and proteinase K pre-treatment followed by SLE provided better recovery compared to other preparation methods. The activated charcoal protocol effectively eliminated the presence of our compounds in non-spiked plasma samples. In addition, tests using different injection methods revealed that better results were obtained with the trap-elute mode compared to direct injection.

Poster 18 - Unfolding the Truth: α-Synuclein and A-Syn 1 Aptamer as a Model System to Explore Binding and Unfolding Dynamics in the gas phase

Benonit, Maxime [a]; De Pauw, Edwin [a]; Eppe, Gauthier [a]; Far, Johann [a]

[a] Mass Spectrometry Laboratory, MolSys Research Unit, Quartier Agora, University of Liège, Allée du Six Août 11, B-4000 Liège, Belgium

Alpha-synuclein (α-syn) is a 140-amino acid protein implicated in several neurodegenerativediseases, including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. The abnormal aggregation of this protein into toxic oligomers and fibrils is a central pathological mechanism in these synucleinopathies. A-syn-1, a 66-mer aptamer, has recently been described in the literature for its ability to efficiently bind to alpha-synuclein and inhibit its fibrillation; however, its binding mechanisms remain poorly understood.

The size of the aptamer–protein supramolecular assembly approaches the limits of conventional structural elucidation by nuclear magnetic resonance (NMR), while cryo-electron microscopy and X-ray crystallography are limited in providing information about the dynamic events that occur during binding.

The use of collision-induced unfolding (CIU) is proposed to explore the structures and dynamics of the aptamer–α-syn complex in the gas phase. Ion mobility mass spectrometry (IM-MS) coupled with CIU can reveal the energy landscape explored during unfolding, while energy-resolved collision-induced fragmentation (also known as breakdown curve experiments) offers insights into the complex's resilience to dissociation and fragmentation.

The mechanisms underlying CIU will be further explored by combining other activation methods, such as electron capture dissociation (ECD) and photodissociation (using ultraviolet and infrared photons). Special attention will be given to the effects of energy input, either via photon irradiation (PhotoSynapt, MS-LaserLab, VU Amsterdam) or thermal denaturation (IMS–IMS, ILM, Université Lyon 1), to better understand the structural transitions observed in the gas phase.

For practical reasons, MS data and CIU heatmaps of proteins are typically produced using positive ionization mode, while negative ionization mode is preferred for nucleic acids. The question of the most appropriate ionization mode for MS and CIU analysis of protein–aptamer complexes remains open.

Our preliminary results were obtained under non-denaturing conditions using electrospray ionization (ESI) and IM-MS with both ionization modes applied to alpha-synuclein and the aptamer. Surprisingly, the charge state distributions of α-syn and A-syn-1 were quite similar in both positive and negative ionization modes. In contrast, their conformer distributions, as determined by IM-MS, differed. Optimization is currently underway using both ESI and nano- ESI to improve detection of the α-syn–A-syn-1 complex in both ionization modes.

Poster 19 - Novel semi-targeted mass spectrometry imaging approach using the SELECT SERIES MRT mass spectrometer with DESI XS source

Jan Claereboudt [a]; Emmanuelle Claude [b]

[a] *Waters NV/SA – Antwerp, Belgium,*
[b] *Waters Corporation – Wilmslow, United Kingdom*

Introduction

Multi-reflecting time-of-flight (MRT) technology enhances mass resolution and mass accuracy by extending flight times, although this can reduce the duty cycle and therefore signal intensity. The SELECT SERIES™ MRT mass spectrometer addresses this with encoded frequency pushing (EFP), performing multiple pushes within a discrete time interval, decoded into a coherent spectrum, improving the duty cycle by two orders of magnitude. However, low-intensity peaks can be missed if they don't reach a threshold to be considered real ions. We present a semi-targeted method that increases the duty cycle over specified mass ranges, enhancing sensitivity on the SELECT SERIES MRT mass spectrometer and demonstrate the improvements of detection of low-intensity ions in DESI MSI experiments.

Methods

MSI experiments were carried out using the DESI XS source mounted on a SELECT SERIES MRT mass spectrometer in positive and negative ionisation mode. DESI spray conditions were set at 1-2 µL/min, 95:5 MeOH: water v/v and the N2 nebulising gas pressure was set at 15 psi. The system was equipped with a modified instrument control software allowing narrow and wider selections of mass range to be enhanced by setting different pulse width (narrow 6 µs and wider 15 µs). Modifying the pulse delay increased different discrete m/z range.

Preliminary Data or Plenary Speakers Abstract

Initial experiments were conducted using rodent brain tissue sections. Fresh frozen tissue sections were sectioned using a cryostat (Lecia), at 18 µm for DESI. The first series of experiments were performed analysing a 1.5 x 1.5 cm area of a mouse brain tissue section acquired at 25 µm pixel size in negative ionisation mode, to particularly detect small endogenous metabolites, such as pyruvate, taurine, glutamate etc... Enhanced duty cycle (EDC) width was tested allowing a ~10-15 Da discrete mass range window to be transmitted with a pulse delay of 6 µs. A range of EDC pulse delay parameters from 1974 µs down to 1961 µs were tested to evaluate the increased intensities within the specific mass range selected. Intensities were on average 10-20 times increased with some as high as 40 times for the lowest m/z metabolites. A second series of experiments were performed with an EDC width setting of 15 µs which allowed an enhanced transmitted mass range of ~100 Da. This mode of acquisition was compared to narrow 6 µs EDC and to standard MRT mode with low threshold EFP decoding. 28 endogenous m/z were detected, giving an average of 2-3 tissue-based metabolites per 10-20 Da. In the case of narrow EDC (width settings of 6 µs), 39 metabolites were detected in the mass range m/z 70-90. In the case of the wider EDC (width settings of 15 µs), between 100-150 tissue metabolites were detected across a mass range of 100 Da. Further experiments will be performed analysing drug dosed tissue sections to evaluate the benefits of employing the novel EDC semi-targeted MSI acquisition mode for the visualisation of drugs and metabolites.

Novel Aspect

Recent developments in the Multi Reflecting Time-of-Flight technology to increase sensitivity for semi-targeted MSI applications.

Poster 20 - MICROBoost: HILIC-FLD-MS/MS characterization of prebiotic oligosaccharides

[a b] Lucia, Cocorullo; [b] Thomas, Tilmant; [b] Maxime, Gavage; [c] Adrien, Boes; c Patrice, Filée; [b] Nathalie, Gillard; [a] Moreno, Galleni.

[a] *Center for Protein Engineering (CIP), Biological Macromolecules, InBioS, University of Liège, 4000 Liège, Belgium;*
[b] *Analytical Laboratory, CER Groupe, 6900 Marloie, Belgium*
[c] *Laboratory of Immunobiology, CER Groupe, 6900 Aye, Belgium.*

The ambition of the MICROBoost project is to improve the nutritional, functional and organoleptic quality of food and beverage developing approaches based on the microbiota. One of the objectives of the project is the enzymatic synthesis of new prebiotics (xylo-oligosaccharides, XOS and/or galacto- oligosaccharides, GOS) and the development of MS-based analysis methods for the characterization of the produced mixtures. Oligosaccharides (OS) were synthetized by glycosyl hydrolases through a transglycosylation reaction that results in a mixture of OS that can vary for their degree of polymerization (DP) and type of glycosidic linkage. A HILIC-FLD-MS/MS (hydrophilic interaction liquid chromatography- fluorescence- tandem mass spectrometry) method was set-up for the characterisation of reducing sugars. Prior to the analysis, OS were derivatised via reductive amination, in which a fluorescent tag such as 2-aminobenzamide or procainamide is covalently attached to the reducing end. This chemical modification not only stabilises the open-ring form of the sugar, thereby preventing α/β anomer separation, but also greatly enhances the analytical performance of HILIC-FLD- MS/MS. Furthermore, fluorescence detection significantly improves sensitivity, enabling the detection of low-abundance sugars and allows relative quantification. The results showed that the developed technique allows OS separation based on their DP and, to some extent, also according to their glycosidic linkages. Partial identification of OS was achieved by comparing with reference standards and converting retention times to Glucose Units (GU), which were subsequently matched against an open-access GU database. Tandem mass spectrometry verified the proposed compositions. However, the structural diversity of OS and the limited availability or the prohibitive cost of authentic standards continue to hamper confident identification. Moreover, oligosaccharides such as GOS and XOS are poorly represented in GU databases, which are designed for glycan identification without the use of MS/MS data. To address this limitation, we have begun developing an internal database dedicated to prebiotic OS, which integrates both GU-based identification and fragmentation spectra. The goal is to continuously expand and enrich this resource over time.

Poster 21 - Introducing the first prototype of preparative native ion mobility mass spectrometer for mass and shape selected pseudomolecular ions for ion soft landing built from a substantially modified Waters Synapt G2s ("SoftSynapt")

Steven Daly [a] , Jerre van der Horst [a], Jan Commandeur [a], Gauthier Eppe [b], Edwin De Pauw [b], Johann Far [b]

[a] *MS Vision , Televisieweg 40, 1322 AM Almere, The Netherlands*
[b] *Laboratory of Mass spectrometry (MSLab), Allée du six Août 11 - Quartier Agora - 4000 Liège, Belgique*

Despite recent advances in computational chemistry, the structural elucidation and dynamics of biological systems resolved at the atomic level are of utmost importance, e.g. for drug docking, proteins folding or aggregation. X-ray crystallography and multidimensional nuclear magnetic resonance (NMR) are massively used for this purpose but fail for system that cannot be crystallized and for systems too large to be resolved by NMR. Ion Beam Deposition (IBD) of electrosprayed samples coupled with cryo Electron Microscopy (cryo-EM) were proposed as an alternative. More recently, native mass spectrometry (native MS) of mass selected proteins for purifying the ion beam was coupled to ion soft landing (preparative mass spectrometry) before the structural elucidation using cryo-EM or scanning tunneling microscopy (STM). The conservation of the structures in the gas phase during the time-frame of the experiments by native MS is still under debate. The lack of thermodynamics and kinetics data that can be retrieved from such experiments prevent to conclude the debate and need to be consolidated using different approaches. Here we propose instead the use of a new type of instrumentation under development combining preparative native ion mobility mass spectrometry and variable temperature cryo Atomic Force Microscope (cryo AFM) for single molecule imaging operating from cryogenic temperature to room temperature in dry or wet conditions. Supervised ion cooling and ion heating by collision induced unfolding of mass selected (quadrupole) and shape selected (ion mobility slicing) of the ion beams is required to evaluate the lifespan and the resilience of the conformations.

Here is the hardware description under evaluation of our modified traveling wave ion mobility mass spectrometer Waters Synapt G2s that we named the "SoftSynapt":

- native MS using a 32KHz RF generator for high mass selection
- gas selection and gas pressure tuning using divert valves and gas restrictors
- traveling wave ion mobility slicing for shape selection
- optional charge reduction and non ergodic fragmentation by electron capture dissociation (ExD, eMSion) implemented in a modified transfer CID cell
- ion soft landing device at variable kinetic energy with transfer airlock for easy retrieval of the collected ions installed after the ExD cell
- electrometer (picoampermeter) for ion density estimation and measurement of charge dissipation
- NanoWizard XP4 and cryo-stage module cryoAFM interrogating the shape of collected ions by single molecule imaging (contact or tapping mode) and single molecule force spectroscopy at variable temperature (-150°C to room temp. and higher)

Poster 22 - Plasma proteome characterization: each step is to be optimized

Blandine, CHAZARIN[a+] ; Lise, DEMULIES[a+] ; Ruddy, WATTIEZ[a]

[a] *Proteomic and Microbiology Department, UMONS, Mons, Belgium*

[+] Equal contributions

Analytical strategy based on mass-spectrometry proteome characterization moves toward clinical application to decipher pathology development. The most requested clinical sample to analyze is plasma sample because it is easy to collect through non-invasive protocol and allows long-term monitoring. However, the dynamic range (characterized by the concentration difference between the less and the most abundant proteins) reaches an amplitude of 9 orders of magnitude in plasma. That makes it challenging to analyze to acquire a broad overview of the plasma proteome. Starting a project on plasma proteome characterization can be challenging and each step must be optimized for in-depth analysis.

In this work, we aim to present the steps of optimizations that can increase the proteome coverage of plasma samples analyzed by liquid chromatography coupled to mass spectrometry DIA acquisition (LC-MS). In order to decipher the impact of each technical step, we will present comparative results from different strategies/settings from the sample preparation to the data interpretation, including different quality control monitoring methods.

Regarding the sample preparation, we compared two strategies and demonstrated the difference of the quantified proteome, as well as the simple quality controls that must be taken in account to monitor proteolytic digestion efficiency and protein sequences coverage. Furthermore, the peptide sample decomplexification by liquid chromatography was optimized by comparing gradient settings over 75 minutes method. We demonstrated that mobile phases proportions alone can increase protein and peptide identification rates, as well as the protein sequence coverage. Furthermore, we demonstrate how the peptide sample load can affect the detection of the analytes, that is a crucial step to compromise between a satisfying proteome coverage and protect the instrument from saturation. The next stage was to select a bioinformatic pipeline allowing the higher number of identification, including the selection of the database to use in library-free search on DIA-NN and the use of MBR (Match Between Runs). We show that increasing the number of entries in the fasta files by including unreviewed proteins can be detrimental to the final output and that MBR algorithm is not recommended for non-equivalent sample loads.

Finally, we will optimize the DIA windows for broader selection of precursors and increasing the identification rate, and test enrichment strategy allowing the selection of low abundant proteins. Then, we will integrate automated sample preparation to increase reproducibility which is crucial for clinical research projects.

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Poster 23 - Parallel targeted and untargeted metabolite analysis of mouse plasma samples using a benchtop multi-reflecting time of flight mass spectrometer

Jan Claereboudt [a]; Adam King [b]; Richard Lock [b]

[a] *Waters NV/SA – Antwerp, Belgium*
[b] *Waters Corporation – Wilmslow, United Kingdom*

Introduction

Metabolomic profiling of biological matrices is routinely performed under two categories; targeted, where known metabolites are analyzed for quantification, and untargeted, where unknown molecules of biological significance are identified. Currently, these types of analyses are performed independently on different MS platforms and data analysis is carried out through different processing workflows. This is due to the specific performance attributes of the instruments employed. Instrument advancements have narrowed the gap on sensitivity between high resolution and tandem quadrupole instruments, coupled with faster scanning capabilities and the reduction of interscan delays, both approaches on one platform becomes a practical solution for metabolic profiling. Combining both within a single sample injection dramatically reduces the impact on instrument resourcing and sample volume requirements.

Methods

Plasma samples (n=48) from mice treated with fenclozic acid or vehicle, were prepared for analysis using the Waters Kairos amino acid kit, which contained amino acid calibrants, internal standards and reagents for protein precipitation and amino acid derivatization using AccQTag reagent. MS data were acquired on the Xevo MRT mass spectrometer in positive mode. Chromatographic separation was achieved over a 9-minute reversed-phase gradient. The MS acquisition method was constructed from multiple Tof MSMS functions for each target amino acid (n=65). Untargeted data was collected from two additional MS functions with either a low (6 eV) or elevated collision energy ramp (20-45 eV) applied. Data were processed using Progenesis QI and UNIFI software for untargeted and targeted respectively.

Preliminary Data or Plenary Speakers Abstract

Fenclozic acid is a drug previously removed from the market due to evidence of liver toxicity and therefore can still be useful in helping further the understanding of drug induced liver toxicity (DILT). The dysregulation of plasma amino acids has previously been linked to liver damage so utilizing methods to accurately quantifying their changes in levels can provide important metabolic information. The fast-scanning capabilities of the Xevo MRT allowed the targeting of 65 amino acids and internal standards alongside the generation of high-quality untargeted data for biomarker discovery, providing the required specificity for accurate quantitation and identification of unknowns. Of the 65 amino acids, 20 were fully quantified against stable isotopically labelled standards (SILS) whilst the remaining 40 amino acids were quantified against the SILS based on retention time. Amino acids were quantified over the concentration range of 5 – 1000 µM where calibration curve linearity for each had a R2 correlation coefficient >0.99 and QC standards had 67 % of injections within 15 % of the nominal concentration. Simultaneously, untargeted data were acquired, converted to mzML data format and processed using MZmine software, which performed peak picking and normalization. Extensive statistical analysis including assessing pooled QC quality and group separation were visualized by PCA and significant features between sample groups were highlighted through OPLS-DA and subsequent S-plots. Targeted Quan data highlighted changes in branched chain amino acid concentration and increases in some aromatic amino acids alongside much dysregulation. Untargeted data highlighted changes in bile acid metabolism when comparing vehicle and drug dosed mice. Combining these to acquisition approaches in a single analysis showed little compromise on data quality for either analysis strategy. The fast-scanning MSMS capabilities allowed a high number of data points across the chromatographic peak for the quantitation workflow, maintaining high resolution accurate mass untargeted analysis data.

Novel Aspect

Fast scanning, high mass resolution, single injection targeted and untargeted analysis for metabolite/biomarker quantification and discovery MetID/Metabolomics.

Poster 24 - From Folk Remedy to Discovery: An HPLC-HRMS Untargeted Metabolomics Workflow Unveil Species-Specific Metabolites in Sideritis herba

Jason Fauquet[1], Mathilde Wells[2], Tania Karasiewicz[3], Pierre Duez[1], Bertrand Blankert[2], Amandine Nachtergael[1]

Corresponding author: Jason Fauquet, Jason.FAUQUET@umons.ac.be

[1] *Laboratory of Therapeutic Chemistry and Pharmacognosy, Research Institute for Health Sciences and Technology, Research Institute for Bioscience, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons (UMONS), Belgium*

[2] *Laboratory of Pharmaceutical Analysis, Research Institute for Health Sciences and Technology, Faculty of Medicine, Pharmacy and Biomedical Sciences, University of Mons (UMONS), Belgium*

[3] *Laboratory of Proteomics and Microbiology, Research Institute for Bioscience, Faculty of Sciences, University of Mons (UMONS), Belgium*

Sideritis herba (Ironwort), as defined by the Committee on Herbal Medicinal Products (HMPC/EMA), comprises the aerial parts of four species: Sideritis scardica Griseb., S. clandestina (Bory & Chaub.) Hayek, S. raeseri Boiss. & Heldr., and S. syriaca L. Those species are traditionally used in Europe for alleviating coughs associated with colds and for soothing mild gastrointestinal disorders. Despite numerous phytochemical investigations, the literature remains incomplete, and certain species within the mixture, particularly S. clandestina, have been insufficiently explored. Furthermore, no untargeted metabolomics approach incorporating a dereplication strategy has been applied to any of these species. Our objective is to demonstrate that a strategy integrating multivariate statistical analyses and molecular networking can effectively distinguish the four species, rank their characteristic metabolites, and prioritize potentially novel and bioactive compounds. To evaluate this approach, we prepared individual crude methanolic extracts of the four species and analysed them using HPLC-ESI-MS/MS with a Sciex ZenoTOF 7600 in Data Dependent Acquisition (IDA mode). Following data (pre)-processing on the Workflow4Metabolomics (W4M) platform, PCA, PLS-DA, and OPLS-DA analyses successfully discriminated the species and identified the most contributive variables to their differentiation (VIP). In the subsequent phase, we intend to process the same data with MZmine software and generate a molecular network in MetGEM. This step is expected to facilitate the visualization of common and specific chemical entities, the rapid annotation of known compounds, and, by elimination, the prioritization of those that remain novel or lack database matches. Future integration of molecular network results with identified statistical markers should yield to a shortlist of promising targets for isolation, structural elucidation, and biological evaluation.

Poster 25 - Affinity-Capture Mass Spectrometry for the Discovery of Receptor-Targeting Peptides in Animal Venoms

Lou Freuville[a], Rudy Fourmy[b], Aude Violette[b], Romulo Araoz[c], Alain Brans[d], Nicolas Gilles[c], Loïc Quinton[a]*

[a] *Mass Spectrometry Laboratory, MolSys Research Unit, Department of Chemistry, University of Liège, Allée du Six Août, 11 – Quartier Agora, 4000, Liège, Belgium.*
[b] *Alphabiotoxine Laboratory srl, Montroeul-au-bois,7911, Belgium.*
[c] *CEA, Département Médicaments et Technologies pour la Santé (DMTS), Université Paris Saclay, SIMoS, Gif-sur-Yvette, France.*
[d] *Centre for Protein Engineering, University of Liège, B-4000 Liège, Belgium.*

Animal venoms are complex mixtures of bioactive peptides, many of which display high affinity and selectivity toward membrane receptors such as G protein-coupled receptors (GPCRs) and ligand-gated ion channels. Despite their pharmacological promise, the systematic discovery of receptor-targeting venom peptides is hampered by venom complexity and the low-throughput nature of existing screening approaches.

We present a versatile and potentially high-throughput methodology combining receptor-based affinity capture with mass spectrometry and proteomic analysis to directly identify toxins binding to membrane receptors, without any extensive purification of the venom. In this study, cell membranes overexpressing target receptors were incubated with crude or fractionated venom samples. Toxins exhibiting receptor affinity were captured on membranes, while non-binders remained in solution. Bound peptides were analyzed by MALDI-MS and LC-MS/MS proteomics, enabling unambiguous identification of receptor-specific toxins.

The approach was first validated using the human vasopressin type 2 receptor (hV2R) and the green mamba venom peptide mambaquaretin-1, a known GPCR ligand. We then extended the method to nicotinic acetylcholine receptors (nAChRs), successfully fishing α-neurotoxins and cytotoxins directly from crude Naja kaouthia. This demonstrates the method's capacity to isolate clinically and pharmacologically relevant neurotoxins directly from native venom mixtures.

Our results confirm that receptor-guided affinity capture combined coupled with proteomics enables the direct discovery of membrane receptor-targeting toxins from HPLC venom fractions and even from unprocessed venoms. This strategy paves the way for scalable, high-throughput screening of venom libraries against a broad array of therapeutic targets.

Poster 26 - Analysis of Per-and-polyfluoroalkyl substances in soil and wastewater/groundwater using a benchtop multi-reflecting Time-of-flight Xevo MRT mass spectrometer

Jan Claereboudt [a]; Hania Khoury-Hollins [b]; Adam King b; Jayne Kirk [b]

[a] *Waters NV/SA – Antwerp, Belgium*
[b] *Waters Corporation – Wilmslow, United Kingdom*

Introduction

The widespread use of per and polyfluoroalkyl substances (PFAS) in industrial processing and manufacturing over the last decades has led to global environmental and health concerns. As the number of these compounds continues to increase, it is becoming a challenging task to monitor PFAS using the traditional targeted methods. A screening workflow using High Resolution Mass Spectrometry (HRMS) is an ideal bridge for monitoring the regulated PFAS and detecting non-regulated PFAS. Here, soil and wastewater/groundwater samples from different locations in the United Kingdom were tested for PFAS content. Combined Non-target screening (NTS) and quantitative workflows were pursued for the identification and quantification of PFAS in samples. PFOS, PFHxS, 6:2 FTS and PFHxA were identified in soil and wastewater with mass measurement accuracy <500 ppb.

Methods

10 mg of soil was extracted with 10 mL of methanol. After 20 minutes of incubation samples were spun, the supernatant was diluted (1:1) with water/0.2% formic acid. River and wastewater were collected from different sites in the UK. After a solid-phase-extraction (SPE) step1, samples were dried down and reconstituted in methanol. Extracts were diluted 1:1 with H2O+ 0.2% formic acid before injection.

Preliminary Data or Plenary Speakers Abstract

- The benchtop Xevo MRT MS instrument demonstrated a RMS of mass measurement accuracy < 500 ppb and high mass resolution < 75K for m/z 556.2765 (Leu-enkephalin).
- The linearity and sensitivity of all 32 studied PFAS was assessed with 26 of the compounds achieving a LOQ < 5ng/L.
- PFAS was identified and quantified in groundwater, river water (Figure 5 and Figure 6) and soil.
- PFOS was detected in soil samples with mass measurement accuracy <0.7 ppm. 41 ± 3 pg/mg of PFOS were quantified in one soil sample (data not shown).
- NTS workflow using the UNIFI application in waters_connect software platform is a single software solution for nontarget screening, discovery, and quantitative analysis.
- The UNIFI screening and discovery workflows offer numerous options from mass defect filtering, mass retention time filtering

Poster 27 - Gas Chromatography–Trapped Ion Mobility Mass Spectrometry: A Highly Specific and Ultra-Sensitive Platform for Quantifying Sub-ppt Levels of Dioxins and PCBs in Food

Muller, Hugo B.; Scholl, Georges; Eppe, Gauthier

Mass Spectrometry Laboratory, University of Liège, 4000 Liège, Belgium

In this study, we evaluated the quantitative capabilities of a GC-APCI trapped ion mobility (TIMS) TOF platform for the determination of ultra-trace levels of polychlorinated dibenzo-p dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) in complex food matrices. Using a Bruker timsTOF Pro 2 instrument, three fat-based proficiency test samples - fish oil, palm oil, and milk fat - were analyzed and compared to a validated GC-sectorHRMS method, in accordance with the criteria set by Commission Regulation (EU) 2017/644. Linearity was confirmed across broad dynamic ranges, with relative response factor (RRF) precision consistently below 20%. Instrumental limits of quantification (iLOQs) reached the 100–500 fg/µL range for dioxins and low pg/µL for PCBs, while method LOQs (mLOQs) were typically in the high fg/g to low pg/g range. Intermediate precision (RSDR) remained below regulatory thresholds in 97% of cases. Furthermore, 80% of PCDD/Fs and over 87% of PCB measurements met trueness requirements, with summed WHO2005-TEQ and NDL-PCB concentrations in close agreement with sectorHRMS values. Importantly, the addition of the ion mobility dimension provided distinct benefits. It enabled the separation of coeluting isomers (e.g., 2,3,7,8-TCDD), resolved isobaric interferences (e.g., PeCDD and HxCB 169), and allowed for high-confidence analyte identification using collision cross section (CCS) values. CCS differences between native and ^{13}C-labeled standards were consistently below 0.5%, supporting its use as an additional identification criterion. Overall, while the method showed slightly reduced performance for ultra-trace dioxins, it demonstrated accurate quantification and enhanced selectivity, highlighting the strong potential of TIMS-TOF technology for contaminant analysis in food safety applications.

Poster 28 - Development of a novel non-targeted approach in mass spectrometry for PFAS imaging

Cabrera-Tejera, Bastien[a] ; De Pauw, Edwin[a]; Eppe, Gauthier[a]

[a] *Mass Spectrometry Laboratory, MolSys Research Unit, Quartier Agora, University of Liège, Allée du Six Août 11, B-4000 Liège, Belgium.*

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds widely used for their resistance to water, oil, and heat. However, their persistence, potential for bioaccumulation, and toxicity to both humans and ecosystems raise growing concerns. Although many studies report PFAS presence and adverse effects in the environment, critical knowledge gaps remain regarding their behavior, especially in complex samples where detection and identification are challenging. These limitations hinder accurate risk assessment.

This project aims to address these challenges by developing a non-targeted analytical approach for PFAS analysis in environmental and biological matrices. We will combine mass spectrometry imaging (MSI) with ion mobility spectrometry (IMS) to map the spatial distribution of PFAS in samples. This IMS-MSI strategy allows not only the identification of various PFAS but also the precise localization of their accumulation in biological tissues and soils, offering insights into their bioaccumulation and metabolic transformations.

The first objective is to optimize MSI desorption/ionization techniques, focusing on MALDI and nanoDESI, to improve sensitivity and spatial resolution, enabling detection of PFAS even at low concentrations. A first round of optimization has been conducted on MALDI, showing promising results in terms of matrix selection and signal enhancement for key PFAS standards. These optimized methods will then be applied to Daphnia magna, a model organism for ecotoxicological studies, and zebrafish. By exposing them to a mixture of historical and emerging PFAS, we aim to visualize their tissue-specific accumulation and metabolism.

Then the same approach will be used to investigate PFAS migration in soil and their transfer to plant roots, providing valuable data on their environmental mobility and the risk of trophic transfer. Understanding how PFAS move from soil to plants is crucial to evaluate their potential for food chain contamination.

Finally, advanced data processing techniques will be applied, including Kendrick mass defect filtration and apparent density trends, to improve PFAS identification and differentiate them from other matrix components.

Overall, this project will deliver a method capable of offering valuable insights into the toxicological behavior of PFAS by mapping their distribution and metabolism in exposed organisms and environmental compartments, thereby contributing to a more comprehensive assessment of their ecological and health risks.

Poster 29 - Thin layer chromatography coupled to MALDI in source decay imaging (TLC/MALDI-MSI-ISD) for whole sequence coverage of oligonucleotides

Charles Delvaux[1], Axel de Monts de Savasse[1], Clara Whyte Ferreira[1], Eppe Gauthier[1], Quinton Loic[1], Johann Far[1], Edwin De Pauw[1]

[1] *Laboratory of Mass spectrometry (MSLab), Allée du six Août 11 - Quartier Agora - 4000 Liège, Belgique*

Oligonucleotides are short nucleic acid polymers (typically less than 100 mers) with numerous uses e.g. in biology, therapeutic agents and drug delivery, digital storage, chemical sensors, and aptamers. Chemical modifications of oligonucleotidess such as conjugation or modified nucleotides provide new interesting properties (stability, activity, affinity) but require impurities profiling before to be FDA-Approved Oligonucleotides. The sequence of short length oligonucleotides containing (modified) nucleotides (or mer) such as those typically used as si-RNA therapeutics agents (or therapeutics candidates) cannot be retrieved with the classical Sanger methods. The sequencing of the full-length products (FLP) and the impurities profiling are still analytical challenges, despite the use of liquid chromatography coupled with Electrospray MS/MS that provide incomplete sequence coverage. The impurities profiling based on reverse phase liquid chromatography heavily relies on the use of poly and perfluorinated organic modifiers that being reviewed for possible future ban. To face these analytical challenges, we revisited the use of high-performance thin layer chro- matography (HP-TLC), and we developed TLC on-chip for separation of oligonucleotides and their impurities combined with the use of MALDI mass spectrometry imaging (MALDI MSI) for multiplexed detection. Additionally, the use of MALDI directly on TLC plates using sprayed matrices inducing in-source decay (ISD), i.e. using in-source fragmentation driven by radical reactions chemistry, allowed the detection and the sequencing of the oligonucleotides in a single experiment , while being compliant with the green chemistry paradigms. Our data support the efficiency of MALDI-ISD for full-length sequencing of pure si-RNA like oligonucleotides in the mass range of therapeutic candidates directly from C18-grafted HP- TLC plates, after their separation. In the case of chemically modified oligonucleotides, interesting insights about the mechanistic of the ISD fragmentation processes will be presented. The first TLC-MALDI-ISD image were successfully obtained and the optimization of the elution of oligonucleotides without the use of perfluorinated compounds is a work in progress.

Poster 30 - Novel heated inlet for in-source activation of native protein complexes

Anisha Haris, Jakub Ujma, Kevin Giles, David Bruton, Keith Richardson, Bradley Davis, Madison Turner, and Siavash Vahidi

Waters Corporation

Introduction

Understanding the structure and interactions of native protein complexes is crucial for elucidating their mechanisms of action, potentially leading to innovative drug strategies for treating human diseases. However, the intrinsic and extrinsic heterogeneity and increasing size of these species can pose significant characterization challenges. Charge Detection Mass Spectrometry (CDMS), utilizing an Electrostatic Linear Ion Trap (ELIT), enables direct mass measurement of individual ions by simultaneously determining their mass-to-charge ratio (m/z) and charge (z). CDMS overcomes the limitations of conventional mass spectrometry (MS) methods. In this work, we describe the use of a novel heated inlet for activating various native protein complexes, which results in their structural elucidation and conformational characterization. This advancement enhances our ability to understand complex biological systems and supports the development of targeted therapeutic strategies.

Methods

Prior to CDMS analysis, all samples were buffer exchanged into ammonium acetate solution (pH 6.8) using either Micro Bio-Spin columns (Bio-Rad) or molecular weight cut-off filters (Amicon). Ions were generated in positive ion mode using nanoelectrospray ionisation (nESI) and mass analysis was performed using a Waters prototype CDMS instrument. The inlet on the instrument was heated to various temperatures, up to 300 °C for ion activation experiments. Signal processing and data visualization were performed using software developed in-house. Ions were trapped for 100 ms and the frequency and amplitude information were converted to m/z and z values respectively, and ultimately mass (m/z x z). These data were subsequently binned to generate the corresponding spectra (histograms).

Preliminary Data

We analyzed various analytes ranging from hundreds of kilodaltons (kDa) up to 3 megadaltons (MDa) in mass, including a proteasome activator, a proteasome, and DNA plasmids. For the bacterial proteasome activator (Bpa), complex dissociation was induced via inlet heating. Experiments with the heated inlet showed charge-reduced Bpa dodecamers, consistent with asymmetric charge partitioning of the Bpa:HspR complex due to HspR ejection. CDMS was also used to probe the structure of the non-covalent 28-subunit 20S proteasome, where the observed intact mass of this native complex was in good agreement with the expected mass of the 20S. A loss of charge was clearly observed when the CDMS inlet was heated above 250°C. Consequently, a higher m/z envelope around m/z 16,000-20,000 with resolved charge states was observed, indicating the loss of an individual alpha subunit from the 20S proteasome. CDMS can also be used for conformational analysis. Measurements of megadalton-sized DNA ions showed two distinctly different charge distributions for both pUC19 (z = 80 and z = 400-600) and pBR322 (z = 90 and z = 500-1000), attributed to the compact form (lower charge population) and the supercoiled form (higher charge population) of the plasmids. Heating the CDMS inlet improved desolvation of these species, resulting in narrower peak widths and shifts to lower mass by tens of kDa in the mass spectra. Additionally, there was a four-fold improvement in the detection of the higher charge populations for both plasmids compared to when no heating was applied. Utilizing a heated inlet for ion transfer demonstrates the ability to measure the mass of high-mass ions with improved desolvation, enabling conformational analysis of DNA, and facilitating ion activation and dissociation of proteasome and proteasome activator complexes. This supports efforts to further understand the structures and mechanisms of these biologically important non-covalent protein complexes.

Novel Aspect

A novel heated inlet on a prototype CDMS enhances desolvation, structural characterization, and protein complex dissociation

Conflict of Interest Disclosure

Anisha Haris, Jakub Ujma, Kevin Giles, David Bruton, Keith Richardson are employees of Waters Corporation.