5th NVMS-BSMS Conference on Mass Spectrometry

Book of Abstracts

28th of April — 30th April 2024

Rolduc, Kerkrade, The Netherlands

Welcome to the 5th NVMS-BSMS conference on mass spectrometry

Welcome at Rolduc2024 !

After a corona-related void and successful IMSC2022, it is our pleasure to welcome you again to the Rolduc2024 conference on Mass Spectrometry. The NVMS and the BSMS have established a tradition of jointly organizing this International Conference on Mass Spectrometry in the abbey of Rolduc in Kerkrade (NL). Our previous conferences in 2009, 2012, 2016, and 2019 have demonstrated that our collaborative efforts can lead to memorable events that are highly appreciated by our members and international guests.

Rolduc Abbey provides an ideal setting for high-level scientific discussions and informal gatherings in a serene and stimulating environment. We have done our best to maintain the high level of scientific presentations. The positive feedback from previous meetings has guided us to retain the general program structure, featuring internationally renowned keynote speakers, and oral and poster contributions from students and researchers from both academia and industry.

We would like to thank everyone who has invested their valuable time in preparing for this meeting: making the reservations long ahead of the meeting, selecting abstracts for the oral and flash presentations are essential tasks for a successful event. Finally, during and after the meeting helping hands and workers behind the scenes are indispensable. We would like to express our special thanks to our generous sponsors. Their support is instrumental in making this event possible. We encourage all participants to visit their booths and engage with the representatives.

If this is your first interaction with the NVMS, youngNVMS or BSMS, we hope that this meeting will inspire you and makes you join us again in the future. For our regular attendees, we hope you appreciate and enjoy connecting/catching up with colleagues involved in mass spectrometry, whether during the scientific program or during the evening social hours at the bar. We wish everyone a productive and enjoyable stay at the Rolduc Abbey.

Jef Rozenski (chair BSMS) & Anouk Rijs (chair NVMS)

Organizing and programme committees

NVMS (incl. young-NVMS): Anouk Rijs, Jan Commandeur, Eef Dirksen, Ariadni Geballa Koukoula, Arjen Gerssen, Annemieke Kolkman, Isabelle Kohler, Christel Kuik, Guinevere Lageveen-Kammeijer, Pieter Langerhorst, Lidia Molina Millán, Martin Pabst, Richard Scheltema, and Bram Snijders.

BSMS: Jef Rozenski, Filip Cuyckens, Pascal Gerbaux, Loïc Quinton, and Ann Van Eeckhaut.

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General information

Conference information

The conference starts with a welcome program on Sunday afternoon.

The rooms where lectures, poster presentations, exhibitions and general assemblies will take place are:

- Plenary lectures: Aula Major
- Parallel scientific sessions: lecture halls 1, 2 and 3
- Manufacturers' exhibitions: corridors
- Poster presentations: corridors
- General assembly NVMS: lecture hall 1

For your convenience, we have included a detailed map of the Rolduc congress centre with the areas clearly marked on the next page.

For those presenting a poster: please mount your poster on the board indicated by your individual poster number on Monday 29th of April after 8:30 and use only the material provided by the congress organization at the registration desk for mounting your poster. All posters should be removed before 16:30 on Tuesday 30th of April.

Presenting authors are kindly requested to present their poster during the specified poster session either on Monday (**odd numbers**) or Tuesday (**even numbers**).

In addition there will be short 'flash' introductions to the posters by young researchers during a specific session on Monday (10:00).

For those presenting an oral: please make sure that your presentation is uploaded on the computer of the appropriate lecture room 20 minutes before the session starts.

Meals and coffee/tea will be served at the following locations:

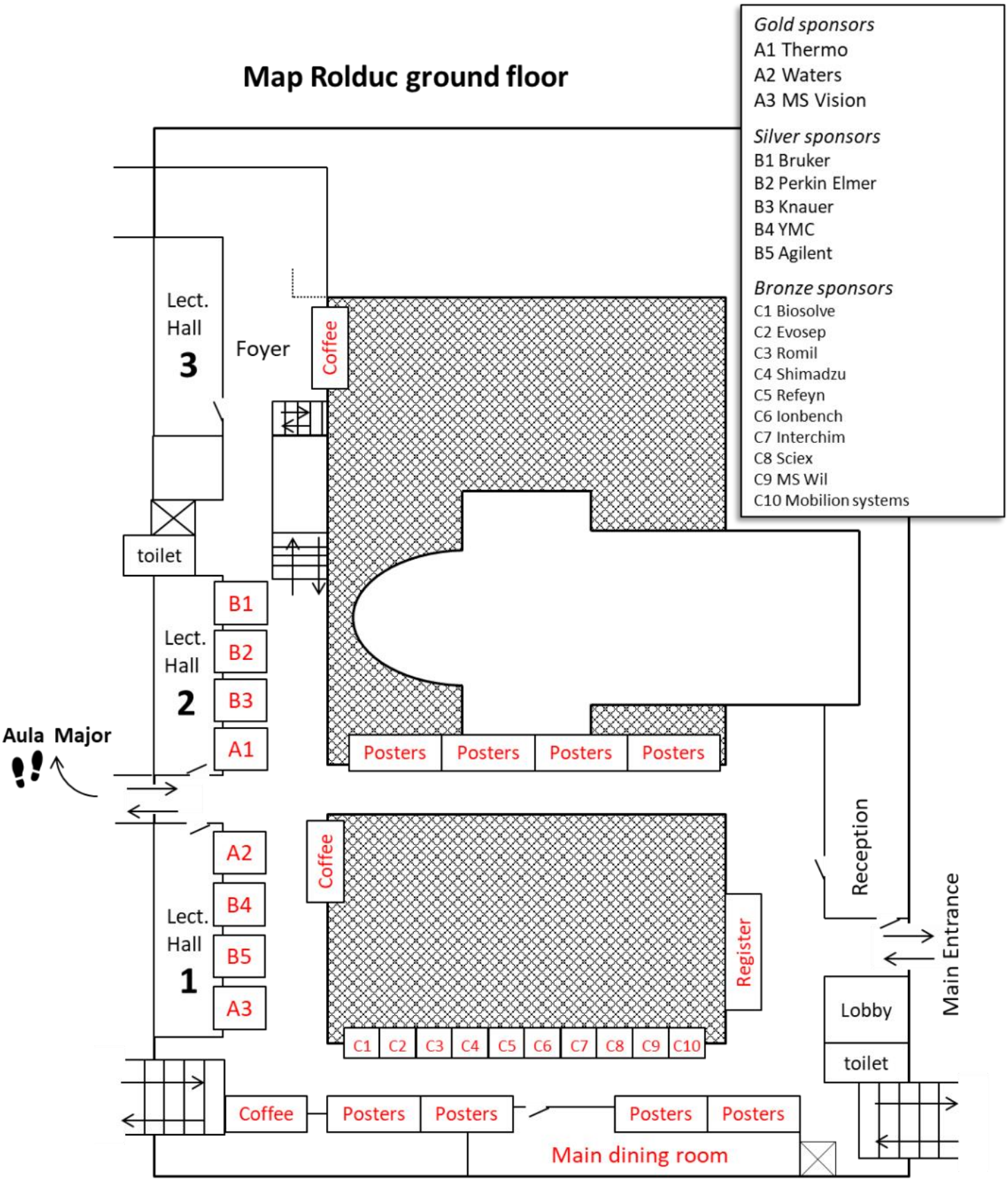
- Breakfast, lunch and dinner: main dining room
- Coffee and tea: foyer and corridor

Conference Registration and Secretariat

The registration desk for the conference is open from 14:30 on Sunday 28th of April and is located near the Rolduc reception in the corridor. For your one or two night stay at Rolduc please register at the Rolduc reception.

If you need assistance, please contact one of the members of the organizing committee.

Map Rolduc ground floor



Conference Programme

Sunday, April 28th, 2024

Aula Major
16:15 - 16:35 Opening
Anouk Rijs (Chair NVMS) & Jef Rozenski (Chair BSMS)

16:35-17:20 K1**-** Plenary Lecture:
Kathrin Breuker (University of Innsbruck)
Stoichiometry-resolved native top-down mass spectrometry for the study of RNA-small molecule interactions

17:20-18:05 K2- Plenary Lecture:
Martin Giera (Leiden University Medical Center)
The Neurolipid Atlas – Mapping lipidomic changes in neurodegenerative diseases

18:15-22:00 Buffet Dinner and Welcome Party (De verloren zoon)

Monday, April 29th, 2024

9:00-9:15 Aula Major Opening

9:15-10:00 K3- Plenary Lecture:
Simona Francese (Sheffield Hallam University)
Fingerprints, from catching criminals to catch cancer

10:00-11:10 Flash Poster Presentations (PhD Students)

11:10-11:40 Coffee Break

3 parallel sessions, **lecture rooms 1, 2 and 3**

Topics: Large Molecules / Small molecules / New developments
11:45-12:05 Lectures O1, O8, O15
12:05-12:25 Lectures O2, O9, O16

12:25-14:00 Lunch and Poster session (odd numbers)

Monday afternoon: 3 parallel sessions, **lecture rooms 1, 2 and 3**

Topics: Heck Session / Small Molecules / Large Molecules

14:00-14:20 Lectures O3, O10, O17
14:20-14:40 Lectures O4, O11, O18
14:40-15:00 Lectures O5, O12, O19

15:00-15:30 Coffee Break

Topics: Large molecules / New Developments / Small Molecules

15:30-15:50 Lectures O6, O13, O20
15:50-16:10 Lectures O7, O14, O21

Aula Major
16:15-17:00 K4**-** Plenary Lecture:
Claire Eyers (University of Liverpool)
What do we really know about +80 Da mass shifts?

17:00-18:00 K5- Plenary lecture:
K5- Matyas Bittenbinder (Naturalis / VU Amsterdam)
The good, the bad and the venom cocktail – what can we learn from these bioactive cocktails?

18:00-19:00 Young NVMS PubQuiz (Hall 2)

19:00 Conference Dinner

20:30 Conference Party (Boerenkelder and foyer)

Tuesday, April 30th, 2024

9:00-9:30- Annual assembly NVMS (Hall 1)

Aula Major
9:30-10:15 K6- Plenary lecture:
Tim Causon (BOKU University)
New opportunities and tools for using IM-MS in analytical methods

10:15-11:00 K7- Plenary lecture:
Frank Vanhaecke (Ghent University)
Use of inductively coupled plasma – mass spectrometry (ICP-MS) as a versatile tool in the biomedical sciences

11:00-11:30 Coffee Break

3 parallel sessions, **lecture rooms 1, 2 and 3**

Topics: Large Molecules / New Developments / Small Molecules

11:30-11:50 Lectures O22, O26, O30
11:50-12:10 Lectures O23, O27, O31

12:10-13:40 Lunch and Poster session (even numbers)

Topics: Large Molecules / Small Molecules / Large Molecules

13:40-14:00 Lectures O24, O28, O32
14:00-14:20 Lectures O25, O29, O33

Aula Major
14:30-15:10 K8- Plenary lecture:
Rawi Ramautar (Leiden Academic Centre for Drug Research)
Charge to move forward in volume-restricted Metabolomics

15:15 Poster award ceremony and goodbye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Sunday			
14:30 - 16:30	Registration		
16:15 - 16:35	Aula Major	Opening by Anouk Rijs (*Chair NVMS) & Jef Rozenski (Chair BSMS)*	
16:35 - 17:20	Aula Major	***K1- Kathrin Breuker,*** *University of Innsbruck* Stoichiometry-resolved native top-down mass spectrometry for the study of RNA-small molecule interactions	*Chair: Anouk Rijs*
17:20 - 18:05	Aula Major	***K2- Martin Giera,*** *Leiden University Medical Center* The Neurolipid Atlas – Mapping lipidomic changes in neurodegenerative diseases	*Chair: Guinevere Lageveen-Kammeijer*
18:15 - 22:00	Buffet dinner & Welcome party		

Monday			
09:00 - 09:15	Aula Major	Opening by Anouk Rijs (*Chair NVMS), Jef Rozenski (Chair BSMS), Pieter Langerhorst (YoungNVMS)*	
09:15 - 10:00	Aula Major	***K3- Simona Francese,*** *Sheffield Hallam University* Fingerprints, from catching criminals to catch cancer	*Chair: Jef Rozenski*
10:00 - 11:10	Aula Major	***Flash presentations (PhD students)***	*Chair: Eef Dirksen*
11:10 - 11:40	Coffee break		
Room	*Hall 1*	*Hall 2*	*Hall 3*
Topic	***Large molecules - Proteomics 1***	***Small molecules - Oligosaccharides***	***New developments - Ion mobility***
Chair	Guinevere Lageveen-Kammeijer	Pascal Gerbaux	Jan Commandeur
11.45 - 12:05	***O1- Eva Smit,*** *Sanquin* Proteomics Reveals Distinct Protein Patterns To Postpartum Development In Small For Gestational Age Preterm Infants	***O8- Julia Vreugdenhil,*** *Utrecht University* *De novo sequencing of human milk oligosaccharides using IMS-MS*	***O15- Raya Sadighi,*** *VU Amsterdam* Novel strategies for the characterization of the early-stages of aggregate formation of α-Synuclein using hyphenated mass spectrometry
12:05 - 12:25	***O2- Dario A. T. Cramer,*** *Leiden University Medical Center* Exploring Interpersonal Variability in Serum Glycoproteins: a Quantitative Analysis of Glycoforms in Healthy Donors.	***O9- Jitske van Ede,*** *TU Delft* Exploring the diversity of nonulosonic acids in pathogenic microbes: targets for the development of novel antimicrobials	***O16- Hugo Muller,*** *University of Liège* Sliding windows in ion mobility (SWIM): a new approach to increase the separation power in trapped ion mobility-mass spectrometry hyphenated with chromatography
12:25 - 14:00	Lunch and poster session for odd numbers		

Monday			
Room	*Hall 1*	*Hall 2*	*Hall 3*
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Chair	Albert Heck	Arjen Gerssen	Eef Dirksen
14:00 - 14:20	***O3- Evolène Deslignière,*** *Utrecht University* The Good, the Bad, and the Ugly: Analysis of mRNA Molecules by Mass Spectrometry and Mass Photometry	***O10- Liam Nestor,*** *VU Brussel* Metabolic profiling of epileptic hippocampal tissue upon chemogenetic modulation of astrocytes using CE-HRMS	***O17- Amber Rolland,*** *Utrecht University* Fab profiling reveals plasma IgA1 clones can co-occur in both monomeric and J-coupled dimeric forms
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15:00 - 15:30	Coffee break		
Room	*Hall 1*	*Hall 2*	*Hall 3*
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15:50 - 16:10	***O7- Tanja Habeck,*** *TU Darmstadt* Studying homo- and hetero-oligomers with native ion mobility – mass spectrometry to understand the conformational interplay of different amyloid β peptides	***O14- Steven Daly,*** *MS Vision* Teaching Old Dogs New Tricks: Extending the Utility of your Mass Spectrometer	***O21- Jan Jordens,*** *VITO* Unraveling the PFAS puzzle: towards a workflow for total PFAS screening
16:15 - 17:00	Aula Major	***K4- Claire Eyers,*** *University of Liverpool* What do we really know about +80 Da mass shifts?	*Chair: Richard Scheltema*
17:00 - 18:00	Aula Major	***K5- Matyas Bittenbinder,*** *Naturalis / VU Amsterdam* The good, the bad and the venom cocktail – what can we learn from these bioactive cocktails?	*Chair: Anouk Rijs*
18:00 - 19:00	Hall 2	yNVMS pubquiz	
19:00	Conference dinner		
20:30	Conference party (Boerenkelder and foyer)		

Tuesday			
09:00 - 09:30	Hall 1	**Annual assembly NVMS**	
09:30 - 10:15	Aula Major	***K6- Tim Causon,*** *BOKU University* New opportunities and tools for using IM-MS in analytical methods	*Chair: Ann van Eeckhaut*
10:15 - 11:00	Aula Major	***K7- Frank Vanhaecke,*** *Ghent University* Use of inductively coupled plasma – mass spectrometry (ICP-MS) as a versatile tool in the biomedical sciences.	*Chair: Filip Cuyckens*
11:00 - 11:30	Coffee break		
Room	*Hall 1*	*Hall 2*	*Hall 3*
Topic	***Large molecules - Proteomics 2***	***New developments***	***Small molecules - Lipidomics***
Chair	Pieter Langerhorst	Richard Scheltema	Pascal Gerbaux
11:30 - 11:50	***O22- Ramon van der Zwaan,*** *TU Delft,* Pushing the limits of proteomics: characterizing microbial processes in wastewater treatment plants	***O26- Victor C. Yin,*** *Utrecht University* Development and Applications of Ultra-long Transients for Orbitrap -based Single Ion Mass Spectrometry	***O30- Michel van Weeghel,*** *Amsterdam UMC* Unraveling mono genetic disorders with complex pathologies through advanced 4D-lipidomics: Insights into ALD and BTHS
11:50 - 12:10	***O23- Christel Kuik,*** *M4i* The proteome response of Staphylococcus Aureus to titanium biomaterial surface roughness	***O27- Ian Anthony,*** *M4i* Advancements in fast mass microscopy speed, molecular information, and data processing	***O31- Tim Hendriks,*** *M4i* Unveiling Lipidomal Dynamics in Glioblastoma Recurrence: Single-Cell MALDI-MSI of Patient-Derived Cell Lines
12:10-13:40	Lunch and poster session for even numbers		
Room	*Hall 1*	*Hall 2*	*Hall 3*
Topic	***Large molecules - CE-MS***	***Small molecules - Food & Flavors***	***Large molecules - Proteoforms and complexes***
Chair	Guinevere Lageveen-Kammeijer	Martin Pabst	Bram Snijders
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14:00 - 14:20	***O25- Elena Domínguez Vega,*** *Leiden University Medical Center* Unravelling functional changes in antibody proteoforms using affinity CE-MS	***O29- Mudita Vats,*** *M4i* Mass Spectrometry Imaging Reveals Essential Oil Treatment Effects on Downy Mildew in grapevine *(vitis vinifera L.)*	***O33- Edvaldo Vasconcelos Soares Maciel,*** *TU Darmstadt* Chemical vs. collision-induced disruption of artificially-induced ternary protein complexes.
14:30 - 15:10	Aula Major	***K8- Rawi Ramautar,*** *Leiden Academic Centre for Drug Research* Charge to move forward in Volume-restricted Metabolomics	*Chair: Ann van Eeckhaut*
15:15	Aula Major	***Poster award ceremony & goodbye***	*Chair: Jef Rozenski*

Keynote abstracts

K1- Stoichiometry-resolved native top-down mass spectrometry for the study of RNA-small molecule interactions

Kathrin Breuker

Institute of Organic Chemistry, University of Innsbruck, Innrain 80/82, Innsbruck, Austria

Top-down mass spectrometry (MS) of ribonucleic acids (RNA) is an emerging field of research with applications ranging from the characterization of posttranscriptional and synthetic modifications [1-3] to the determination of binding sites of proteins and small molecule therapeutics [4-6]. Here we show how data from top-down MS experiments on a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer can be used to localize and relatively quantify posttranscriptional and synthetic RNA modifications, and how the same approach in combination with native electrospray ionization can identify RNA binding motifs and their occupancy with ligand. For the 40 nt aptamer of a neomycin sensing riboswitch functional in yeast and a 47 nt construct of rev response element (RRE) RNA from human immunodeficiency virus 1, we demonstrate that the binding of aminoglycosides to RNA is surprisingly intricate and can involve multiple binding sites and complex stoichiometries. We are currently extending our native top-down MS approach to other classes of RNA and ligands with the ultimate goal of establishing general principles of RNA recognition to guide the development of drugs that target RNA.

[1] M. Taucher, K. Breuker: Characterization of modified RNA by top-down mass spectrometry (2012) *Angew. Chem. Int. Ed.* 51, 11289-11292

[2] H. Glasner, C. Riml, R. Micura, K. Breuker: Label-free, direct localization and relative quantitation of the RNA nucleobase methylations m^6A, m^5C, m^3U, and m^5U by top-down mass spectrometry (2017) *Nucleic Acids Res.* 45, 8014-8025

[3] M. Palasser, K. Breuker: RNA chemical labeling with site-specific, relative quantification by mass spectrometry for the structural study of a neomycin-sensing riboswitch aptamer domain (2022) *ChemPlusChem* 87, e202200256

[4] E.M. Schneeberger, K. Breuker: Native top-down mass spectrometry of TAR RNA in complex with a wild-type tat peptide for binding site mapping (2017) *Angew. Chem. Int. Ed.* 56, 1254-1258

[5] E.M. Schneeberger, M. Halper, M. Palasser, S.V. Heel, J. Vušurović, R. Plangger, M. Juen, C. Kreutz, K. Breuker: Native mass spectrometry reveals the initial binding events of HIV-1 rev to RRE stem II RNA (2020) *Nat. Commun.* 11, 5750

[6] S.V. Heel, K. Bartosik, F. Juen, C. Kreutz, R. Micura, K. Breuker: Native top-down mass spectrometry uncovers two distinct binding motifs of a functional neomycin-sensing riboswitch aptamer (2023) *J. Am. Chem. Soc.* 145, 15284-15294

K2- The Neurolipid Atlas – mapping lipidomic changes in neurodegenerative diseases

Martin Giera[a]

[a]Leiden University Medical Center / Albinusdreef 2, 2333ZA, Leiden, The Netherlands

Emerging genetic, epidemiological, and cell biological data has implicated lipid and cholesterol metabolism in a variety of neurodegenerative diseases. However, lipid biology remains an understudied area of research, and there are technical complexities with the application of lipidomic methods to human tissues. The Neurolipid Atlas project analyzes the lipidome of human induced pluripotent stem cell (iPSC)-derived neurons and glia, gene-edited for specific disease variants and aimed at generating the first map of disease-specific, genotype-specific and cell type-specific changes in the human lipidome associated with neurodegenerative diseases. The project involves the establishment of a publicly available homepage (www.neurolipidatlas.com) including the development of dedicated data visualization and analysis software ready to use with shotgun lipidomics data. As examples we will dissect astrocyte specific lipidomic changes and pathways associated with the Alzheimer's disease relevant APOE4 gene variant as well as neuron specific changes of the Amyotrophic Lateral Sclerosis (ALS) inducing mutation C9orf72. We will highlight the technical implementation of comprehensive quantitative shotgun lipidomics with iPSC derived human cell technologies and the use of lipidomics and metabolomics technologies in dissecting patho-physiological mechanisms. Furthermore, we will present our novel software tool called SODA (Simple Omics' Data Analysis) developed for the Neurolipid Atlas project. Finally, we will discuss potential rescue mechanisms and therapeutic approaches based on the molecular insights obtained.

K3- Fingerprints, from catching criminals to catch cancer

Simona Francese

Sheffield Hallam University, Howard Street , Sheffield, UK

In the last decade fingerprint analysis by mass spectrometry and mass spectrometry imaging has brought about significant forensic opportunities to both profile and identify a suspect. Particularly Matrix Assisted Laser Desorption Ionisation profiling and Imaging (MALDI MSP and MSI) have been investigated to provide chemical and biometric information on an individual from their fingerprints[1].

The overall method that we called "molecular fingerprinting" has been reported in the Fingermark Visualisation Manual, edited by the Home Office and the Defense Science and Technology, Laboratory UK, and has been already used in casework Nationally[2] and Internationally.

However, MALDI MSI has also shown significant potential to use fingerprints and fingerprint smears to non-invasively detect pathologies[3,4], thus locating fingerprints at the interface between forensic science and clinical diagnostics.

In this presentation, both these applications will be discussed through the journey that has led to bridge the gap between these two fields.

1. Francese S, Criminal profiling through MALDI MS based technologies – breaking barriers towards border free forensic science, 2019, Australian Journal of Forensic Sciences, DOI: https://doi.org/10.1038/s41598-023-29036-710.1080/00450618.2018.1561949

2. Bradshaw R., Denison N., Francese S., Implementation of MALDI MS profiling and imaging methods for the analysis of real crime scene fingermarks, 2107, Analyst, 142:1581

3. Heaton C, Witt M, Cole L, Eyre J, Tazzyman S, McColm R, Francese S " Detection and mapping of haemoglobin variants in blood fingermarks by MALDI MS for suspect “profiling”, 2021, Analyst, 46:4290

4. Russo, C., Wyld, L., Da Costa Aubreu, Bury, C., Heaton C., Cole, L.M., Francese S. Non-invasive screening of breast cancer from fingertip smears—a proof of concept study, 2023, https://doi.org/10.1038/s41598-023-29036-7

K4- What do we really know about +80 Da mass shifts?

Claire E. Eyers

Centre for Proteome Research, Faculty of Health & Life Sciences, University of Liverpool, United Kingdom

Post-translational modifications (PTMs) are ubiquitous and key to regulating protein function. Understanding the dynamics of individual PTMs and their biological roles requires robust characterisation, with mass spectrometry (MS) being the method of choice for their identification and quantification. Protein phosphorylation is one such dynamic PTM that is extensively characterised by MS, based on observation of an 80 Da mass shift. We have recently begun to explore the diversity of protein phosphorylation and the implications of other 80 Da mass shifts in MS data.

This presentation will explore strategies for the enrichment and site localisation of phosphorylated (canonical and non-canonical) peptides. I will also discuss approaches for the discrimination of phosphorylated peptides from those that contain sulfate, which also induces an 80 Da mass shift, and how these new workflows are advancing biological understanding of PTM-mediated regulation of biological processes. Finally, we will consider the potential implications of mis-identification of peptides containing multiples of 80 Da modifications in the context of PTM discrimination, and differentiation from e.g. pyrophosphorylation.

K5- The good, the bad and the venom cocktail – what can we learn from these bioactive cocktails?

Mátyás Bittenbinder [a,b]

[a] AIMMS Division of BioAnalytical Chemistry, Vrije Universiteit Amsterdam, De Boelelaan 1085, 1081HV, Amsterdam, The Netherlands

[b] Naturalis Biodiversity Center, 2333 CR Leiden, The Netherlands;

Animal venoms contain a rich source of bioactive molecules that, over the course of millions of years, have evolved into highly efficient 'biochemical weapons' used for prey capture or self-defence. Worldwide, there are over 100,000 venomous animal species, each with a unique cocktail of toxins. The clinical effects following envenomation can be categorised into three main pathologies, including neurotoxicity, haemotoxicity, and tissue-damaging effects, with some venoms causing a combination of these. Venomous bites and stings, mainly from snakes and scorpions cause a major global crisis affecting millions of people worldwide. The combined annual number of people that fall victim to envenomation by snakes and scorpions surpasses 1.5 million. Snakebite envenoming is particularly relevant, with annual mortality rates estimated to range between 81,000 – 138,000. Snake venoms may cause a range of local and systemic effects in bite victims, with some being life-threatening while others are permanently debilitating. The potency and selectivity of these venom cocktails on the other hand makes them highly interesting from both a biochemical and biological perspective. The study of animal venoms has successfully led to the development of billion-dollar biopharmaceuticals such as Aggrastat and Captopril but has also advanced our knowledge on (haemostatic) disease mechanisms and generated important diagnostic tools which are being used in clinical laboratories worldwide, some already for many decades. To date, we are using only a fraction of all compounds found in animal venom for the treatment of human disease. During my talk, I will share with you some of the most incredible venomous animals and tell you why we should not be afraid of them but cherish them instead.

K6- New opportunities and tools for using IM-MS in analytical methods

Sangeeta Kumari[a], Stephan Hann[a] and Tim Causon[a]

[a]University of Natural Resources and Life Sciences Vienna, Muthgasse 18, Vienna, Austria

Ion mobility-mass spectrometry (IM-MS) as a commercial analytical technology has been on the market from multiple vendors for more than 10 years. During this time, breakthroughs in instrument performance capabilities and an increasing range of analytical applications have been realized. However, along with this research and development activity come new challenges for standardization of measurement practices, more accessible analytical workflows, and data processing tools that utilize the full range of IM-MS data available. Furthermore, new application directions can now be sought with new high-resolution (HR)IM-HRMS developments. However, using HRIM-HRMS successfully in analytical applications will require a major shift in the balance between the timescales of pre- and post-ionization separations requiring working toward goals that contradict one another: i.e., harnessing the high-resolution IM separation and allowing sufficient acquisition time for obtaining high-quality data; no longer relying on slow LC separation and still delivering high sensitivity signals free of ion suppression. Using a new commercial *Structures Lossless for Ion Manipulation* (SLIM)-HRMS platform, we are currently exploring the analysis of isomeric systems where the potential of HRIM-HRMS can be realized. Finally, examples of our recent research in (HR)IM-HRMS applications and software development particularly for metabolomics and small molecule applications will be highlighted including collaborative work in the new MSCA Doctoral Network MobiliTraIN (www.mobilitrain.eu).

K7- Use of inductively coupled plasma – mass spectrometry (ICP-MS) as a versatile tool in the biomedical sciences.

Frank Vanhaecke

Ghent University, Department of Chemistry, Atomic and Mass Spectrometry – A&MS research unit, Campus Sterre, Krijgslaan 281 – S12, Ghent, Belgium

Inductively coupled plasma – mass spectrometry (ICP-MS) is the most powerful technique for (ultra)trace element analysis and is routinely used in clinical labs for the quantification of both essential and toxic elements in body fluids, like whole blood, serum, and urine. However, ICP-MS is capable of so much more in the context of research in the biomedical sciences.

In its standard configuration, ICP-MS is intended for the analysis of liquid samples. Solid materials have to be taken into solution, which is typically accomplished using (microwave-assisted) acid digestion. The digest thus obtained needs to be appropriately diluted and the aqueous sample solution thus obtained is introduced into the ICP ion source using pneumatic nebulization. By using laser ablation (LA) as a means of sample introduction, solid samples can be analyzed directly (no digestion required). By scanning the deep-UV laser beam line per line over a thin section of tissue, LA-ICP-MS enables the distribution of elements to be revealed in 2 (and even 3) dimensions. With the set-up used at UGent-A&MS, these elemental maps can be obtained at a pixel acquisition rate up to 1 kHz, while the minimum beam diameter is 1 µm. When using an ICP-MS instrument equipped with a scanning mass analyzer (quadrupole filter or sector-field mass spectrometer), only one ion signal can be monitored at any given time. When using an ICP-MS instrument based on a time-of-flight (ToF) analyzer, however, a(n almost) complete elemental mass spectrum can be obtained for every laser shot / map pixel.

ICP-MS provides sufficient detection power for determination of the content of either exo- or endogenous elements in individual cells of plant, animal, or human origin. This requires the nebulization of a dilute suspension of cells into the ICP and monitoring the short transient signal observed whenever a cell is reaching the ICP and is giving origin to a burst of ions. While bulk analysis provides the average cell content only, single-cell ICP-MS also provides insight into the corresponding distribution, providing a deeper insight into, e.g., drug penetration into cells.

Finally, with multi-collector ICP-MS (MC-ICP-MS), isotope ratios of essential mineral elements in body fluids and tissues can be determined with extremely high precision (down to 0.001% RSD measurement precision). It has been shown that isotope ratios are either more sensitive in picking up changes in metal metabolism in case of a disorder or/and contain information that is not embedded in element concentrations. Isotopic analysis can provide insight into changes in biochemical reactions occurring upon development of a disease and show promise as a diagnostic/prognostic tool.

This presentation will use applications carried out by the A&MS team at Ghent University to illustrate the capabilities described above.

K8- Charge to move forward in volume-restricted Metabolomics

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In metabolomics, advanced analytical workflows are needed to study biochemical changes in small-volume biological samples, notably for samples originating from 3D microfluidic cell culture models, individual zebrafish larvae and neonatal clinical blood sampling. Recent work from our lab revealed that capillary zone electrophoresis-mass spectrometry (CE-MS), regardless of utilizing a sheath-liquid or sheathless interface, is a strong analytical tool for probing polar and charged metabolites in biological samples with a good reproducibility. Moreover, in a simulated metabolomics study, CE-MS was able to find the right set of differential metabolites between controls and cases. These studies clearly indicate the value of CE-MS for biomarker discovery and comparative metabolomics studies.

Given our ambition to address volume-restricted biomedical questions with metabolomics, we report in this presentation on the development of new CE-MS-based analytical workflows for the highly efficient and sensitive analysis of polar (endogenous) metabolites in neonatal plasma and individual zebrafish larvae. As only nanoliters of samples are consumed by a single CE-MS analysis, multiple injections/assays can be performed on the same valuable volume-limited sample allowing for technical replicates and/or probing different classes of ionogenic metabolites. We show how these new CE-MS-based workflows can be employed in a reliable way for the quantitative analysis of creatinine, and many more endogenous compounds, in neonatal plasma samples using a starting amount of less than 5 microliter, whereas gold standard clinical chemistry approaches require often a minimum of 100 microliter for only creatinine determination. Hence, the proposed CE-MS-based workflow will contribute to minimizing both the amount and frequency of blood collecting required for diagnostic purposes in a neonatal setting.

We also demonstrate the utility of a new CE-MS workflow for the profiling of metabolites in extracts from individual zebrafish larvae and pools of small numbers of larvae. More than 70 endogenous metabolites could be observed in a pool of 12 zebrafish larvae, whereas 29 endogenous metabolites were detected in an extract from only 1 zebrafish larva. So far, zebrafish has proven to be a very effective model for stress research, in particular for studies on the effects of cortisol, with a clear role of the glucocorticoid receptor during stress. However, the role of the mineralocorticoid receptor (MR) on mediating the effects of cortisol is less known. By using wild-type (WT) and ubiquitous MR-knockout (MRKO) zebrafish larvae exposed to exogenous cortisol treatment, our CE-MS-based metabolomics workflow revealed the implication of metabolic pathways solely activated via MR. Taken together, CE-MS has the potential to identify novel pathways and mechanisms of action in zebrafish larvae and is a viable analytical approach for volume-restricted metabolomics.

Abstracts Orals

O1

Proteomics Reveals Distinct Protein Patterns To Postpartum Development In Small For Gestational Age Preterm Infants

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Preterm infants, especially those born small for gestational age (SGA), are at risk of various short-term and long-term health complications. Characterization of changes in circulating protein postpartum in preterm infants may provide valuable fundamental insights into changes in the major biological systems this population, as little is known regarding how these circulating protein change after birth. Mass spectrometry (MS)-based serum profiling is an attractive tool to study circulating proteins in this vulnerable population, as it allows for the identification of hundreds of proteins from limited sample volumes.
Here, we explored the potential of MS-based serum profiling to establish postpartum developmental patterns during the first four weeks and identify protein signatures that deviate with clinically relevant conditions, focusing on SGA infants and appropriate for gestational age (AGA) infants. Longitudinal serum samples from 67 very preterm infants were analyzed using unbiased MS-based proteomics. To identify biological processes associated with postnatal age, we classified protein abundance changes over time and related these proteins to molecular processes. Protein profiles of SGA and AGA infants were compared through statistical analysis of protein abundances in cord blood and longitudinal monitoring of group-specific changes.
In this study, we showed age-related changes in circulating protein levels after birth for 314 out of 833 quantified serum proteins. In addition to previously described changes in immunoglobulins, hemoglobin subunits and acute phase proteins, we found additional postpartum changes, most notably in apolipoproteins (e.g. APOA4) and proteins belonging to the terminal complement cascade (e.g. C9). While we found limited differences at birth between SGA and AGA infants, longitudinal monitoring revealed 69 proteins with deviations in protein level trajectories over time. This included the fat-accumulating hormone adiponectin (ADIPOQ) as well as 24 proteins with an annotated function in adaptive and innate immunity. In conclusion, we show the potential of MS-based serum profiling in defining circulating protein trajectories in the preterm infant population and its ability to identify longitudinal alterations in protein levels associated with SGA, one of the major risk factors for health complications in preterm infants.

O2

Exploring Interpersonal Variability in Serum Glycoproteins: a Quantitative Analysis of Glycoforms in Healthy Donors.

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Glycosylation is a highly prevalent post-translational modification among all kingdoms of life. Specifically, *N*-linked glycosylation plays a crucial role in the functional modulation of human serum glycoproteins and is associated with a range of conditions and diseases. The human serum "*N*-glycome" has been the subject of many studies on the released glycan level. By enzymatically releasing the glycans, changes in glycosylation patterns have been described not only as a result of disease states, but also in healthy populations due to homeostatic factors like age, sex and BMI. Examples related to lifestyle include aberrant glycosylation found in non-alcoholic fatty liver diseases and type 2 diabetes, affecting large populations. In both, increase in antennation and fucosylation or other glycoform changes can be found. Similarly, immunoglobulin G *N*-glycosylation changes with BMI and is related to the inflammatory role of immunoglobulins in obesity. Observing changes in glycosylation can thus potentially serve as a biomarker in conditions that are manifested before they are symptomatic. As a result, many specific glycoforms have become a target of both therapeutic and fundamental interest.

However, knowledge on the exact identities of the glycoproteins involved in these altered glycosylation states is still limited, as peptide-location specific information is lost in glycan release. Often, the role of glycoproteins in these processes is studied on isolated proteins with a case-by-case approach through glycopeptide or intact glycoprotein analysis. The wide variety of techniques and sample sets used in these studies further complicates a comparative analysis of the abundances and types of glycoforms in serum. Thus, the need for a serum wide characterization and quantification of glycopeptides is apparent.

Here, we present the results of a serum-wide glycopeptide study on a cohort of 20 healthy individuals. We characterized and quantified glycopeptides after enrichment and tryptic digestion using LC-MS^2 with HCD and EthCD fragmentation. We describe a range of glycopeptides and uncover a discrepancy between reported and observed glycoform abundances. Additionally, we show how specific glycoforms can show a wide range of abundance between individuals, from remaining practically unchanged to large detectable differences between donors. With this work, we aim to bring additional insight into serum glycoproteoform profiling work, provide a relevant point of reference and increase awareness of interpersonal glycoform abundance variability.

O3

High mass analysis of intact mRNAs by mass spectrometry and mass photometry: The Good, the Bad, and the Ugly

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mRNA-based vaccines recently emerged as a favorable alternative to conventional vaccines against various infectious diseases, as demonstrated by the regulatory approval and success of two highly efficient mRNA-based vaccines encoding for the SARS-CoV-2 spike protein. Analytical approaches must keep pace to support the surge in the development of mRNA therapies. Intact mass analysis of mid-to-large mRNA molecules (>1000 nucleotides) poses significant analytical challenges due to mRNAs' size, heterogeneity, and instability. So far, their characterization has been mostly restricted to digestion-based workflows. Here, we demonstrate how single-particle Orbitrap-based charge detection MS (CDMS) and mass photometry, can rapidly and accurately measure the mass of various intact high mass capped mRNAs, in size up to 9400 nucleotides (~3 MDa).

First, ensemble MS experiments evidenced that mRNAs bear fewer positive charges in nESI than similarly sized proteins. Charge distributions in high m/z ranges could be distinguished, albeit peaks were still broad and 'ugly' likely due to the presence of multiple polyA tail variants and counterions. Although ensemble MS provided a first mass approximation for mRNAs <2000 nucleotides, it failed to provide information for larger samples, for which unresolved distributions were obtained.

By recording single ions in the Orbitrap, drawbacks of ensemble MS could be circumvented. Using CDMS, masses could be extracted not only for mid-sized mRNAs (800 – 2000 nucleotides), but also for mRNAs in the megadalton range (up to 9400 nucleotides), although 'bad' unstable ion behavior made measurements challenging. Even more interesting was the possibility to study highly charged unfolded populations enhanced by the addition of methanol. Performing CDMS on higher charges (~100z) is particularly beneficial as it directly translates into better S/N and reduced charge uncertainty, thus improving mass accuracy (mass error decreasing from 10 to 3 %).

Lastly, we explored mass photometry to characterize large mRNAs in solution. Binding events for mRNAs molecules were readily observed. Varying molecule-to-molecule contrasts revealed low amounts of mRNA fragments and dimers as production byproducts that can be overlooked in CDMS, highlighting the complementarity of the two approaches. We could also detect extremely large mRNAs with high accuracy by mass photometry, as differences of only 0.5% compared to the theoretical values were obtained (9412 ± 67 nt *vs* 9432 nt), which is remarkable in this mass range (>3 MDa).

Overall, CDMS and mass photometry clearly outperform ensemble MS, enabling the study of even heterogeneous mRNA samples in the megadalton range without requiring a digestion step or online separation. Considering the growing interest in mRNA-based vaccines, therapeutic development would strongly benefit from both approaches.

Single Molecule Mass Spectrometry - Democratization of Native MS

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In Native MS, macromolecular complexes are brought into the gas phase and ionized while retaining their non-covalent interactions, preserving a native-like structure. This enables the investigation of the molecular architecture of said complexes and can give detailed insights into their composition like binding of small ligands and the presence of post-translational modifications on individual subunits.

Such analysis can answer unique biological questions, often elusive to other structural methods. However, despite the wide range of applicable biological systems, native MS is, opposed to other flavours of mass spectrometry like bottom-up, still mainly employed by experts. One reason therefore is the lack of robust methods which can be applied to a wider range of analytes but the need to optimize sample preparation, data acquisition, and processing for most experiments, especially when analytes get bigger.

Single molecule mass spectrometry is a technique, where instead of recording the combined ensemble signal for all ions from a given analyte, each individual molecules m/z and charge are measured separately. It has been shown that large ions exhibit a surprising level of stability in the Orbitrap™ mass analyzer and are particularly well suited for such experiments. Aside from increasing sensitivity and reducing sample consumption, this method offers also a unique opportunity of tackling existing bottlenecks in native MS. Particularly, automated data processing and the possibility to subject the samples to online buffer exchange have the potential to increase throughput in native MS and open the technique for a much broader userbase.

O5

Multifaceted Conformational Characterization of Native Therapeutic Antibodies by structural mass spectrometry approaches

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Conformational characterization of native therapeutic monoclonal antibodies (mAbs) is essential for understanding their structure-function relationships and optimizing their therapeutic efficacy. In recent years, structural mass spectrometry (MS) techniques offer a comprehensive toolkit for elucidating the complex mAb conformational landscapes.

Native mass spectrometry (nMS) enables the analysis of intact mAbs and related complexes, preserving their native-like conformations in the gas phase, providing insights into their composition, stoichiometry, and higher-order structure. Ion mobility spectrometry coupled to MS (IM-MS) further extends the capabilities of structural MS by separating ions based on their size, shape, and charge. IM-MS offers insights into the collisional cross-sections and gas-phase stabilities of mAbs, elucidating their structural shape and compactness. When ion activation is added prior to IM separation, collision-induced unfolding (CIU) experiments allow to monitor the extend of unfolding in so-called CIU unfolding profiles, that reveal the hierarchical stability of mAb domains and subunits. CIU experiments provide valuable insights into the structural integrity of native mAbs and their susceptibility to conformational changes induced in different conditions (storage, upon conjugation process, stress, etc.).

More recently, mass photometry (MP) has been applied to complement native MS by providing single-molecule analysis of mAbs in solution, without extensive sample preparation. By measuring the mass of individual antibody molecules in real-time, MP offers direct information on size distributions, oligomeric states, and conformational dynamics. Integration of MP with native MS and IM-MS enhances the understanding of antibody folding, stability, and assembly, providing a holistic view of their conformational landscape. These multidimensional insights into antibody structure-function relationships pave the way for the design of next-generation antibody therapeutics with enhanced efficacy and specificity.

Here the synergistic integration of native MS, mass photometry, and ion mobility-based strategies for comprehensive conformational characterization of therapeutic mAbs will be illustrated through different examples, with emphasis on continued advancements in instrumentation, methodology, and data analysis over years.

O6

Revisiting Protein Gas-Phase Unfolding: Contribution of the Mobile Proton Model through Chemical Modifications and Ion Mobility

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Mass spectrometry coupled with ion mobility (IM-MS) can monitor the evolution of ion shapes of intact proteins transferred into the gas phase using collision unfolding experiments (CIU). CIU achieves multiple gas-phase low-energy collisions (slow heating) of ions at increasing accelerating voltages against argon, nitrogen, or helium to explore their energy landscapes and (meta)stable conformers through the change of collision-cross sections. Some studies suggest a link between the mobile proton model and protein gas-phase unfolding. Here, we propose to chemically modify (covalent labeling) peculiar side chains to affect the proton affinity (PA) of charged residues and evaluate how the proton transfer in the gas phase would disturb the CIU heatmaps and CID survival yields.

Cytochrome c and ubiquitin have been modified with various NHS-esters derivatives to turn lysine side chains, having a high proton affinity due to its primary amine moiety, into acetylated amines or amine substituted with various aromatic organic groups. The influence of the incorporated chemical modifications was monitored by CIU and CID on a traveling wave ion mobility mass spectrometer (Synapt G2 Si HDMS from Waters, U.K.) under non-denaturing or denaturing conditions. The lack of regioselectivity of the NHS-ester reagents for primary amines produced a set of labeled proteins at various lysine positions.

Our preliminary data suggest that the modification of lysine PA affects the energy landscape of the investigated proteins. The acetylation of cytochrome c lysines leads to the progressive loss of the most extended conformations during CIU experiments. When all the lysines are acetylated, the range of observed conformers during CIU becomes highly restricted. Proteins fully labeled act as homopolymer undergoing CIU, e.g. not extending, suggesting the presence of a uniform or isotropic PA all along the polypeptide chain. Interestingly, the modification of lysine using aromatic NHS-esters amplifies the phenomenon probably due to electronic or steric effects. Density Functional Theory performed on a model tripeptide suggests that the PA of an acetylated lysine is similar to the PA of the peptide backbone, and possibly affects the migration of mobile protons due to the change of proton affinities of the chemically modified residues.

Ubiquitin possesses lysine residues involved, in solution, in structuring salt bridges. CIU reveals that the lysine acetylation favored the compact conformation(s) upon ion heating instead of lowering the threshold of CIU energy leading to ion unfolding. In contrast, survival yield experiments (CID) indicate that the acetylation of lysine side chain decreases the amount of collision energy inducing the fragmentation of ubiquitin. Non-covalent interactions involving lysines should play a role in the gas-phase unfolding but look like to be model dependent.

O7

Studying homo- and hetero-oligomers with native ion mobility – mass spectrometry to understand the conformational interplay of different amyloid β peptides

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Amyloidogenic peptides are associated with various neurological conditions and are potential targets for therapeutic intervention. Among these peptides, amyloid beta (Aβ) is of particular interest as it is linked to neurodegeneration and plaque formation in Alzheimer's disease. The process of plaque formation and the cause of cytotoxicity are still active areas of research, as important questions remain unresolved. Sequential cleavages of the amyloid precursor protein by secretase enzymes produce different-length isoforms of Aβ peptides in humans. We used native ion mobility in conjunction with high-resolution mass spectrometry (IMMS) to examine and compare the conformational variations of different Aβ species. Additionally, the interaction of these peptides with one another in mixed solutions was investigated, with IMMS enabling the precise elucidation of conformations of homo- and hetero-oligomers relevant to the initial stages of the aggregation process and the identification of structural variations.

We used a Waters Synapt XS instrument with a travelling-wave ion mobility cell in positive ion mode. All peptides were prepared in water as stock solutions (50 µM) and diluted to 30 µM in 10 mM ammonium acetate without further purification for measurement. To investigate the influence of Aβ isoforms on one another, the peptides were mixed in equimolar amounts. In addition, control samples were included using a non-cytotoxic scrambled sequence and a less aggregation-prone rodent form of Aβ42.

Clear differences in the arrival time distribution and collision cross section were observed among the various Aβ monomers. Moreover, our high-resolution data revealed that a signal on the *m/z* axis, attributed to overlapping Aβ42 oligomeric species in previous literature reports that used low-resolution MS, actually originated from a conformationally diverse dimer without contributions from higher-order oligomers. The conformation of this dimer was compared between isoforms with varying levels of toxicity associated with their aggregation. Hetero-oligomeric species were also studied, supporting the hypothesis that interactions between Aβ peptides influence oligomerization processes.

To the best of our knowledge, this is the first study of the conformational interplay between different Aβ species using native IMMS, a method that is uniquely suited to study the conformation(s) of individual oligomers with different stoichiometries. Furthermore, the combination of different Aβ species based on the production process of Aβs *in vivo* provides new insights into early oligomerization processes. As soluble oligomers are hypothesized to play a crucial pathogenic role, our findings may be valuable in enhancing our understanding of Alzheimer's disease.

O8

De novo sequencing of human milk oligosaccharides using IMS-MS

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Human milk is the gold standard for an infant's nutrition. It consists of bioactive substances such as lipids, proteins, and human milk oligosaccharides (HMOs). These HMOs are crucial for protection against infections and for the development of the intestinal microbiome. To establish structure-function relationships and exploit glycoscience for the development of future nutraceuticals and therapeutics, it is essential to determine exact HMO structures in human milk. Currently, accurate mass measurements are used for compositional assignment and additional MS/MS fragmentation experiments can provide structural information. Due to their isomeric nature, however, the assignment of exact HMO structures from MS/MS spectra remains very challenging. Furthermore, the lack of well-defined standards is another major hurdle for exact structure identification. In this work we present an ion mobility-MS de novo sequencing methodology, that can unambiguously determine isomeric HMO structures in the absence of synthetic standards.

A limited set of chemoenzymatically synthesized isomeric HMOs was used to develop a drift tube ion mobility spectrometry (DTIMS)-MS methodology and a reference library with common terminal glycan epitopes, including Lewis and human blood group epitopes, for de novo sequencing of isomeric HMO structures. The DTIMS-MS methodology uses high□resolution demultiplexing to obtain high resolution arrival time distributions (ATDs; up to 240 Ω/ΔΩ) and accurate mass and CCS values of intact isomeric HMOs and their fragments.

The DTIMS-MS methodology was used for the elucidation of HMO structures with undefined carbohydrate sequence and linkages in neutral and acidic fractions of five different milk donors. Fragments of HMOs were identified by their accurate mass and CCS values, using fragment ion entries from the reference library, and used for de novo sequence assembly to elucidate the HMO structures. The CCS values of elucidated intact structures were then added to the reference library and used to identify larger and more complex isomeric structures, creating a self-expanding reference library in the absence of further standards. This resulted in the elucidation of 34 exact HMO structures, ranging from DP 2-9 in the samples and >200 new (fragment) ion entries for the reference library. The structure assignment by DTIMS-MS was validated with well-defined standards and revealed 100% correct assignment by the de novo sequencing method.

This new methodology can also be applied to other glycan classes and will allow the glyco-workfield to rapidly and unambiguously identify exact structures in biological samples, independently of reducing end labels, without the need for synthetic standards.

O9

Exploring the diversity of nonulosonic acids in pathogenic microbes: targets for the development of novel antimicrobials

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Pathogenic microbes are frequently covered with complex sugars known as nonulosonic acids (NulOs, sialic acids). This diverse group of nine-carbon sugars displays a remarkable molecular-level diversity that has not been fully explored to date. In humans, these sugars are involved in signalling and cellular recognition. In bacteria, they have been linked to pathogens, such as *C. jejuni*. An infection with this bacteria is one of the leading causes of gastroenteritis worldwide. While the specific virulence mechanism of *C. jejuni* is still unknown, it is believed that cellular components modified with NulOs, such as the flagella, play a crucial role[1].

However, the chemical diversity of NulOs in microbes presents a challenge in profiling these sugars. Therefore, we recently established a new sialo-omics approach based on selective labelling and small mass window fragment ion scanning[2]. This approach not only revealed the wide spread occurrence of these sugars in microbes, but also their potential for "charge-balancing," alteration of hydrophobicity, and stereoisomeric recognition.

Here, we demonstrate the expansion of this approach to analyse activated NulOs, which are the direct precursors in the biosynthetic routes of these sugars and their glycoconjugates. Our established method involves a porous graphitized carbon separation followed by continuous MS2 small mass window scanning (with alternating collision energy). Additionally, we developed a data processing tool for the fully untargeted identification of these compounds. This employs a large sugar composition database (containing over 2200 theoretical compositions[3]) in order to link the precursors with their marker fragment ions.

To demonstrate the approach, we analysed the NulO profiles of a wide range of microbes, including several pathogens such as *C. jejuni* and *L. pneumophila*. Our findings not only showcase the significant differences in their potential to produce diverse glycoconjugates, but also reveal previously unknown forms. Understanding the biosynthetic routes of NulOs allows for the development of novel antimicrobials and vaccine candidates. Furthermore, identifying biological sources of these sugars is crucial for the discovery of novel biocatalytic routes, as their chemical synthesis is often not feasible.

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O10

Metabolic profiling of epileptic hippocampal tissue upon chemogenetic modulation of astrocytes using CE-HRMS

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Astrocytes are a major glial cell type in the central nervous system, actively involved in healthy brain functioning through metabolic support and ion buffering. In addition, astrocytes directly react to synaptic activity through intracellular Ca^{2+} signaling and in turn release gliotransmitters. Emerging evidence has indicated a role for neuron-astrocyte interactions in epilepsy. This neurological disorder is characterized by spontaneous seizures and affects up to 65 million people worldwide. One-third of the patients suffer from drug-resistant epilepsy. This shows that research into the disease mechanism and possible new drug targets remains important. Previous studies have found increased astrocyte Ca^{2+} signals in mouse models for epilepsy, but it has been difficult to disentangle how these drive or mitigate seizures. To specifically target astrocytes, chemogenetic modulation can be used. This technique involves designer receptors exclusively activated by designer drugs (DREADD), enabling precise spatiotemporal modulation of a cell type. In this study, chemogenetics was used to specifically modulate astrocyte Gq and Ca^{2+} signaling and investigate the metabolic effects hereof in hippocampal epileptic tissue in the intrahippocampal kainic acid mouse model for epilepsy.

Mice were intrahippocampally injected with kainic acid or 0.9% NaCl, to obtain epileptic and control mice. Two weeks later, an adeno-associated viral vector encoding for the Gq-DREADD or its control was injected. After 3 weeks, mice were injected with the DREADD agonist clozapine N-oxide (CNO) and 24h later, mice were sacrificed and the hippocampus was dissected. This timepoint was selected based on previous experiments demonstrating a significant reduction of seizures in mice that express the Gq-DREADD in hippocampal astrocytes 24h after CNO injection. An internal standard mixture in water/acetonitrile was added before tissue homogenization. After centrifugation, filtration and evaporation to dryness, the precipitate was reconstituted in water. Samples were analyzed by capillary electrophoresis (CE) coupled to high-resolution mass spectrometry (HRMS) in electrospray positive and negative modes. Altered metabolites were selected using multivariate chemometric tools.

Currently, we focused on the effect of Gq-DREADD modulation in the epileptic tissue. Thirty-one metabolites were identified as altered, including nucleotides in their different phosphorylation states, but also intermediates of their synthesis, products of cellular breakdown and peptides, among others. We are further investigating the effects of DREADD modulation in epilepsy and healthy controls to gain understanding of the role of astrocytes in the epileptic disease mechanism.

O11

Waters SELECT SERIES: Unrivalled Ion Mobility Performance and High Mass Resolution Independent of Scan Speed

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The SELECT SERIES Cyclic IMS combines novel cyclic ion mobility separation with high performance, Time-of-Flight mass spectrometry, enabling researchers to unlock the potential in scientific discovery. This talk will cover the unique capabilities of the instrument and how single and/or multi-pass separation data, with the flexibility of ion-guiding technology, provides benefits and flexibility for a wide range of applications, including routine pharma, imaging, HDX and biomedical research.

The SELECT SERIES MRT helps you to accelerate your research and comprehensively identify analytes in complex samples through state-of-the-art Multi Reflecting Time-of-Flight (MRT) technology. Providing a combination of ultra-high resolution and specificity, independent of scan speed, and with superior ppb mass accuracy, the system gives unambiguous compound identification. This talk focuses on the fundamentals of the technology and the advancements it provides in both LC and imaging experiments, including DESI XS (Desorption Electrospray Ionisation). The system is uniquely positioned, giving a combination of unequivocal compound identification/elucidation with multi-modal spatial imaging, to understand the behaviour and distribution of a wide range of molecular species, from biomarkers through to drug molecules.

O12

Metal-ligand equilibria in solution: Quantitative insights from isotopic labelling

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Investigating the speciation and exchange kinetics of labile ligands is essential to understand how metal complexes react in solution[1]. Mass spectrometry coupled with Electrospray ionization is often used to study the speciation of metal complexes with labile ligands, regardless of the nature or spin state of the metal, or to follow reactions catalysed by metal–organic complexes[2]. However, the difficulty of correlating the abundance of ions in the gas phase with the concentration of their precursors in the solution is a pitfall of MS methods. Quantitative measurements, such as determining binding constants and reaction rates, thus become challenging.

The delayed reactant labelling (DRL) approach is designed to bridge the gap between the solution and the gas phase experiments[3]. This method involves the spiking of a reaction mixture by an isotopically labelled reagent, enabling the generation of isotopically labelled reaction intermediates and products. Because isotopologues share the same ionization efficiency regardless of matrix effects, their relative intensities in a mass spectrum directly relate to their relative concentrations in solution, thus making it possible to obtain quantitative information on the reaction kinetics in solution.

While DRL has been used to map reaction paths for gold-catalysed reactions[3] or organocatalytic transformations[4], we show that DRL quantitatively captures the solution-phase equilibria of labile pyridine ligands coordinated in the cavity of macrocyclic porphyrin cage complexes. Rate constants and activation parameters for ligand dissociation in the solution can be derived for selected species, thereby providing mechanistic insights that are not easily obtained from traditional solution-phase techniques[5].

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O13

Fast and accurate disulfide bridge detection

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Introduction

Disulfide bridges are reversible in nature allowing cleaved bridges to reform. Recent findings suggest that these bridges can serve a regulatory function, influencing protein activity upon cleavage or formation. This specific category of regulatory disulfide bridges is referred to as allosteric disulfide bridges. Plasma proteins such as VWF and plasminogen are implicated in being regulated through this mechanism. However, the current understanding of disulfide bridges is labor-intensive and mostly relies on structural models. Here we developed a novel approach for the direct detection of disulfide bridges that utilizes optimizations in sample preparation, state-of-the-art mass spectrometry, and software improvements to the XlinkX/PD data analysis environment. We show we can extract the disulfide bridges and accurately quantify their occupancy.

Methods

Proteins were hydrolyzed with microwave assisted acid hydrolysis (MAAH) and normal cleanup was performed. All data was recorded through a reverse Thermo Scientific™ Vanquish™ Neo system connected to an EASY-Spray™ PepMap™ RSLC C18 column (0.075 mm x 250 mm, 2 µm particle size, 100 Å pore size (Thermo Fisher Scientific)) at 250 nL/min flow rate. The analytical column was interfaced to an Orbitrap Eclipse™ Tribrid™ mass spectrometer coupled with a FAIMS Pro Duo interface. The mass spectrometer was set to perform fragmentation events with EthcD for precursors at charge state 3 and above. All data was analyzed in Proteome Discoverer with optimized XlinkX data analysis nodes.

Preliminary Data

We show that the hydrolysis method produces ideal length peptides of 10-15 amino acids, that the disulfide bridges remain intact, and that the disulfide bridged peptides can be identified from the sample. A large degree of chemical noise is however present in the measurements that masks the signal of the disulfide bridges. As this chemical noise is present mostly at charge state 1, the background can effectively be removed by integrating the FAIMS interface. By using EthcD we, like others, can produce highly informative spectra combining peptide backbone fragments (HCD) with the cleavage of the disulfide bridge (ETD). From the data, we observe that FAIMS integration leads to the complete eradication of charge state 1, almost complete removal of fragmentation events at charge state 2 (which result from mis-interpretations at realtime), and for low to intermediate complexity samples brings the full range of signals into view. For the identified spectra we observe that the integrated HCD step gives excellent sequence coverage for both peptides across a wide range of spectral qualities. As protein hydrolysis by MAAH is rather unspecific, we made steps to optimize the data analysis search of XlinkX/PD by integrating an open search option. Here, the search engine attempts to identify the first (dominant) peptide from the spectrum and uses a normal crosslinks search to locate the second peptide. Although this works out of the box, we found that integrating knowledge of the disulfide bridge cleavage vastly improves performance. Finally, we improve on the FDR control by extending our multi-stage FDR control (at the level of the fragmentation and crosslink tables) with a repeat FDR that weeds out all remaining false positives. Occupancy levels are automatically calculated, and we show are on-par with published work.

Novel Aspect

A novel combination of sample preparation, advanced mass spectrometry, and data analysis for disulfide bridge identification

O14

Teaching Old Dogs New Tricks: Extending the Utility of your Mass Spectrometer

Steven Daley

MS Vision

Mass spectrometry is a ubiquitous tool in a multitude of areas of research, and has a huge industry to supply instruments to research institutions around the globe. There are a multitude of commercially available instruments that can do almost anything you can desire. However, one of the downsides of this is that although there are many mass spectrometers available, individual mass spectrometers are typically targeted for a specific application. Therefore, the mass spectrometer you have may not be able to do exactly what you want them to do. In this presentation, modification of mass spectrometers to be able to perform new experiments will be discussed.

One such set of modification is the extension of mass spectrometers to be able to perform something that is currently unavailable on commercially available mass spectrometers. Two examples of this are IR/UV spectroscopy and soft landing. In both these cases, the capability of a Waters Synapt instrument to prepare mass selected ions and separate them by ion mobility is leveraged to prepare mass and shape selected ion beams, which can then either be trapped for spectroscopic measurements which can be used to elucidate structure, or to land them on a surface which can then be removed from the vacuum and studied by alternative structural techniques such as electron microscopy.

Alternatively, the mass spectrometry itself is fine for the application, but additional functionality can help with interpretation of better performance of experiments. One example of this would be the study of large proteins or protein assemblies containing PTMs or glycan modifications. This can lead to an extremely dense spectrum that is difficult to convolute. Here, addition of charge reduction can help to deconvolute the spectrum and aid assignment of peaks. Another example is the modify a mass spectrometer so it is more suitable for performing native mass spectrometer, by improving desolvation and extending the range of the quadrupole allowing for mass selection of higher m/z ions.

In conclusion, modification of existing mass spectrometers can significantly extend the utility and lifetime of mass spectrometers, allowing them to perform experiments not possible on current commercially available machines.

O15

Novel strategies for the characterization of the early-stages of aggregate formation of α-Synuclein using hyphenated mass spectrometry

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Parkinson's Disease (PD) is the second most common and fastest-growing neurodegenerative disease at this moment. This neurodegenerative disorder is characterized by protein aggregates comprised of α-synuclein, where soluble proteins transform into insoluble fibrils. However, our understanding of this molecular process is limited, which significantly hampers the development of (new) treatment for Parkinson's Disease. Here, we aim to develop a multidisciplinary MS-based approach that allows us to understand the mechanism of oligomer formation of α-synuclein from humans expressed in *Escherichia coli* at the molecular level. Subsequently, we will employ our MS-based approach to evaluate the 26F1 antibody from Syngle Therapeutics as a potential new drug for PD treatment[1]. This antibody is designed to have a high affinity to capture oligomers over monomers and amyloid fibrils.

The initial step involves establishing an analytical workflow to separate and characterize α-synuclein aggregation. We employ two different strategies to characterize early-stage aggregation. The first is native low-flow size exclusion chromatography (SEC) with mass spectrometry (MS). Native SEC facilitates the separation of aggregates based on size, while simultaneously preserving their structural integrity preceding electrospray ionization mass spectrometry (ESI-MS). The second approach entails the application of ion mobility mass spectrometry, particularly utilizing trapped ion mobility mass spectrometry (TIMS-MS). In this approach, fresh and formed α-synuclein aggregates are analyzed by their mass-to-charge distribution and shape (3D structure). Essential advantages of the TIMS-MS workflow is the possibility to separate and identify early-stage oligomers[2] and the compatibility with a nanoESI source, ensuring gentler ionization and thus preservation of the fragile oligomeric structures. Using this TIMS-MS approach, fresh α-synuclein shows two distinct distributions, i.e. a folded structure with a main charged state of +6 and an unfolded charge state of +14, while we can measure up to hexametric assembly for the aggregated sample.

Subsequently, the α-synuclein oligomers are subjected to incubation with antibodies, with careful optimization of incubation duration and antibody concentrations. Employing the TIMS-MS method, it is demonstrated that upon incubation with the 26F1 monoclonal antibody (mAb), oligomers preferentially form complexes, whereas lesser binding is observed towards the monomeric species.

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O16

Sliding windows in ion mobility (SWIM): a new approach to increase the separation power in trapped ion mobility-mass spectrometry hyphenated with chromatography

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In recent years, the separation capability of commercially available IMS instruments has improved drastically. Advanced linear IMS technologies, including the TIMS, cyclic cTWIMS, and SLIM, have demonstrated impressive resolving powers exceeding 300. However, achieving such high resolving power in TIMS typically requires extended measurement times or analysis of ions over a restricted ion mobility range, which can pose significant challenges when coupling the IMS-MS instrument with front-end chromatography and analyzing compounds with a wide range of collision cross section (CCS) values. This can be particularly problematic in the analysis of environmental pollutants, where a broad range of CCS values are typically present.

We have developed a novel approach for improving the resolving power of trapped ion mobility spectrometry (TIMS) when coupled with gas chromatography, called "Sliding Windows in Ion Mobility" (SWIM). This method was applied to a challenging mixture of 175 persistent organic pollutants (POPs). Our measurements were performed using a Bruker TIMS TOFpro II mass spectrometer equipped with a GC-APCI II source for sample separation and ionization, prior to TIMS-MS analysis.

The resolving power in TIMS has been demonstrated theoretically and experimentally to be dependent on several parameters, including the scan rate β_v, which represents the rate at which voltage is decreased during the elution step. When the analysis is performed over a shorter ion mobility range (ΔV_{ramp}) and/or with a longer analysis time (t_{ramp}), the scan rate decreases ($\beta_v = \Delta V_{ramp}/t_{ramp}$), resulting in improved resolving power for the analytes of interest. However, in standard TIMS operation, the ion mobility range must be large enough to trap and analyze all the compounds of interest, while the analysis time must be short enough to cope with the time scale of the front-end separation technique. This limitation impedes the use of slow ramp speed and significantly restricts the achievable resolving power in chromatography hyphenated TIMS applications.

Our SWIM approach builds upon the exceptional ability of TIMS to selectively trap ions within a specific range of ion mobilities. Instead of the standard TIMS mode, which employs a broad and constant IM analysis range, the SWIM mode uses narrow and mobile ion mobility windows that are continuously scanned and adapted to the ion mobility range and elution time of the targeted analytes throughout the gas chromatographic run. For example, in the analysis of POPs, the ion mobility windows were initially set to target early eluting, low halogenation degree pollutants with the lowest CCS values, and then gradually increased to analyze pollutants with increasing halogenation degree and higher CCS values. This approach enabled the SWIM mode to achieve significantly improved resolving power (~40%) compared to the standard mode, significantly improving the separation of several critical GC coeluting isobaric and isomeric pairs.

O17

Fab profiling reveals plasma IgA1 clones can co-occur in both monomeric and J-coupled dimeric forms

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IgA is the most abundant antibody class in mucosal secretions, such as milk and saliva, and has the second-highest concentration in serum after IgG1, making it the most abundant immunoglobulin in the human body overall. Like IgM, IgA molecules feature a tailpiece which enables polymerization via interaction with the J-chain. In serum, IgA is predominantly monomeric with a smaller population of J-coupled dimers, but in secretions IgA forms oligomers from dimers up to pentamers. The composition of IgA assemblies varies between these different compartments of the immune system, as likely does their function and antigen-specificity, the latter of which is determined by the variable amino acid sequence contained in the Fab arms. Previously we established a method for intact LC-MS profiling of immunoglobulin IgA1 Fab clones from various donor biofluid samples, including breastmilk and serum. Application of this method to paired breastmilk and plasma samples revealed that IgA1 Fab clonal repertoires are unique to each donor but are also shared to some degree between the systemic and mucosal immune compartments, with the source of this overlap most likely being J-chain-coupled dimeric IgA1. Here, we build upon this work by revealing the clonal repertoires of monomeric and dimeric IgA1 subpopulations retrieved from healthy individuals' human plasma.

Mass photometry measurements of total IgA captured directly from individual plasma samples shows that monomers are the predominant form. However, the relative abundance of dimeric IgA can vary significantly between individuals, somewhat between 5 to 30%. To investigate the composition of human plasma IgA in greater detail, with clonal resolution, we used offline size exclusion chromatography to separate monomeric and dimeric IgA prior to intact LC-MS profiling of IgA1 Fab clones from selected SEC fractions. This revealed three distinct populations of IgA present in human plasma, with clones either originating only from monomeric or dimeric IgA1 as well as clones that were present in both monomeric and dimeric assemblies simultaneously. Monomer-only clones accounted for more than half of the total unique clones identified in each donor plasma sample, while the number and proportion of dimer-only clones varied between individuals.

Our detailed investigation into the composition of IgA in human plasma revealed significant variation in the proportion of dimers both at the total as well as clonal level. Importantly, clonal overlap between monomeric and dimeric IgA implies shared (J+) B cell origins for these IgA. As J-chain-coupled IgA can be transported into mucosal secretions by pIgR, these results highlight potential sources of crosstalk and coordination between the systemic and mucosal immune compartments. While further details—such as whether these shared clones are present in serum only as dimers or as both monomers and dimers—remain to be elucidated, the present work clearly illustrates that IgA dimers in plasma cannot be ignored.

O18

A multiplexed LC-MS/MS approach to quantify the immunoglobulin landscape in blood: from concept to clinical validation

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Immunoglobulins (Ig) are amongst the most abundant plasma proteins and play an essential role in humoral immunity. They are structurally divers, consisting of 5 isotypes (IgG, IgA, IgM, IgD, IgE), which can further be subdivided in classes (IgG1-4, IgA1-2). Monitoring Ig concentrations can aid in diagnosis of immunodeficiencies and hematological malignancies, monitoring of disease activity, and provide mechanistic insights into B-cell development. Ig concentrations are usually quantified using immune-based methods, such as ELISA or nephelometry. However, these methods lack molecular specificity and their quantification accuracy can be influenced by sequence polymorphisms and other proteoforms. Additionally, the multiplexing possibilities are severely limited.

To circumvent the use of immune-based methods we have designed a multiplexed parallel reaction monitoring (PRM) LC-MS/MS method to quantify all isotypes and subclasses Ig in blood. To this end, Ig isotype and class specific peptides were selected based on known genetic variations from the IMGT database. Peptides were measured using a high resolution mass spectrometer (Orbitrap Fusion) operated in PRM mode in combination with a highly robust LC system (Vanquish Neo) operated at nanoflow. To quantify the Ig's we designed a quantification strategy based on stable isotope labeled-peptides and an external calibration curve.

The multiplexed LC-MS/MS method was first analytically validated for linearity and accuracy. We showed for the majority of Ig's a linear signal over 3 log scales (R^2>0.95) and the quantification accuracy fell within range (80-120% range). Next, we clinically validated our method in a cohort of 30 Waldenstrom Macroglobulinemia (WM) patients. WM is a B-cell malignancy characterized by the presence of a monoclonal IgM, which can lead to high, supraphysiological concentrations of IgM in the blood. The 30 WM patients expressed a wide variety of circulating IgM concentrations (0.1 – 57.02 g/L) and additional aberrations in the Ig landscape, including decreased abundance of IgG and IgA. We demonstrated an excellent concordance between our method and immune-based methods. Additionally, due to multiplexed nature of our assay we were able to provide a comprehensive overview of the entire Ig landscape and associated proteins, including CD5L and J-chain.

To conclude, we designed a multiplexed targeted LC-MS/MS method to quantify the Ig landscape in blood and performed an initial analytical and clinical validation in WM patients. This method has potentially great benefits in both fundamental and (pre)-clinical studies.

O19

Antibody Fc profiling of IgG, IgA and IgM by light chain capturing coupled with nanoLC-MS analysis

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The constant domain (Fc) of antibodies is responsible for binding to effector cells and activate component mediators therefore triggering downstream immune responses. Differences in the amino acid sequence of the Fc define the antibody isotype (with the most abundant being IgG, IgA and IgM) all sharing regions of the light chain with two potential variations (kappa and lambda light chain). Furthermore, antibodies exhibit post-translational modifications, with glycosylation as one of the most important, which can modulate their biological functions. Characterization of antibody Fc structure contribute to understand (altered) immune responses. Up to now, endogenous antibodies are characterized using bottom-up approaches resulting in a loss of combinational information of multiple PTMs such as multiple N-glycans of IgA and IgM.

With new developments of mass spectrometry, intact or middle-up protein analysis are increasingly applied for glycoform characterization study, since it provides comprehensive structural information compared to peptide analysis. In middle-up analysis, antibodies are cleaved via specific proteases into two subunits, constant domain (Fc) and variable domain (Fab). So far, only IgG has been studied using middle-up approaches for Fc characterization. For endogenous IgGs, normally the antibodies are captured by FcXL beads which bind to the Fc of IgG, followed by a hinge-region cleavage by IdeS and elution of the Fc subunits under acidic conditions. For IgA or IgM there is no analytical platform for middle-up Fc profiling yet. In this project, we have developed a middle-up analysis platform for sequential Fc profiling of IgG, IgA and IgM.

To allow capturing of all antibody isotypes a light chain affinity capturing, using a mixture of kappa and lambda light chain beads, was established. After capturing, the Fc/2 subunits of IgG, IgM and IgA1 were sequentially released by specific IgG, IgM and IgA proteases providing directly the Fc portions and eliminating the elution step required in Fc affinity strategies. The Fc/2 subunits of each isotype were individually analyzed by nanoRPLC-MS. The IgG Fc/2 profiles showed no bias between the developed light chain capturing method and the classical FcXL capturing. For IgM Fc/2 subunits, very complex glycosylation profiles containing 2 and 3 N-glycosylation sites were observed. Next to glycosylation, other modifications such a c-terminal tyrosine truncation were detected for IgA and IgM. The mass spectra of IgA and IgM Fc/2 glycoforms was annotated by integrating the intact subunit and the site-specific bottom-up information. In addition to Fc/2 subunits, the corresponding joining chains from IgA and IgM were detected and annotated with different glycoforms. The proposed method was applied to the characterization of the Fc/2 subunits of three independent donors resulting in different profiles, therefore illustrating the potential of the approach to study antibody Fc/2 changes.

O20

Identification and quantification of plastic particles in human blood using non-targeted pyrolysis-GC-MS

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Micro- and nanoplastics (MNPs) have been recognized as an environmental threat and there is global concern about their presence in humans. To investigate human exposure and the toxicological effects of MNPs a method to identify and quantify these plastic particles is essential. Pyrolysis-GC-MS (Py-GC-MS) has recently emerged for the analysis of MNPs in human blood, as it provides polymer-specific MS fingerprints and semi-quantitative data. However, pyrolysis also introduces complex effects, such as secondary reactions between different polymer types and matrix compounds, which complicate quantification based on single MS fingerprints. To address this potential vulnerability, all pyrolysis products can be measured to obtain a comprehensive understanding of the pyrolysis process, which leads to more robust identification and quantification.

In this work, we employ such a non-targeted approach to MNP characterization using double-shot Py-GC-MS in scan mode. Explorative experiments using mixtures of polyethylene terephthalate (PET), polyethylene (PE) and polyvinylchloride (PVC) standards provided promising results. MS fingerprints were extracted from this data using chemometric approaches (PARAFAC2 and ROIMCR) and subsequently fed into a mixing-model (POCHEMON) to identify polymer-specific markers as well as markers originating from the co-pyrolysis of different polymers. Subsequently, an analogous strategy was applied to human whole blood spiked with varying amounts of PET, PE and PVC. The performance of multivariate quantification, using random forest and partial leas squares regression, was compared to traditional linear regression based on a single marker.

The multivariate quantification (using >400 features) outperformed single-marker regression, especially for the quantification of PVC. Recursive feature elimination (RFE) was used to select an optimal subset of markers, which further decreased the quantification error by 15% compared to single-marker quantification. Inspection of the most reliable markers revealed that the pyrolysis of PE is relatively unaffected by the blood matrix. For PET, however, more complex interactions were observed, as markers originating from the co-pyrolysis with blood were most relevant for quantification. Interestingly, for PVC the presence of PE appears to increase formation of PAHs during the pyrolysis, causing an overestimation of PVC quantities.

In conclusion, this study highlights the importance of considering secondary effects in pyrolysis for the identification and quantification of MNPs in blood. By relying on a non-targeted approach and using multivariate analyses these effects can be taken into account, leading to improved quantification. Moreover, the identification of relevant makers provides valuable chemical insight into the pyrolysis process, which can potentially be exploited in further development of the experimental method.

PFAS in soil: workflow towards total PFAS screening.

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It needs no further introduction that PFAS are a widespread environmental problem. Increasing PFAS sources, contaminated regions or contaminated matrices are being discovered. On one hand, this leads to an ever increasing amount of samples. On the other hand, most often analysis is only performed on a limited subset of the known PFAS. We propose a new PFAS analysis workflow that allow a selection of samples to be analyzed with increasing depth. This workflow will be illustrated with an example of soil samples.

When mapping the PFAS contamination in a certain area it is necessary to collect soil (or another environmental matrix) at different spots around the site of interest, at different depths and possibly at different time points. This leads to an increasing amount of samples, not necessary all useful to obtain a decent image of the PFAS contamination. When the soils arrive in the lab they are first screened using ambient ionization (DART-MS) by picking up the soil with a glass capillary and holding it in front of the DART. The obtained mass spectra are analyzed using data treatment steps (like mass defect filtering) and peak identification using an in-house developed database. Based on these results a fast selection can be made between soils with a high, medium or low PFAS content as well as an initial screening of the type of PFAS present, allowing differentiating by different types of PFAS contamination.

The selected samples are then extracted, prepared and analyzed by a targeted LC-MS/MS method to determine the amount of target PFAS present (list of 43 PFAS). The preliminary information obtained by ambient ionization determines the level of dilution that is required for this analysis. To get a more global overview of the PFAS content the samples can be analyzed in 2 ways. First, they can be analyzed using EOF (and/or AOF for liquid samples) to determine the total organic fluor in the samples. Secondly they can be analyzed using the TOP assay to determine the precursors present that degrade to one of the target PFAS.

Based on the results obtained by the ambient ionization measurement and the difference in PFAS concentration found using the targeted approach and the total organic fluor approach combined with the TOPA results it can be decided to select samples for an in depth non target analysis. With this non target analysis more detailed information about the precursors, degradation products as well as PFAS that are not yet incorporated in the targeted list can be determined and semi-quantified based on the EOF and TOPA results.

With the described workflow it is possible to get a detailed overview of the PFAS contamination, present at a site of interest and to use the data to make correlations and predictions using smart preliminary sample selection.

O22

Pushing the limits of proteomics: characterizing microbial processes in wastewater treatment plants

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The life science industry increasingly employs multi-omics approaches to study natural and engineered ecosystems, such as the gut microbiome or microbes used for cleaning our environment. Of these multi-omics approaches, microbial community proteomics is gaining increasing popularity, because it allows to measure the expressed metabolic pathways of individual microbes. These insights are especially relevant for the optimization of industrial process.

Of the many applications in industrial biotechnology, microbial wastewater treatment is one of the fastest-growing areas. Currently, more than 350 trillion liters of wastewater are generated globally each year. With the continuously growing world population, this urgently asks for advancements in water purification strategies.

In biological wastewater treatment, a complex consortium of microbes purifies wastewater from pathogens and excess nutrients such as nitrogen and phosphorus to prevent eutrophication of our surface waters[1]. However, in contrast to DNA, proteins cannot be amplified prior to proteomic analysis, and peptide sequencing is performed consecutively rather than in parallel. Therefore, the depth of information that can be obtained by proteomics strongly depends on the microbial complexity and the effort taken to sequence these microbial communities. Furthermore, the sample preparation procedures used to lyse the cells and extract the proteins have a strong impact on the outcome.

Here, we demonstrate how proteomics on complex microbial mixtures directly sampled from wastewater treatment plants allows to reveal the underlying microbial processes[2], which subsequently inform on the optimization of purification strategies. We demonstrate the challenges that microbial community proteomics faces, including protein extraction from such complex matrices and the proteome coverage for low-abundant proteins and microbes.

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O23

The proteome response of Staphylococcus Aureus to titanium biomaterial surface roughness

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Introduction: Biomaterials are engineered substances to support, replace, or enhance tissue functioning. However, implants can lead to bacterial adhesion and infections, causing implant failure.[1] The biofilm-forming pathogen Staphylococcus aureus is a leading cause of implant-associated antibiotic-resistant infections.[2] The surface roughness of, e.g. titanium biomaterials significantly impacts their integration with the surrounding tissue. Rough titanium surfaces promote better cell and tissue integration and facilitate biofilm formation and bacterial adhesion. This study aims to investigate the bacterial proteome response to titanium surface roughness using an LC-MS-based proteomics workflow. Samples were analysed in both DDA and DIA mode, providing insights into the cellular response of the bacteria to the surface.

Methods: S. aureus (ATCC 25923) biofilm was grown on titanium disks with different topographies (polished, corundum, and plasma-pore) for 2 and 7 days. The biofilm was sonicated in PBS and centrifuged at 3500x g to separate the bacterial cells from the supernatant. Three replicates per condition were obtained by collecting the pellets from six biofilms. The pellets were dissolved in ABC containing urea (5M) and 0.1% n-Dodecyl-β-D-maltoside, followed by beating and freeze-thaw. Proteins were quantified by Bradford assay, and 42 µg of protein was used for automated Single-pot, solid-phase-enhanced sample preparation (SP3) protein digestion. The digested samples were injected and separated on an LC C18 column (2 µm, 75 µm × 500 mm, 100 Å), hyphenated with an ESI- Orbitrap MS Q-Exactive. Samples were analysed in data-independent acquisition DIA and data-dependent acquisition (DDA) mode, and the Raw files were processed using proteome discoverer, DIANN, and Perseus for protein identification, abundance, and fold change calculations using Swiss-Prot bacteria databases.

Results: Label-free proteomics analysis was performed to investigate the differences in protein expression between the biofilms grown on different titanium surfaces during biofilm formation (day two and day 7). Individual biofilms contained an average of $2x10^7$ and $5x10^7$ CFU on days 2 and 7, respectively. An optimised DDM protocol and an automated Sp3 workflow detected 1835 and 1121 proteins in DIA and DDA mode, respectively. Showing an increased protein count between the methods due to increased dynamic range. Among these data sets, statistically differentially expressed (p-value≤0.05; FC 1.5-fold) proteins were found between the surfaces and the stages of biofilm formation. 138 Proteins were found to be significantly upregulated in day two using the DIA method and 51 with the DDA method. Besides, in day seven, biofilms 179 and 29 were upregulated with the DIA and DDA methods, respectively. This analysis showed an upregulation in the pyrimidine biosynthesis pathway and cell wall organisation in day two biofilms. In day two biofilms, several metabolic and catabolic processes were increased.

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O24

Higher order structures of biomolecules investigated by capillary electrophoresis and ion mobility MS

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The fine characterization of samples requires identification and quantification of the content, the determination of the stoichiometry and connectivities (primary structures) and interaction domains (higher order structures) of (supra)molecular assembly. The elucidation of structure-activity relationships holds paramount significance in evaluating and comprehending the underlying processes involved in various applications such as therapeutic oligonucleotides (ONs), aptamers, functional foods, and bio-active compounds, including pre and probiotics. Mass spectrometry plays a pivotal role in the comprehensive analysis of organic samples, even at minute trace levels. The exploration of higher order structures relies on numerous analytical methods tailored for relatively pure and concentrated samples, typically utilizing spectroscopy methods in solution. However, the utilization of mass spectrometry (MS) and ion mobility mass spectrometry (IM-MS), both of which are vacuum-based methods, is increasingly important, primarily due to its fast screening capabilities for trace amounts in non-pure samples. It is highly tempting to extrapolate data obtained from mass spectrometry (MS) and ion mobility mass spectrometry (IM-MS) to deduce structural information in solution based on the assumptions of native mass spectrometry. However, it is imperative to validate these assumptions for each investigated model to ensure their applicability and accuracy. Nonetheless, there is still a way to circumvent these problems and create a shortcut. Here we propose the concomitant use of the different modes of Capillary Electrophoresis (CE) on-line coupled with IM-MS. CE is a separation method in solution presenting different operation modes which separates analytes according to the shapes, averaged charge states, and hydrodynamic radii (capillary zone electrophoresis, CZE), the relative electrophoretic mobilities (µe) in solution (transient isotachophoresis, t-ITP), or even on the affinity and binding constant of host-guest systems (kinetic capillary electrophoresis, KCE). Different models, e.g. peptides, naturally occurring and chemically modified oligonucleotides, and G-quadruplexes were investigated by CZE, t-ITP, and KCE coupled with ion mobility mass spectrometry, energy-resolved collision induced dissociation (breakdown curve and V50), and collision induced unfolding (CIU). Advantages and drawbacks of CE-IM-MS coupling will be addressed from the analytical point of view. The adequacy of parameters extracted from CE experiments and the ion mobility constant (K) obtained from IM-MS will be also addressed from the physical chemistry point of view.

O25

Unravelling functional changes in antibody proteoforms using affinity CE-MS

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Antibodies recruit immune responses via interaction with different Fcγ receptors (FcRs). These interactions are strongly influenced by structural features of the antibody, including glycosylation. Unfortunately, common approaches, such as SPR provide an overall affinity response for all different glycoforms and assessment of their individual binding require tedious production or enrichment of specific forms. In particular, assessment of the influence of Fab glycosylation in binding represents a huge challenge due to the complexity of generating homogeneous forms.

In our lab, we have exploited the capabilities of affinity CE-MS to study the binding of antibodies and FcRs in a proteoform-resolved fashion. To this end, the FcR receptors were added to the background electrolyte whereas the mixture of antibody glycoforms were injected in the CE. We will show that the proposed approach is able to determine the relative binding affinity of different glycoforms based on the shifts on their mobility. For FcγRIIIa, the obtained affinity profiles were benchmarked towards affinity LC using the same constructs providing similar results. Due to the low amounts of receptor required in affinity CE-MS, the developed platform was ideal for testing a variety of FcRs, namely FcγRIIa, FcγRIIb, FcRn and FcγRIIIa and including different allotypic variants. As anticipated, Fc glycosylation was key for the binding. Hemiglycosylated antibodies showed strong decrease in the binding affinity towards the FcγRs while non-Fc glycosylated forms showed near no binding. Fc-glycoforms behaved differently between receptors with clear differences for afucosylated and high mannose variants. Interestingly, different receptor allotypes also revealed glycan-sensitive differences. Furthermore, we explored the potential of the approach to investigate the influence of Fab glycosylation in FcR binding. Our results showed an altered binding for the Fab-glycosylated variants. As Fab glycans are far from the binding site, the change in binding properties most likely correlates to a change in protein conformation in the interaction surface.

O26

Development and Applications of Ultra-long Transients for Orbitrap-based Single Ion Mass Spectrometry

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Single ion mass spectrometry is an emerging class of experimental methods that capitalize on the sensitivity of modern mass analyzers to trap and detect individual molecules. One such approach is Orbitrap™-based charge detection mass spectrometry (CDMS), which leverages this capacity to directly measure both the *m/z* and *z* of ions, allowing mass information to be extracted from even extremely heterogeneous, intact macromolecular assemblies.

As with any FT-MS technology, the resolution and S/N can be enhanced by recording longer transients. For Orbitrap mass analyzers the maximum recording times are typically 1-2 seconds, although in most proteomics experiments much shorter transients of ~32 ms are used due to duty cycle considerations. Single ion MS uniquely benefits from prolonged transients, as most mechanisms of signal decay are not relevant. We therefore aimed to develop a methodology for recording ultra-long transients, i.e. an order of magnitude longer, with the goal to improve the S/N, mass resolving power, and accuracy of Orbitrap-based CDMS.

By installing an external high-performance data acquisition system alongside several software alterations to a Q Exactive™ UHMR™, we can now successfully record transient times up to 25 seconds, with sufficient platform stability for these extended scans to be sequentially recorded for more than several hours (***Nat. Methods*, In Press**). Record improvements in both S/N and resolution were obtained. For the 800 kDa protein complex GroEL, we can achieve a *m/z* resolution of 1,100,000 at 11,786 *m/z*, corresponding to an effective resolution of ~6,000,000 at 400 *m/z*¸ using absorption-mode FT. S/N ratios were also enhanced 5-fold compared to standard transients, allowing for more sensitive and accurate charge detection. Single ions as small as native cytochrome c (12 kDa, 6+) can now be successfully detected. These enhancements directly translate to improved performance and accuracy in Orbitrap-based CDMS, with mass errors of < 1% now achievable using ultra-long transients independent of spectral complexity, allowing separation of species that were previously not resolvable.

An additional benefit of our ultra-long transient platform is the new capacity to reliably track the evolution of individual ions over prolonged time periods with high *m/z* resolution. This has enabled the development of new experimental approaches in single ion MS, such as tracking desolvation processes and fragmentation of individual complexes.

O27

Advancements in fast mass microscopy speed, molecular information, and data processing

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"Microprobe mode" mass spectrometry imaging (MSI) collects data pixel-by-pixel [1] usually at fewer than 500 pixels per second. This speed, at 1 µm pixels requires 23 days to image a half microscope slide (1 billion pixels). In contrast, fast mass microscopy (FMM) collects many MSI pixels simultaneously using a fast, pixelated, event-based detector and a continuously-moving stage. FMM enables orders of magnitude faster imaging — i.e., scanning a half-slide in 33.5 minutes [2]. However, the previously reported FMM study did not demonstrate the full capabilities of FMM. Here we describe advancements in FMM including a tenfold increase in speed, the implementation of matrix-assisted laser desorption/ionization (MALDI)-FMM, and new strategies for processing FMM data.

A fast mass microscope [2] was modified with a custom laser interface (optics: Thorlabs; vacuum flange: Kurt J. Lesker) and laser (Explorer One 349-120, Spectra-Physics), linear encoders (LS 477, Heidenhain) connected to a digitization card (NI6612). Test samples were prepared on ITO slides using metal standards of holmium and gadolinium, table salt and a thin lemon slice for SIMS-based analyses, crystal violet for LDI-based analyses, and sprayed DHB and cetrimonium chloride (TM, HTX Technologies LLC) for MALDI-based analyses. TEM grids (Agar Scientific) were used for testing. Some samples were sputter-coated with gold (0.5 to 2 nm) (SC7640, Quorum Technologies). All chemicals were provided by Sigma Aldrich.

The added stage encoders improved construction accuracy of images at any speed and allowed imaging at the stage's maximum speed, an equivalent of 5 million pixels per second at 1 µm pixel size.

The added laser interface enabled LDI and MALDI-based stigmatic imaging at speeds of at least 2,000 pixels per second at 3 µm pixel sizes. Higher throughputs of at are likely possible by increasing laser repetition rate from 1.2 kHz to 5 kHz, imaging with a larger field of view, or by using fewer laser shots than 1,000 per position.

Incremental principle component analysis (IPCA) is an approach that allows for processing potentially unlimited numbers of mass spectrometry pixels with a finite amount of RAM. This contrasts with standard PCA that cannot be performed easily on large data sizes where pixel counts exceed a few hundred million. Such processing on data produced by FMM is highly valuable due to the large number (often >10 million) of pixels in a single FMM image. We have validated the use of IPCA on a FMM image of >250 million pixels.

McDonnell, L. A., Heeren, R. M. A. 2007 Mass Spect. Rev. 26, 4, 606-43

Körber, A., Keelor, J. D., Claes, B. S. R., et al. 2022 Anal. Chem. 94, 14652–58.

O28

Flavor mapping by HS-SPME GC-HR-MS to improve flavor molecular information and understand flavor formation

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The flavor (taste and odor) of food is recognized in many studies to be the most important driver for consumer liking. Therefore, to increase overall consumer acceptance of plant-based (meat) alternatives, a better understanding of flavor development is required. It is important to understand the global flavor systems in order to provide unique and high-performing solutions.

In this lecture, the overall approach in flavor mapping will be represented from start till the end. The steps (typically in a design-build-test-learn cycle) involve untargeted on-line SPME sampling followed by desorption and Gas Chromatography High Resolution Mass Spectrometry (GC-HRMS) analytical method development, data processing, data annotation, coupling to the dsm-firmenich flavor library, and finally the data interpretation.

While we are stepping up in automated peak annotation and broadening the analytical toolbox so that more and more flavor compounds can be profiled by GC-HRMS (Orbitrap), it is important to distinguish which molecules in the data set are relevant and which are not. This will improve and speed up data interpretation.

For flavor mapping of a portfolio of products, a reliable and reproducible sensory test has been set-up using a trained panel. By using references, a scaling method has been implemented and next to that, the repetition of specific samples in different sets (so called anchors) helped to compare data over different sensory trials.

A dsm-firmenich flavor library was also applied which contains flavor molecule characteristics like odor description, presence in (types of) food, compounds class and molecular weight. This database was coupled to the annotated peaks after the volatiles profiling.

O29

Mass Spectrometry Imaging Reveals Essential Oil Treatment Effects on Downy Mildew in grapevine (*vitis vinifera L.*)

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Transcriptomic studies revealed that oregano oil treatment reduced downy mildew sporulation and up-regulated salicylic acid, jasmonic acid, and ethylene. However, the accumulation patterns of these compounds in the leaves are not known. Mass spectrometry imaging (MSI) can visualize the interactions and spatial relationships of molecules within the same sample. Therefore, this study employs MALDI-MSI to visualize the localization of compounds triggered in grapevine leaves post- essential oil treatment and infection in control conditions and in the vineyards.

Control leaves, infected and essential oil treated leaves were collected. Sample preparation involved two methods, mounting the leaf discs on the ITO slides with double-sided adhesive copper tape and preparing cross-sections. Subsequently, the mounted leaves were sprayed with 10mg/mL DAN matrix using M3+ sprayer. MSI measurements were conducted using the Bruker Tims-TOF instrument in negative ion mode, at 30 µm spatial resolution and a mass range of m/z 100-1000.

The data was imported into SCiLS lab and the distribution of key compounds was studied. Tentative identification was given based on literature which including viniferin (*m/z* 453.0), resveratrol (*m/z* 227.0), and stilbenes (*m/z* 230.0), among others. Viniferin was found to be localized on the leaf veins of essential oil treated leaves. An unidentified compound at *m/z* 604.4 was only detected in the leaf blade of infected leaves. Cross sections unveiled the leaf palisade responsible for photosynthesis and vascular bundles for inter-tissue signaling. Sucrose (*m/z* 381.07) was localized in the vascular bundles of the infected leaf cross sections. While a chlorophyll peak (*m/z* 871.5) was localized in the palisade of un-infected leaf cross-sections. Principle component analysis was conducted to study the variance among the different samples (control, essential oil treated, infected and essential oil treated + infected). All the four samples showed separation on the PCA plot providing an evidence of changes in the metabolic profile of plant under different conditions. In summary, this study demonstrates the potential of MALDI-MSI in elucidating the spatial distribution of key compounds in grapevine leaves under essential oil treatment and infection conditions, offering valuable insights into plant-microbe interactions and metabolic responses.

O30

Unraveling mono genetic disorders with complex pathologies through advanced 4D-lipidomics: Insights into ALD and BTHS

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Adrenoleukodystrophy (ALD) and Barth Syndrome (BTHS) are genetic disorders of lipid metabolism with complex pathologies. ALD is caused by mutations in the ABCD1 gene and is characterized by the accumulation of very long-chain fatty acids (VLCFA) throughout the body. Clinical symptoms can include spinal cord disease, adrenal insufficiency and lesions within the white matter of the brain. BTHS involves cardiac and skeletal muscle myopathy, among other symptoms, due to mutations in the TAFAZZIN gene affecting cardiolipin (CL) remodeling. Despite significant progress, the precise pathophysiological mechanisms underlying these diseases remain elusive.

To unravel the intricate lipidomic changes associated with these disorders, we employed a cutting-edge 4D-lipidomics approach utilizing the timsTOF Pro mass spectrometer. This method involved four LC runs on two analytical columns, normal phase and reverse phase, in positive and negative ionization modes. Compounds were ionized using Vacuum Insulated Probe – Heated ESI (VIP-HESI) for enhanced sensitivity. Parallel Accumulation Serial Fragmentation (PASEF) enabled the detection and fragmentation of ions, while lipids were annotated using a sophisticated in-house build bioinformatics workflow in conjunction with MetaboScape (Bruker).
Through the 4D-lipidomics approach, we achieved comprehensive lipid profiling, particularly focusing on the incorporation of saturated VLCFA and fatty acid (FA) composition alterations within complex lipid species. By leveraging ion mobility and high-quality MS/MS data provided by PASEF, we enhanced the confidence level of lipid annotation.

In ALD, our results demonstrate that saturated VLCFAs are incorporated into multiple complex lipid species including phosphatidylcholines, lysophosphatidylcholines, cholesterol esters and triglycerides. Furthermore, a reduction of polyunsaturated variants of these lipids was observed. These systemic changes in the lipidome might underlie the disease mechanisms that result in the multiform pathology of ALD. Similarly, in BTHS specific fatty acids are incorporated into CL which might contribute to the disease pathology. Finally, we demonstrate the benefit of 4D-lipidomics using VIP-HESI for the analysis and annotation of lipids compared to our established lipidomics platform.

Our study represents a novel application of 4D-lipidomics, incorporating VIP-HESI, trapped ion mobility, and PASEF, to investigate lipidomic alterations associated with mono genetic disorders with complex pathologies such as ALD and BTHS. This approach provides unique insights into the lipid metabolism dysregulation underlying these diseases.

O31

Unveiling Lipidomal Dynamics in Glioblastoma Recurrence: Single-Cell MALDI-MSI of Patient-Derived Cell Lines.

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Single-cell matrix assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) is a powerful analytical approach that allows for the high-spatially resolved investigation of molecular profiles within individual cells. In this study, we applied MALDI-MSI to analyze single cells derived from patient-specific glioblastoma cell lines, encompassing both primary and in some cases recurrent tumors from the same patients. The primary objective was to delineate the molecular changes occurring at the single-cell level and discern alterations associated with tumor development and tumor recurrence.

Patient-derived glioblastoma cell lines were grown on indium tin oxide (ITO)-slides and MALDI-MSI was employed to acquire detailed molecular information within single cells. The investigation focused on elucidating the spatial distribution of lipids, shedding light on the intricate cell-heterogeneity characteristic in glioblastoma.

Comparative analysis of primary and recurrent tumors revealed distinctive molecular signatures within individual cell types. These signatures not only highlighted inherent heterogeneity within the tumor microenvironment but also provided insights into the changes associated with tumor recurrence.

The integration of MALDI-MSI with patient-derived cell lines allowed for the identification of potential biomarkers and molecular pathways associated with glioblastoma recurrence. These findings contribute to a deeper understanding of the molecular underpinnings of glioblastoma progression, creating opportunities for targeted therapeutic interventions and personalized treatment strategies.

In conclusion, this study showcases the utility of MALDI-MSI in unraveling the complexity of patient-derived glioblastoma cell lines at the single-cell level. The observed molecular changes provide valuable insights into the biology of recurrent tumors, offering a foundation for further investigations into the mechanisms driving glioblastoma recurrence and progression.

O32

Going big: non-denaturing HRMS- hyphenated separations unleash the analysis of complex proteoform mixtures over 100 kDa

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In the last decade, significant progress has been made in native mass spectrometry (MS), enabling the characterization of large proteins and protein complexes in application areas such as biopharmaceutical and structural biology studies. Yet, to date, many studies that apply native MS use purified samples and direct infusion with nanospray sources, reducing the application potential of this technique. Hyphenating native separations to native MS has several potential advantages. The chemical separation in the liquid phase prior to MS detection allows the measurement of complex samples, resolving proteoforms according to specific mechanisms (and therefore aiding identification) and increasing the dynamic range of the measurement. However, the approaches to perform native MS hyphenated separations require relative large amount of samples (e.g. conc of 1-2 mg/mL injecting 10s of µgs of purified protein) and need to be more sensitive for biological studies.

In our research, we aimed to extend the application of native separation methods to study intact proteins and complexes in microscale format to allow for the analysis of biological samples. The separations were developed at nanoflows (< 1 µL/ min), facilitating desolvation during electrospray ionization and increasing MS detection sensitivity.

In this presentation, we will discuss our results obtained using non-denaturing capillary zone electrophoresis (in collaboration with Aalen University), nanoflow size exclusion chromatography, and nanoflow ion-exchange chromatography, and their hyphenation to native MS. Focus of the talk will be in particular the use of nanoflow cation exchange chromatography. Results from the analysis of reference proteins between 10 and 150 kDa, a model cell lysate and serum immunoglobulin G by a salt-mediated pH gradient using volatile additives will be discussed. Proteins presented non-denatured MS with low charge states and low detection limits were achieved (0.22 pmol of monoclonal antibodies). Excellent chromatographic separations were obtained, including the resolution of different proteoforms for large proteins (over 140 kDa). The proposed native hyphenated separations setup shows great potential for analyzing diverse proteins in native top-down proteomics and provides unprecedented opportunities for clinical applications.

O33

Chemical vs. collision-induced disruption of artificially-induced ternary protein complexes.

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The development of new disease treatments is challenging, in part because of the incompatibility between traditional small molecule therapeutics and so-called “undruggable” protein targets, which are characterized by a lack of defined pockets that can be affected by conventional ligands. This affects approximately 80% of the human proteome, explaining why non-traditional drug modalities are so important nowadays. Targeted protein degradation offers an innovative approach and acts via the induction of a complex of the target with an E3 ligase, resulting in ubiquitination and degradation. Specifically, proteolysis-targeting chimeras (PROTACs) and molecular glues (MGs) are two types of molecules used to induce the aforementioned complexes. Despite significant interest in these new drug modalities from medicinal chemists, however, only a few research groups have studied PROTAC- and MG-induced complexes with structural mass spectrometry.

It is well established that gas-phase collision-induced dissociation (CID) of naturally-occurring multiprotein complexes usually leads to ejection of peripheral subunits, providing insights into the complex architecture. Conversely, not so much has been reported about how PROTAC- and MG-induced complexes behave under similar conditions. As the geometry of these complexes is crucial for ubiquitination of the target to occur, this is a research area of considerable potential interest. The capability of MS to assist in such structural elucidation in a straightforward manner is very attractive.

We used native MS to study the dissociation pathways of two artificially-induced protein complexes: one induced by a molecular glue, and one by a PROTAC. The targets were FK506-binding protein complexes pertinent to drug discovery. In addition to studying both main types of molecules (PROTACs and MGs) for targeted protein degradation this way (i.e., natively), we compared gas-phase CID after electrospray ionization from a native-like solution (200 mM aqueous NH_4OAc) to solution-phase chemically-induced breakdown of the same complexes. The latter was achieved by stepwise addition of acetonitrile to the sample solution. Despite the PROTAC or MG being located at the interface between proteins, we found that CID consistently induced a dissociation pathway in which the small molecule was ejected, and a [protein1]:[protein2] complex remained. This allowed us to accurately measure the mass of the ejected molecule in the low-*m/z* range, which offers perspectives for identification or validation of a successful PROTAC or MG from a mixture. Conversely, chemically-induced disruption in solution more accurately reflected the solution-phase assembly pathway, by preferentially losing the less tightly bound protein monomer, leaving a [protein1]:[ligand] complex. Gas-phase CID and chemically-induced breakdown therefore seem to be complementary methods for characterizing the structure and stability of these assemblies, and we expect that structural MS methods will play an increasingly important role in the development of these new drug modalities in the future.

List of Posters

P1- Investigation of the higher order structure of peptides by capillary electrophoresis coupled with ion mobility mass spectrometry.

Evan Seyssens, Cédric Delvaux, Simon Godaux, Edwin De Pauw, Gauthier Eppe, Johann Far

P2- Structural elucidation of plant metabolites using infrared ion spectroscopy.

Teun van Wieringen, Matthias Vink, Jos Oomens, Simon Perry, Agisiloas Chantzis, Saeed Mansoor and Jonathan Martens

P3- DIA analysis evolution: Exploring the use of high resolving power multi-reflecting TOF spectrometry for metabolite identification.

Michael McCullagh, Stephen Griffin, Emma Marsde-Edwards, David Eatough, Martin Palmer and Dale Cooper-Shepherd

P4- Rapid pre-screening RP-LC-MS/MS method for amino acids in complex food products

Wilma du Chatinier, Hans-Gerd Janssen

P5- Accurate mass library for PFAS analysis in environmental samples using high resolution GC/Q-TOF

Remko van Loon, Carola Damen, Sofia Nieto, Luann Wong, Gabrielle Black and Thomas Young

P6- Enhancing the mobility resolution for co-eluting compound classes during plasma characterisation using multi-sequence IMS n acquisitions.

Adam M King, Christopher Jurtschenko, Dale A. Cooper-Shepherd, Martin Palmer, Darren Hewitt, Emma Marsden-Edwards, Perry Derwig

P7- Discovery Lipidomics and Mapping of Exogenous Fatty Acid Incorporation into the HeLa Lipidome Using LC-IMS/MS/MS/MS

Johannes Morstein, Andrew Baker

P8- Preferred protonation site of aromatic amines: elucidation via IR ion spectroscopy

Laura Finazzi, Lara van Tetering, Jonathan Martens, Giel Berden and Jos Oomens

P9- Comprehensive LC-PAD/MS Analysis of N-glycans using SweetSep™ HPAEC Column

Jean-Pierre Chervet, Christian Marvelous, Hendrik-Jan Brouwer, Martin Eysberg, Valentina Valentini

P10- Venomics and antivenomics from the ADDovenom project, towards a new generation of antivenoms based on virus like-particles.

Damien Redureau, Fernanda Gobbi Amorim, Thomas Crasset, Stefanie Menzies, Nicholas Casewell and Loïc Quinton

P11- Rapid discrimination of black truffles and annotation of unknown marker compounds by DART-QTOF-MS

Erik van Balderen, Ilona Nordhorn, Klemens Losso, Mathias Rainer and Carsten Baessmann

P12- Hydrogen Carrier Gas use in GC/MS/MS Analysis - Achieving the MRLs for 200 Pesticides in Produce

Remko van Loon, Carola Damen, Danny van Oevelen, Katrien Sabbe and Anastasia Andrianova

P13- Analysis of intact pectins by MS-based approaches

Yasmin van der Velden, Constantin Blöchl, Christoph Gstöttner, Elena Dominguez-Vega, Leon Coulier, Adriana Carvalho-de-Souza, Yuri van der Burgt, Simone Nicolardi and Manfred Wuhrer

P14- A Technical View: Enabling Ultra-long Transients on an Orbitrap Mass Spectrometer

Arjan Barendregt, Victor C. Yin, Evolène Deslignière, Eduard H.T.M. Ebberink, Amber D. Rolland, Tobias P. Wörner, Konstantin O. Nagornov, Anton N. Kozhinov, Kyle L. Fort, Yury O. Tsybin, Alexander A. Makarov, Albert J.R. Heck

P15- Exploring Clusterin Glycosylation in Alzheimer's Disease: Insights from Human Plasma and Cerebrospinal Fluid Analysis

Amalia Kontochristou, Nika Simicic, Anouk M. Rijs, Melissa Baerenfaenger

P16- Characterizing modified oligonucleotides by combining Mass Spectrometry with Ultra-Violet Photon Dissociation

Kevin Hes, Maxime Benonit, Sjors Bakels, Govert W. Somsen, and Anouk M. Rijs

P17- Solving the PFAS Challenge: Comprehensive Screening of Environ-mental Samples against 1000s of Compounds in a Single Run

Rob van der Heijden, Arnd Ingendoh, Carsten Baessmann, Andrea Kiehne, Silke Bodendiek and Eva-Maria Niehaus

P18- Improvements in a Multi-Reflecting Tof Mass Spectrometer to Enhance Mass Spectrometry Imaging Specificity

Emmanuelle Claude, William Johnson, Emma Marsden-Edwards, Martin Palmer and Perry Derwig

P19- Wavelength dependence in surface-assisted laser desorption/ionization mass spectrometry

Wendy H. Müller, Alexander Potthoff, Jens Soltwisch, Klaus Dreisewerd

P20- Evaluation of a benchtop infrared ion spectroscopy (IRIS) setup for the analysis of small molecule structural isomers.

Arnaud Lubin, Tom Van Boxtel and Filip Cuyckens

P21- Probing the onset of TDP-43 aggregation using Native Mass Spectrometry.

Behrad Ghavim, Chuck van der Veen, Melissa Bärenfänger, Anouk M. Rijs

P22- Chemical derivatization of polar compounds for analysis in volume-limited biological samples using LC-MS/MS

Liam Nestor, Cinzia Lella, Dimitri De Bundel, Ilse Smolders, Yvan Vander Heyden and Ann Van Eeckhaut

P23- Enhancing microflow LC-MS/MS analysis of neuromedin U through reduced aspecific adsorption

Linus Donvil, Jana Bongaerts, Maria Bjerke, Debby Mangelings, Yvan Vander Heyden and Ann Van Eeckhaut

P24- Workflow development for the characterization of neurofilament light chain (NfL) by LC-MS/MS for clinical applications

Lynn Kalut, Aurélie Ladang, Etienne Cavalier, Caroline Le Goff and Philippe Massonnet

P25- Multiplexed Quantitative Proteomics Ac-IP Tag in Data-independent Acquisition Mode

Xiaobo Tian, Marcel de Vries, Hjalmar Permentier, Rainer Bischoff, Peter Horvatovich

P26- Exposing the Exposome: Global Plasma Profiling with LC-MS

Laurens Heijn, Anne-Charlotte Dubbelman, Amy Harms, Thomas Hankemeier

P27- Comparison of sample preparation for determination of mycotoxins in cannabis and derived products using LC-FLD and LC-MS/MS.

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P60- Expanding the applicability of multidimensional LC-MS for automated bottom-up analysis of biopharmaceuticals by integrating in-solution on-line digestion

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FP65- May post-translational succination be involved in cardiac arrhythmia? A joint experimental and theoretical study combining CIU and molecular dynamics approaches

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FP67- Charge Variants Separation of Intact Monoclonal Antibodies at pH 5 using Nanoflow Sheath liquid CZE-MS

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Aurore Schneiders, Johann Far, Edwin De Pauw, Lidia Belova, Adrian Covaci, Gauthier Eppe

FP69- Towards reliable single cell proteomics data generation and analysis

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FP71- Collecting and analysing pig mucosal scraping samples for mass spectrometry-based proteomics: investigating variation factors

Hanne De Rijcke, Christof Van Poucke, Marc Heyndrickx, Stefaan De Smet, Kris Gevaert and Sam Millet

P1- Investigation of the higher order structure of peptides by capillary electrophoresis coupled with ion mobility mass spectrometry.

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Bioactive Peptides obtained from hydrolyzed proteins are nowadays of great interest in both the food and pharmaceutical industries for their wide range of therapeutical application [1]. These peptides, like proteins, derive their biological activity from their specific structure, including their three-dimensional conformation.

Capillary electrophoresis (CE) separates compounds by (averaged) charges and in-solution shape, described in terms of hydrodynamic radius. CE offers a powerful alternative to liquid chromatography (LC) for the separation of peptides potentially in non-denaturing condition, improving the detection of both highly hydrophilic and hydrophobic compounds while providing insights onto the structure of the analytes in solution [2].

Ion mobility spectrometry (IMS) is somewhat similar to CE but operates in the gas phase at moderate pressure. The ions are also separated by charge, this time induced by the electrospray ion source, as well as the ion shape, described in terms of collision cross section (CCS). Ion mobility coupled to mass spectrometry (IM-MS) improve the peak capacity of any separation methods coupled to MS depending on the degree of orthogonality between the separation technique and ion mobility. The recent development of interfaces allows nowadays for robust hyphenation of CE and ESI-MS and ESI-IM-MS instruments.

Peptides generated from BSA tryptic digest were separated by capillary zone electrophoresis (CZE) at different pH and detected on-line by ESI-IM-MS using a homemade sheath liquid microfluidic interface. We observed that, for some peptides, the conformation in solution and in the gas phase of unique peptide sharing the same charge state were not strictly correlated. Additionally, several conformers of the same peptide could be detected either in the gas phase, either in solution, either both, i.e. solution and gas phase These results suggest that the structure conservation hypothesis, which states that the structure of a specie is preserved by being kinetically trapped during the ESI process might not be true for oligopeptides containing less than 20 residues.

CZE coupled with IM-MS and collision induced unfolding (on helium) experiments were performed on some peptides. Our data suggest that conformation of oligopeptides could indeed be kinetically trapped conformations under our experimental conditions.

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P2- Structural elucidation of plant metabolites using infrared ion spectroscopy.

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In plants, animals and the environment, agrochemicals undergo a variety of chemical and metabolic transformations, producing derivatives that need to be characterised to determine their toxic properties and their persistence in the environment. Furthermore, some plants produce natural toxins, which need to be monitored for food safety. Using a method called infrared ion spectroscopy the structure of these compounds can be determined. The method has the sensitivity and selectivity of mass spectrometry, while giving the structural information obtained from infrared spectroscopy.

A Bruker amaZon quadrupole ion trap was used allowing for the optical access of the FELIX IR free electron laser. The spectrum is constructed from a series of mass spectra by monitoring the fragmentation yield as the IR frequency is scanned. When the frequency of the IR laser is resonant with an absorption band of the trapped ions, photodissociation occurs, and the fragment ions are detected.

Infrared ion spectroscopy acts as a unique fingerprint, giving structural information which is able to distinguish structural isomers. Computational chemistry with density functional theory (DFT) can predict the spectrum, based on the vibrational normal mode analysis.

Using infrared ion spectroscopy, plant metabolites can be characterized with high sensitivity and little sample consumption.

P3- DIA analysis evolution: Exploring the use of high resolving power multi-reflecting TOF spectrometry for metabolite identification.

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Data independent acquistion (DIA) to identify small molecule drug metabolites, has been used to assess the LCMS accurate mass measurement specificity attained using the SELECT SERIES™ MRT (Figure 1), a state-of-the-art hybrid quadrupole Multi Reflecting Time-of[1]Flight mass spectrometer (MRT).1 It provides a unique combination of high resolving power (>200,000 FWHM), and routine ppb mass accuracy, independent of acquisition speed. Using unbiased non-targeted “wide-scope” data acquisition, thousands of detections can be made in a single analysis. High mass resolving power enhances ion selectivity and subsequently the detection of analytes in complex matrices, providing high mass accuracy which enhances analyte identification confidence and facilitates use of more stringent data tolerances during retrospective targeted data analysis. The attained precursor/fragment ion ppb mass accuracy can be utilised to improve identification confidence in research involving small molecules, such as metabolite identification. Metabolite identification is an important part of the drug development process where the metabolic fate of a drug molecule is investigated. This requires mass spectrometry techniques with high specificity for structural elucidation. A urinary screen of a healthy human volunteer was undertaken to identify therapeutic drugs and metabolites. A metabolite identification workflow using LCMS (system resolution >200,000 FWHM) has been implemented.

P4- Rapid pre-screening RP-LC-MS/MS method for amino acids in complex food products

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Given the inherent polarity of amino acids, employing a direct reverse-phase (RP) chromatographic approach for non-derivatized amino acids presents considerable challenges. Traditionally, two primary strategies are employed: utilizing ion pairing reagents or pre-derivatizing compounds before LC-MS/MS injection. However, both methods entail drawbacks. The use of ion pairing agents poses a risk of contaminating the MS system, while derivatization introduces complexity into sample preparation, leading to time-consuming procedures. In practice both routes require dedicated instruments, which can be an obstacle for labs only occasionally performing amino acid analyses.

To address these challenges, non-derivatized amino acid separation is typically accomplished using hydrophilic interaction liquid chromatography (HILIC), yet this method is plagued by poor reproducibility and extended column equilibration times.

Here we present a screening method that allows us to quantify non-derivatized amino acids without the need for special pre-treatment of samples. In the method, samples are spiked with certified isotopically labeled (13C- and/or 15N-) amino acids as internal standards. The amino acids are then extracted from complex food products and subjected to analysis via RP-UPLC-MS/MS. Quantification is performed by determining the analyte/internal standard chromatographic peak area ratios.

P5- Accurate mass library for PFAS analysis in environmental samples using high resolution GC/Q-TOF

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants of increasing concern due to their environmental persistence, toxicity, and capability of bioaccumulation. PFAS include a wide range of chemical classes, therefore, a variety of analytical techniques are necessary for their detection. GC/MS is typically used for detecting volatile and non-polar PFAS compounds. In this study we used a GC/Q-TOF system to take advantage of high resolution for detecting compounds with negative mass defect in complex environmental matrices.

For specific and sensitive PFAS detection, we have first created an accurate mass GC/MS library in both EI and positive CI modes for over hundred fifty GC-amenable PFAS standards. The PFAS compound classes included perfluoroalkyl iodides (PFAIs), fluorotelomer iodides (FTIs), fluorotelomer alcohols (FTOHs), fluorotelomer olefins (FTOs), fluorotelomer acrylates (FTACs), fluorotelomer methacrylates (FTACs) and perfluoroalkyl carboxylic acids (PFCAs) among others.

Accurate mass fragment ions have been automatically annotated with formulas based on accurate mass information and isotope ratios using MassHunter Qualitative Analysis software. The fragment formula annotations were manually verified, corrected when necessary and automatically converted to the theoretical m/z. Kovats retention indices have also been calculated for each compound.

The accurate mass PFAS library was evaluated using soil and water extracts. Soil samples were extracted using dichloromethane. The samples were separated using a 30 m medium polarity 6% cyanopropyl/ phenyl, 94% polydimethylsiloxane GC column with 1.4 µm film thickness. GC/MS analysis was performed using an Agilent 8890 GC coupled to an Agilent 7250 high resolution Q-TOF. Drinking water samples were extracted on a multi-mode SPE (HLB, WAX, WCS, Isoelut ENV) and eluted with different solvent mixtures. The combined extracts were concentrated, solvent exchanged to Ethyl Acetate and diluted 10x.

To identify other contaminants in drinking water samples the GC/Q-TOF Pesticide PCDL as well as NIST 20 library were used.

P6- Enhancing the mobility resolution for co-eluting compound classes during plasma characterisation using multi-sequence IMS n acquisitions.

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Introduction Human plasma comprises of many small molecules including amino acids, organic acids, and larger lipid molecules across many classes. To accurately characterise this biological fluid requires multiple extraction and complimentary LC-MS acquisition methodologies to deconvolve certain compounds not efficiently resolved across methods. When analysing small polar analytes by HILIC based methods, many moderately polar and non-polar compounds like lipids are also present but co-elute in class regions and the solvent front, meaning specific analytical methods must be employed to separate out these features. Coupling ion mobility with mass spectrometry has improved the separation of many of these co-eluting compounds, increase the peak capacity and improving spectral quality. But different compounds require different levels of separation to achieve the desired resolution.

Methods Human plasma samples, obtained from healthy human donors, underwent extraction using acetonitrile and water to obtain small molecule metabolites and lipids. The resulting extracts were analysed in both positive and negative ion ESI modes with the data acquired on the SELECT SERIES ™ Cyclic ™ IMS system using a data independent acquisition (DIA) multi-sequence acquisition mode. This acquisition mode enabled multiple ion mobility sequences to be scheduled in different functions across the chromatographic run with each tailed to the elution of different compound classes and mass ranges. Chromatographic separation was achieved rapidly over 10 mins using a ACQUITY ™ Premier BEH ™ amide (2.1 x 100 mm) column and HILIC gradient elution profile using acetonitrile and water mobile phases.

Preliminary Data A single pass of the Cyclic IMS device provides good broad mobility separation when analysing complex matrices, the unique capabilities of the SELECT SERIES Cyclic IMS allows multiple passes round the device, increasing the mobility path length and the resolution. This higher mobility resolution is achieved over a narrower mobility range when compared to the single pass acquisition. The pooled human plasma sample was initially acquired using a single pass HDMS ^{E} acquisition mode to determine the regions of the chromatogram where compounds requiring additional passes of the Cyclic IMS device were eluting. Due to the hydrophilic nature of the separation, those lipids remaining following the extraction procedure elute based on the polarity of the head group of the class causing them to co-elute. The single pass of the Cyclic IMS device provided sufficient resolution for many small molecules throughout the chromatographic separation, but those regions corresponding to lipids from the triacylglyceride and phosphatidylcholine classes from the beginning and middle of the gradient respectively, required additional passes to separate out these co-eluting features. A multi-sequence HDMS/MS method was then created incorporating multiple HDMS/MS functions each using broad quadrupole isolation windows across specific mass ranges each corresponding to the co-eluting classes and with optimised cyclic multipass sequences. Collision induced dissociation was performed to provide high energy fragmentation spectra to assist in elucidating structural information for the mobility resolved features by applying collision energies of between 20-30 eV. Optimised mobility sequences of 1 – 3 passes were employed with static T-wave heights of 15 V and 25 V for small molecules and larger lipid molecules were used respectively. Employing the multi-sequence analysis for plasma metabolomics assisted in improving the depth of characterisation of the plasma matrix, enhancing the isolation of co-eluting lipid phosphatidylcholine isomers and identification using elevated collision energy spectra.

Novel Aspect Plasma characterisation using the IMS ^{n} multi-sequence acquisition mode to resolve co-eluting lipid class features during HILIC metabolomic analysis.

Conflict of Interest Disclosure All authors are employees of Waters Corporation and the poster is presented on behalf of the company.

P7- Discovery Lipidomics and Mapping of Exogenous Fatty Acid Incorporation into the HeLa Lipidome Using LC-IMS/MS/MS/MS

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Odd-chain fatty acids are not produced endogenously and therefore often used as standards for lipid quantification in lipidomics experiments. However, when added to cells, these fatty acid analogs may be metabolized into a range of phospholipids and sphingolipids. A combination of discovery and targeted lipidomics techniques are used to profile the incorporation of these exogenous lipid probes into cellular membranes. When coupled with liquid chromatography, ion mobility spectrometry (IMS) represents an orthogonal technique that separates ions based on charge, size, and shape. IMS strategies were used to probe the complex mixture of endogenous lipids along with targeted detection of modified lipids present in HeLa cells.

Methods

200,000 HeLa cells were plated and grown to 70% confluence, then were incubated for 4 hours with 50 micromolar solutions of heptadecanoic acid. After incubation, cells were isolated by centrifugation and the cell pellet was extracted using an MTBE: methanol: water extraction protocol. The extracts were dried down and reconstituted in 100 microliters of 60% acetonitrile prior to chromatographic analysis using a water: acetonitrile: isopropanol gradient on an ACQUITY™ Premier CSH™ C18 column (2.1x100 mm, Waters Corporation). The SELECT SERIES™ Cyclic™ IMS instrument (Waters Corporation) was used to collect positive and negative mode LC-IMS MS and MS/MS datasets. Multiple pass experiments were used to increase the mobility resolution for specific classes of lipids.

Preliminary Data

Exogenous fatty acids are thought to be incorporated into cell membranes through the remodeling system (Lands Cycle). The odd-chained fatty acid heptadecanoic acid (C17:0) was rapidly incorporated into phospholipids in HeLa cells. Major phosphatidylcholine (PC) species identified in the HeLa cells incubated with heptadecanoic acid were PC C17:0/C16:0, PC C17:0/C16:1, PC C17:0/C18:1, PC C17:0 C18:2, PC C17:0/C18:3, and PC 17:0/C20:4. As additional confirmation, the measured collisional cross section (CCS) values were compared to modelling results generated using CCS OnDemand software package (Waters Corporation). The experimental CCS were all within 2% of the calculated values. In addition to the PC lipids, low levels of phosphatidylethanolamine (PE) C17:0/C18:0 and PE C17:0C18:1 and sphingomyelin dC18:1 C17:0 were detected in cells incubated with heptadecanoic acid. These phospholipids with exogenous fatty acid content were not detected in control HeLa cell incubations lacking the exogenously added heptadecanoic acid. Using ES-, several other C17 containing lipids were observed including PE 17:0/C18:1, PE C 17:0 C18:2, PE C17:0/C20:4, PI C17:0/C18:1 and PI C17:0 C20:4. Extracted ion chromatograms of the m/z 269.24 ion in the high energy MSE function suggest that there is incorporation of C17 fatty acids in a number of other lipids, but at rather low concentration. SELECT SERIES, Cyclic and (ACQUITY Premier CSH are trademarks of Waters Technologies Corporation

P8- Preferred protonation site of aromatic amines: elucidation via IR ion spectroscopy

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The protonation site of aromatic amines in the gas-phase involves a subtle competition between the higher proton affinity of the amine group and the better charge-delocalization properties of the aromatic moiety. This fundamental question has been at the center of many debates in mass spectrometry [1-3]. Solvents and external conditions influence the environment and, subsequently, the outcome of protonation. Hence, gas-phase environments provide an ideal setting to discern electronic effects from solvation effects with no impact of the external conditions. Nevertheless, establishing the favoured protonation site becomes challenging in the case where multiple possibilities are viable, and several factors, for instance resonance stabilization, come into play. Furthermore, experimental conditions such as ionization source and solvents may influence the species formed and higher-energy tautomers may become kinetically trapped, adding another layer of complexity to the problem [4]. In this study we elucidate where protonation occurs for 1-aminonaphthalene, 2-aminonaphthalene, 1-aminoanthracene and 2-aminoanthracene. The goal of these experiments was to tackle how the position of the amino moiety and the size of the aromatic system (and hence the degree of charge delocalization) influences the competition between protonation on the ring and the amino group. Trapped ion mobility spectrometry (TIMS) experiments revealed the presence of multiple isomeric species, and employing the TIMS unit on a Bruker-FTICR mass spectrometer as a filter we were able to record tautomer-resolved infrared ion spectra and assign the recorded features to the specific protonation isomers.

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P9- Comprehensive LC-PAD/MS Analysis of N-glycans using SweetSep™ HPAEC Column

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In the field of glycomics, understanding the functional aspects and biological implications of glycoproteins requires a comprehensive understanding of their structure, for example, the glycosylation sites or the type of glycan moieties. Despite the significance of these insights, the analysis of glycans in glycoproteins presents a major challenge due to the vast number of different glycan isomers present in glycoproteins, each with varying abundances. Therefore, to improve the structure elucidation workflow in glycomics, there is a need for the development of analytical methods that enable the separation, detection, and quantification of glycans from glycoproteins.

The typical approach for glycan analysis of glycoproteins involves enzymatic release of the glycans from the glycoproteins, followed by chemical derivatization of the glycans. Subsequently, the mixture of glycans can be separated and identified using liquid chromatography such as HILIC with fluorescence and MS detection. High-performance anion exchange chromatography combined with pulsed amperometric detection (HPAEC-PAD) is an alternative analytical technique that allows for the separation and detection of glycans without the need for derivatization. When coupled with MS, HPAEC-PAD becomes a powerful analytical workflow for the high-resolution separation, sensitive detection, quantification, and accurate identification of glycans from glycoproteins.

In our study, HPAEC-PAD/MS was conducted using a SweetSep™ AEX200 anion-exchange column. The AEX200 stationary phase is based on a highly monodisperse 5 µm resin poly(divinylbenzene-co-ethylvinylbenzene) coated with quaternary amine functionalized latex nanoparticles (crosslinking degree of 80%). This stationary phase has a high ion-exchange capacity of 86 µeq per 200×4 mm ID column.

Several glycan standards were successfully separated using HPAEC at high pH using NaOH/NaOAc, followed by parallel PAD/MS detection using post-column flow splitting. Due to the non-volatile nature of NaOH/NaOAc, a desalter was employed for post-column removal of the sodium ion before MS detection. However, the use of a desalter may contribute to peak dispersion and thus loss in resolution. Therefore, an alternative anion-exchange separation approach was evaluated using a volatile buffer system that is typically used for MS, i.e. NH_4OH/NH_4OAc. The use of the volatile buffer eliminates the necessity of using a desalter, thus simplifying HPAEC-PAD/MS. The results highlight the potential of the SweetSep™ column for glycan separation using HPAEC-PAD/MS, enabling both quantification and identification of individual glycans.

P10- Venomics and antivenomics from the ADDovenom project, towards a new generation of antivenoms based on virus like-particles.

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Snakebite, a neglected tropical disease, annually accounts for over 120,000 fatalities and significantly exacerbates disability rates. While antivenom, sourced from hyperimmunized animal serum, remains the conventional therapeutic approach, its efficacy is inconsistent, often accompanied by adverse effects, and production costs are prohibitively high. Addressing these challenges, the multidisciplinary European initiative, ADDovenom, seeks to revolutionize snakebite treatment through the implementation of ADDomer©, a thermally stable synthetic virus-like particle with high toxin affinity, engineered for swift toxin clearance post-envenomation. Employing advanced mass spectrometry and proteomics, the ADDovenom project aims to catalog the toxin profiles of nine highly venomous snakes in sub-Saharan Africa, focusing on five *Dendroapis* and four *Echis* species. Proteomic analyses utilizing the innovative Multi-Enzymatic Limited Digestion (MELD) technique elucidated venom compositions, revealing distinct molecular weight distributions between *Dendroaspis* and *Echis* species. Notably, *Dendroaspis* species predominantly comprised large peptides within the 6-15 kDa range, while *Echis* species exhibited higher molecular weight proteins (up to 120 kDa). Analysis indicated that approximately 46% of *Dendroaspis* venoms comprised toxins, with 3-finger toxins, kunitz-type toxins, and snake venom metalloproteinases as prevalent classes. *Echis* venoms contained nearly 49% toxins and proteases, with snake venom metalloproteinases, C-type lectin-like proteins, and serine proteinases being highly expressed. Common peptide constituents were identified among *Dendroaspis* and *Echis* species, facilitating subsequent bioinformatics modeling for toxin structure determination. Additionally, a preliminary assessment of antivenom potency was proposed, employing immunocapture techniques with antivenom antibodies tethered to magnetic beads. This method promises enhanced understanding of antivenom mechanisms and efficacy, while enabling comparative analyses with innovative ADDobodies/ADDomers constructs. Furthermore, compared to actual antivenomics approach, using microcolumn, the use of magnetic beads and shotgun proteomics allow to decrease significantly the amount of antivenom and venom needed for such experiment and can be automated in 96 well plate.

P11- Rapid discrimination of black truffles and annotation of unknown marker compounds by DART-QTOF-MS

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Food fraud is a major issue in the food industry leading to financial losses for food processors as well as inflicting lasting damage in the trust of consumers. Due to the motivation of financial profit, particularly expensive products are prone to adulteration. Truffles are considered a luxury product with prices ranging up to 1000 - 2000 €/kg for the Périgord truffle (Tuber melanosporum VITTAD.). Other black truffle species, for example the Asian truffle (T. indicum COOKE ET MASSEE) are morphologically highly similar to the Périgord truffle but much cheaper in price. Therefore, identification of adulterated truffle batches is of utmost importance, leading to a demand for analytical solutions that deliver highly reliable results, while simultaneously being easy-to-operate and providing a high degree of automation for high-throughput analyses.

In this study, we developed a comprehensive workflow for the differentiation of different black truffle species utilizing direct analysis in real time (DART) ionization (DART JumpShot source, Bruker Daltonics) in combination with QTOF-MS (Impact II, Bruker Daltonics). Unsupervised and supervised multivariate statistical models built using the software MetaboScape 2023b (Bruker Daltonics) revealed a successful discrimination of the truffle samples according to their species. In the next step, the marker compounds with the largest contribution to the species discrimination were annotated using tools for untargeted unknown identification included in MetaboScape. In detail, annotation was performed based on the information of accurate mass, isotope pattern, and fragmentation pattern, either by a fully automated spectral library search or by a semi-automated annotation workflow comprising elemental composition prediction, structure assignment and in silico fragmentation.

Compared to chromatographic methods, DART-QTOF offers significantly shorter analysis times of 15 s per sample and reduced solvent consumption. All in all, DART-QTOF paired with chemometrics presents a fast, robust, and resource-saving method to counteract adulteration of black truffles.

P12- Hydrogen Carrier Gas use in GC/MS/MS Analysis - Achieving the MRLs for 200 Pesticides in Produce

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Recurring helium shortages and increased prices increase the demand for performing GC/MS analysis with alternative carrier gasses. While helium is the best carrier gas for GC/MS, hydrogen is the second-best alternative. However, unlike helium, hydrogen is not an inert gas. Hence, it could react with target analytes, matrix components, or even solvent resulting in compound degradation, chromatographic problems such as peak tailing, distorted ion ratios in the mass spectrum, poor library matching, and sensitivity loss.

Pesticides analysis can be challenging even with helium carrier gas given the diverse and labile nature of many pesticides and complex matrices, in which they are analyzed. This presentation discusses the key strategies for optimizing pesticides analysis with hydrogen carrier:

When transitioning to hydrogen one of the requirements is to reliably quantitate pesticides down to the levels corresponding to the maximum residue limits (MRL) with the lowest default values set by the U.S. EPA and the European Commission of 10 ppb. The required LODs for the GC/MS method are lower if the sample preparation requires additional dilution. It is frequently observed that transitioning GC/MS analysis from helium to hydrogen carrier gas results in a degradation of the LODs by approximately a factor of 3 -5.

To achieve the required detection limits with hydrogen carrier gas, the injection conditions, including the injection mode, temperature, and injection volume were optimized. Further, the changes made to the MS hardware allowed for preventing undesirable in-source chemical reactions. Those reactions are known to cause ion ratio distortion, leading to poor spectral fidelity, and more importantly, for GC/MS/MS analysis – loss of sensitivity when precursor ions are affected. The ion source optimized for use with hydrogen carrier gas greatly reduces the undesirable in-source reactions, thus, allowing the use of the same MRM transitions that had been developed with helium carrier gas.

The combination of the HydroInert source, method translation, and retention time locking techniques allows the use of the MRM transitions and retention times from the database created with helium carrier gas.

A panel of 200 pesticides was tested in multiple food matrices to evaluate LODs achieved under the optimized GC/MS conditions. It was demonstrated that over 90% of the evaluated compounds were quantitated at least at, and often below, 10 ppb in the QuEChERS extract, even in challenging highly pigmented matrices.

P13- Analysis of intact pectins by MS-based approaches

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Pectins are complex polysaccharides containing a homogalacturonan backbone with side branches consisting of twelve different types of sugars in over twenty different linkages. In the food industry, pectin is used as a gelling, stabilizing or thickening agent in products such as jam. The molecular structure of pectin impacts its functionality and therefore a detailed understanding of pectin structure is key for proper product performance.

The use of mass spectrometry (MS) for the analysis of polysaccharides has been limited, due to their size, heterogeneity, polydispersity and compositional diversity. Instead, nuclear magnetic resonance (NMR), other spectroscopic and chemical methods are used for the characterization of polysaccharides. Additionally, polysaccharides are often chemically or enzymatically fragmented to reduce their complexity. However, fragmenting polysaccharides results in loss of information of polymer's overall size, degree of modification, and the presence of domains including the clustering of certain structural features. Therefore, there is a need for methods to analyze intact polysaccharides which provide such information. MS can potentially be applied to characterize intact polysaccharides, however its use is still very limited due to the structural complexity and low ionization efficiency of these molecules.

Recent work has shown the potential of intact and top-down MS for the analysis of intact polysaccharides and highly glycosylated glycoconjugates. From monodisperse synthetic polysaccharides, a broad-range coverage of fragment ions at relatively high resolution and mass accuracy with in linkage-specific fragments and branching positions has been achieved [1], an dopant enriched nitrogen gas has been shown to boost ionization of highly glycosylated glycoproteins in nano-electrospray ionization [2, 3]. Inspired by these data and combined with previous knowledge, in this project we aim to develop MS-based approaches to analyze heterogeneous polysaccharides, such as pectins. First results from a pectin sample show the potential of wide mass range ultrahigh-resolution matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance (MALDI-FT-ICR) MS for the analysis of this complex polysaccharide mixture.

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P14- A Technical View: Enabling Ultra-long Transients on an Orbitrap Mass Spectrometer

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Over the last 2 decades, native MS has evolved into a powerful tool to determine and characterize intact proteins and their non-covalent biomolecular assemblies. One of the remaining challenges in native MS is the analysis of highly heterogeneous protein complexes (*e.g.* co-occurring stoichiometries, variable sub-complexes, post-translational modifications), due to insufficient instrument resolution and peak interference preventing accurate charge state identification. In principle, the achievable *m/z* resolution can be enhanced by extending the transient recording time, which is normally limited to a maximum of ~1 second. Here, we show the modification of a Thermo Scientific™ Q Exactive™ UHMR™ Orbitrap™ mass spectrometer to enable transient recordings more than an order of magnitude longer than currently possible.

Previous work to extend transients were bottlenecked primarily by memory limitations of the on-board data acquisition (DAQ) electronics, which often led to hardware, firmware, and software instability. To overcome this limitation, we installed an external high-performance DAQ system FTMS Booster X2 (Spectroswiss, Lausanne, Switzerland) to record data externally and independently of the on-board DAQ electronics. To enable prolonged ion trapping periods within the Orbitrap mass analyzer, we utilized a modified acquisition workflow whereby consecutive ultra-long transient scans could be recorded. Using this approach, we are in principle able to record ion signals for more than 100 seconds, corresponding to ion oscillation distances of over 100 km. In practice, remaining instabilities in the central electrode hampers recording of stable ion trajectories to approximately 25 seconds, still an order of magnitude improvement over existing approaches.

We performed several native MS experiments using ultra-long transient acquisition to assess its performance across several analytes. While normal, "ensemble ion" native MS saw only moderate improvements due to limitations such as ion dephasing, massive improvements in resolution were observed for single ion measurements for most (but not all) analytes, particularly for charge detection MS (CDMS) experiments. To better understand their differential behaviors, in-house developed Python scripts were used to monitor ion behavior during ultra-long transients. Our analyses suggest that not all capabilities have been reached during Orbitrap-based analyses using ultra-long transients, and further modifications may further improve its performance.

P15- Exploring Clusterin Glycosylation in Alzheimer's Disease: Insights from Human Plasma and Cerebrospinal Fluid Analysis

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Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder, which is hallmarked by the accumulation of proteins such as tau protein and amyloid β-peptides (Aβ) aggregates, leading to the disruption of several cellular functions and eventually to irreversible neurodegeneration. Despite considerable efforts aimed at therapeutics that target the accumulation of Aβ in the brain, existing therapies have shown limited effectiveness. To better understand the disease pathway, we open a novel course towards therapeutic approaches by focusing on the glycoproteins that directly modulate the amyloid-beta aggregation process.

Central to this study is clusterin, also known as Apolipoprotein J, a multifunctional glycoprotein that acts as a chaperon and binds to Aβ oligomers, affecting their solubility and aggregation dynamics. Multiple studies have correlated the abnormal glycosylation with changes in the functions of the protein, without fully unravelling the mechanism or the exact changes in glycosylation. For example, it has been reported that different glycoproteoforms can either promote or inhibit Aβ aggregation in AD pathology. To unravel the role of clusterin glycosylation in AD we developed a highly sensitive method for the extraction of clusterin from human body fluids and exploit novel mass spectrometry-based techniques to study the glycosylation pattern of this highly glycosylated protein. We intend to apply this methodology to characterise glycosylation abnormalities in AD, to improve our understanding of disease progression and potentially deliver a novel glycan-based biomarker.

Firstly, a novel extraction and purification strategy has been developed to obtain clusterin from human plasma and human cerebrospinal fluid (CSF). Therefore, we tested newly released commercial clusterin affinity beads and developed a protocol for affinity purification. Due to the chaperon nature of clusterin, we observe the co-purification of distinct plasma and CSF proteins, indicating specific interactions between these proteins. To further purify our samples, we evaluated several additional chromatographic separation methods. After successful enrichment, clusterin was subjected to hyphenated mass spectrometry bottom-up approaches using the TIMS-TOF Pro2 (Bruker) and ZenoTOF 7600 (Sciex) for in-depth characterisation. Our initial data confirms the organ-specific nature of protein glycosylation, showing differences in clusterin glycosylation from human plasma and CSF. By elucidating the distinctions between blood-type and brain-type glycosylation of clusterin we demonstrate that our methodology can be used to detect glycosylation differences of human clusterin from clinically relevant samples.

P16- Characterizing modified oligonucleotides by combining Mass Spectrometry with Ultra-Violet Photon Dissociation

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Oligonucleotides offer a promising perspective for targeting undruggable diseases. Oligonucleotides is a collective name for all synthetic DNA and RNA molecules, which are commonly modified to produce a therapeutic effect. These therapeutics display an increased specificity over conventional therapeutics. One of the ways in which this is achieved, is through binding of the antisense oligonucleotide with the mRNA that needs to be marked for digestion by RNases. Moreover, this approach could theoretically be applied to a wide range of diseases. However, to produce such an effect, modifications will have to be made to increase its stability, affinity and nuclease resistance. The synthesis of these oligonucleotides often generates impurities that only slightly differ in structure and mass. Moreover, these modifications introduce certain analytical problems, such as enantiomers, which makes it challenging to fully characterize these modified oligonucleotides.

Analysis and characterization of oligonucleotides is commonly done by using LC-MS approaches. Unfortunately, these LC-based methods are usually insufficient to deal with the previously described challenges. Another option is to use fragmentation methods within high-resolution mass spectrometry. Collision-induced dissociation (CID) is a technique that uses a collision gas to generate a set of specific fragment ions. CID is generally sufficient for full sequence coverage of oligonucleotides. However, the sequence coverage decreases as the complexity of the oligonucleotides increases. Thus, some of these oligonucleotides cannot be fully characterized using standard fragmentation techniques such as CID.

In this research, we propose a novel method to study oligonucleotides; combining Ultra-Violet Photon Dissociation (UVPD) and infrared (IR) action spectroscopy with Mass Spectrometry. UVPD is a technique that uses a UV laser to fragment mass-selected ions. This method works via a fast-heating principle and should generate unique fragments. Here, a 213 nm UV laser is aligned to a Bruker ion trap. The selected precursor ion can be irradiated with a specific amount of UV pulses, generating a unique set of fragments for that precursor probed by mass spectrometry, allowing us to study the potential differences in fragmentation pathways for these oligonucleotides. In an IR-MS experiment, IR spectra of the individual, mass-selected ions can be acquired by measuring the IR-induced fragmentation yield as a function of the IR frequency. The resulting IR signatures can offer diagnostic information regarding specific functional groups, modifications, and substituents. Hyphenating table-top lasers with mass spectrometry can aid in elucidating the structure of oligonucleotides and their impurities.

P17- Solving the PFAS Challenge: Comprehensive Screening of Environ-mental Samples against 1000s of Compounds in a Single Run

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Tot PFAS are known as “forever chemicals” due to their persistent, bio-accumulative, toxic (PBT) properties and ubiquitous presence in the environment and organisms. Ca. 5000 PFAS are marketed worldwide, making their systematic environmental monitoring an extremely challenging task. On top, there is a lack of reference standards or spectral libraries, and there are plenty of isomers. Adding trapped ion mobility spectrometry (TIMS) to UHPLC-HRMS allows for comprehensive monitoring of organic micropollutants in environmental matrices such as water and soil. Presented here is a complete solution for PFAS characterization in environmental samples, combining ion mobility supported target analysis with wide scope non-target screening against the complete set of 5000 compounds.

Water samples were spiked with sets of PFAS compounds or taken from common household and environmental water resources. Data independent acquisition was performed on a high-resolution mass spectrometer equipped with ion mobility. Kendrick mass defect analysis filtered potential PFAS from the matrix background, based on the fluorine content (repeating CF2 units). The sample spectra were compared with either a spectral library of available PFAS standards in a targeted workflow or with the NORMAN network database of 5000 entries for non-targeted analysis. From the Norman database, information for the exact mass, in-silico fragmentation, and CCS prediction for every PFAS compound was derived which could be used for an automated identification.

The ion mobility feature of the system was utilized for several purposes. First, it could separate coeluting isobars and isomers. Second, the TIMS filter resulted in higher sensitivity and lower detection limits of the targeted PFAS as well as significantly higher quality of full-scan MS and bbCID MS/MS spectra. Finally, collisional cross sections (CCS) as additional identification criteria enhanced the identification confidence with was based on retention time, exact mass, diagnostic fragmentation ions and the isotope pattern fit.

The wide-scope suspect screening of real-life samples against the NORMAN network database proved to be a comprehensive approach for a fast and efficient identification and quantification of PFAS against the total set of 5000 compounds in complex environmental matrices. Therefore, it will assist in understanding the chemical universe of PFAS in the environment and protecting environment, wildlife, and human health.

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P18- Improvements in a Multi-Reflecting Tof Mass Spectrometer to Enhance Mass Spectrometry Imaging Specificity

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Mass spectrometry imaging (MSI) provides a method to visualise the spatial distribution of molecules across a surface, with MALDI and DESI being the most common techniques used.

The complexity of MSI data can make accurate identification of analytes a challenge due to overlapping species. Therefore, it is desirable to acquire data at the highest mass spectral resolving power possible to reduce interference. However, MSI experiments that use high spatial resolution often require long acquisition times and can be further limited by the scan speed of the mass spectrometer. Here we employ a method of extending the flight path to enable multiple passes of an MRT analyser that increases the resolving power by >50% enabling >300,000 FWHM at acquisition rates >10Hz.

Experimental and Results

A healthy wild-type mouse kidney and brain were axially cryo-sectioned onto standard glass slides at a thickness of 16 µm.

The sections were analysed by DESI MS on a multi-reflecting Q-ToF mass spectrometer in duplicate employing either a single pass of the analyser or by a novel modification of the analyser to allow a method of extending the flight path to multiple passes of the analyser.

Mainly glycerophospholipids and triglycerides were detected with a mix of potential cation types such as H+, Na+ and K+, increasing the complexity in lipid identification. Further interrogation of the single pass multi-reflecting Tof brain section data revealed a broad peak at m/z 865.6, the image for the spatial location of this ion shows a higher abundance in the hippocampal region of the brain and a similar abundance in the rest of the brain, no identification of the analyte could be performed. When a consecutive section was analysed with the mass spectrometer modified to allow a longer flight path through a second pass of the multi-reflecting Tof analyser, resulting in a resolving power >300,000 FWHM, two ions at m/z 865.6 were partially resolved. These ions were putatively identified as a potassium adduct of an 13C isotope of HexCer(42:2;O3) and a sodium adduct of SM(42:3;O4), the image of the former ion shows strong localisation to the hippocampus, whereas the latter has a similar abundance across the entire brain. A composite image of the two ions showed identical localisation as obtained for the unresolved ion observed in the data acquired with a single-pass of the analyser.

Conclusions

The use of Ultra-high resolving power (>300,000 FWHM) to improve DESI MS imaging specificity has been presented. Multiple examples of ions that were previously unresolved can now be separated resulting in increased imaging specificity, for example, four ions within a 30mDa window were baseline resolving allowing putative identification to be made and images free from interferences. Excellent mass accuracy, 74 ppb RMS, is also observed for a selection of lipids present in the murine kidney section.

P19- Wavelength dependence in surface-assisted laser desorption/ionization mass spectrometry

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Surface-assisted laser desorption/ionization (SALDI) mass spectrometry (MS)[1,2] relies on the use of (usually inorganic) nanostructured surfaces (*e.g.,* coatings of colloidal nanoparticles[3], nanostructured solid supports[4,5] or sputtered metal nanoclusters[6]), instead of organic matrices, as in the conventional matrix-assisted laser desorption/ionization (MALDI) MS technique. While the interplay between the laser wavelength and the absorption profile of the matrix has been widely studied and proved to be a crucial factor in MALDI-MS[7-10], very few, if any, fundamental studies have been carried out in SALDI-MS. Yet, the laser wavelength is a key parameter that needs to be tuned to correspond to the maximum of optical absorption of the assisting material, in order to provide optimal analytical results[7-10]. Indeed, the desorption in SALDI-MS has been proved to be mainly driven by thermal processes, resulting from the heating of the nanosubstrate surface upon absorption of the photon energy[1]. However, almost all SALDI-MS studies use standard laser wavelengths of 337 or 355 nm, even though the peak absorption of the SALDI nanosubstrate might completely differ from these values. Here we employed wavelength-tunable optical parametric oscillator (OPO) lasers to investigate the wavelength dependence in SALDI-MS, using citrate-coated gold nanoparticles (AuNPs) as SALDI nanosubstrates and model analytes (*e.g.,* amino acids). The uniform spray deposition of the AuNPs and analyte ions on Superfrost™ glass slides was performed using an ultrasonic spray coater. We then recorded gold and analyte ion signals as a function of the laser wavelength and laser fluence in the UV-visible range, knowing that the maximum absorption of the colloidal AuNPs is at 529 nm. The results of this study provide new insights into the SALDI desorption/ionization processes and could assist the improvement of the analytical performance of the SALDI-MS technique, through instrumental and methodological adjustments.

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P20- Evaluation of a benchtop infrared ion spectroscopy (IRIS) setup for the analysis of small molecule structural isomers.

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The most challenging task in metabolite identification is the detection and elucidation of trace levels of metabolites in complex matrices. Liquid chromatography tandem mass spectrometry (LC-MS/MS) has imposed itself over the years as the gold standard to tackle such a complex task with its ever-increasing sensitivity and elucidation capabilities. However, despite the democratization of high-resolution mass spectrometry (HRMS), ion mobility and orthogonal dissociation techniques such as ultraviolet photodissociation (UVPD) or electron-activated dissociation (EAD), the complete structure elucidation of certain classes of compounds or structural isomers solely by LC-MS/MS remains challenging. Nuclear magnetic resonance (NMR) spectroscopy is predominantly used to unravel the structure of metabolites impervious to mass spectrometry structural elucidation, but its main limitation is the need for large amounts of purified material (typically in the microgram range) which is time consuming and labor intensive to obtain.

Infrared ion spectroscopy (IRIS) combines the sensitivity and specificity of mass spectrometry with the structural identification capabilities of infrared analysis. In IRIS, ions confined within an ion trap are exposed to tunable infrared light. Varying the frequency of the light and observing the subsequent photodissociation of the ions allows us to gain insights into their vibrational modes and, consequently, their structural features.

We investigate the capabilities of IRIS for the analysis of same class compounds and structural isomers of small hydrocarbon molecules on a benchtop setup comprised of an AmaZon speed ETD quadrupole ion trap mass spectrometer (Bruker) combined with an optical parametric oscillator (OPO), with a power of 2000 mW and a repetition rate of 30 kHz (LaserSpec) in the mid-IR region. In the range provided by the laser (2800 to 3700 cm-1), C-H, O-H and N-H stretching bands are observable, a practical range to study small pharmaceutical compounds and their metabolites.

P21- Probing the onset of TDP-43 aggregation using Native Mass Spectrometry.

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Aggregation of disease-specific proteins is the common pathological hallmark of most neurodegenerative diseases. Studying protein aggregation and the structure of related proteins is not only important for the understanding of disease mechanisms, but can also contribute to the mapping of pathological events and the development of diagnostic tools. Recent studies have shown that the early-stage aggregates (oligomers) significantly contribute to the cytotoxicity of neurodegenerative diseases. Therefore, we investigate conformational changes and oligomer formation to better understand the processes underlying the disease mechanism.

In our study, we focus on TDP-43, a disease-specific protein that is involved in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia-TDP (FTLD-TDP). Aggregation of TDP-43 is the hallmark of these diseases, but they are also characterized by the presence of modified and truncated proteins. Protein fragments and post-translational modifications are increasingly demonstrating their involvement in the toxicity of neurodegenerative diseases. Therefore, we focus on the early stages of oligomer formation of full-length TDP-43 and its truncated and modified forms using native mass spectrometry.

Here, we employ mass spectrometry (MS) combined with ion mobility (IM) and liquid chromatography (LC). MS enables us to directly observe proteins and oligomers and set the first steps by detecting intact and native TDP-43. IM-MS allows us to better observe the initial oligomerization states such as dimers, which helps to separate and visualize different oligomers and to track their formation over time. To be able to do this, we first are developing suitable native LC-MS and nano-ESI MS methods using a set of proteins of similar size, such as myoglobin, protein A and ß2-glycoprotein 1. We assess the potential of different ESI ionization sources and separation modes (in LC-MS) using myoglobin and protein A. The latter is used for further optimization of this methodology as a model protein for TDP-43. ß2-glycoprotein 1 is used to develop ion mobility methods. To date, the developed methodology enabled the detection of intact TDP-43 and low-order oligomeric species.

With the workflows in place, TDP-43 and its truncated forms can be studied under native and aggregated conditions. The aim of this research is to provide new insight into the early-steps of the aggregation process of TPD-43 and to unravel the role of protein fragments and post-translational modifications in the formation of oligomers.

P22- Chemical derivatization of polar compounds for analysis in volume-limited biological samples using LC-MS/MS

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The analysis of extracellular brain metabolites by liquid chromatography (LC) approaches is an important field of research as it gives information about the release and re-uptake of these molecules by different cells. Imbalances in extracellular metabolites are often seen in neurodegenerative diseases such as epilepsy. Microdialysis is an *in vivo* sampling technique for interstitial brain fluid which does not require much sample preparation before LC injection. To investigate rapid changes in the levels of neurochemicals, often a high (spatio)temporal resolution is desired, resulting in low-volume microdialysates. Moreover, the compounds of interest are usually present in low concentrations and possess a wide range of physicochemical properties. We are developing a method using sample derivatization with dimethylaminophenacyl bromide (DmPABr) to increase the retention of polar compounds in a reversed-phase LC assay coupled to tandem mass spectrometry (MS/MS) and to allow the analysis of all compounds in electrospray positive mode. DmPABr reacts with carboxylic acids, primary and secondary amines and thiols. As a result, it allows the analysis of a wide range of compounds in one run in these volume-limited samples.

Experiments were carried out on a miniaturized Acquity UHPLC system coupled to a TQ-XS triple quadrupole system (both Waters), which was operated in electrospray positive mode. The system was equipped with an IonKey source which integrates the analytical column (BEH C18 iKey™ separation device (130 Å, 1.7 µm, 150 µm x 50 mm), Waters), kept at 60°C and an electrospray ionization probe. A gradient program was used, with a flow rate of 3 µL/min. Mobile phases A and B consisted of 0.1% formic acid in water and acetonitrile, respectively. The sample injection volume was 1 µL.

Using a mixture of 8 compounds, i.e. glutamate, γ-aminobutyric acid (GABA), cysteine, spermidine, lactate, butyrate, ornithine and kynurenic acid, LC and MS parameters and the derivatization reaction were optimized. Selected reaction monitoring (SRM) parameters, including precursor and product ion, cone voltage and collision energy were first optimized for all compounds. For each compound, a quantifier and qualifier transition were selected. The quantifier transition always had a product ion with an m/z of 134.1, while we searched for a unique product ion for the qualifier transitions. The optimal capillary voltage was 3.0 kV. Subsequently, some parameters of the derivatization reaction were optimized. Currently, we are evaluating the use of an isotope labelled form of the derivatization reagent as internal standard and the usage of a 'greener' solvent for mobile phase B. After optimization of these parameters, our list of compounds will be expanded with more neurochemicals and validated for the quantification of these compounds in mouse brain microdialysis samples.

P23- Enhancing microflow LC-MS/MS analysis of neuromedin U through reduced aspecific adsorption

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Quantifying neuropeptides in biological samples from preclinical studies is often challenging due to their low concentrations (low picomolar (pM) to femtomolar (fM) range) in volume-limited samples, even for sensitive hyphenated mass spectrometry (MS) methods. Sensitivity is further limited by aspecific adsorption of the target molecules. Peptides are prone to bind non-specifically to the surfaces of tubes, vials, pipette tips and the tubing of the LC system, which impedes accurate quantification. In this study, a microflow ultra-high performance liquid chromatography system coupled to tandem mass spectrometry (UHPLC-MS/MS) (Waters AQUITY UPLC M-class system with CSH C18 130Å 1.7µm 150µm x 50mm iKey separation device coupled to Xevo TQ-XS) was used in multiple reaction monitoring (MRM) mode to optimize a method to quantify mouse neuromedin U (NmU). NmU is a 2.7 kDa neuropeptide belonging to the neuromedin family that is involved in the stress response. Different approaches to combat aspecific adsorption of NmU at the level of sample preparation and analysis were investigated.

In order to prevent aspecific adsorption, solvents with varying water and organic modifier compositions were used to dissolve the lyophilized NmU standard and to perform further dilution steps. The highest responses were obtained by dissolving NmU in H_2O/Acetonitrile/Formic Acid (70:30:0.1 v/v/v) followed by making dilutions with the same solvent composition. The signal could further be increased by adding 10% (v/v) of both acetonitrile and formic acid to the sample in Quanrecovery UHPLC vials before injection. Prior to the analysis of samples, it is crucial that the LC system is passivated by repeatedly injecting a 40 µg/mL bovine serum albumin (BSA) solution, which acts as an adsorption competitor to NmU, saturating all non-specific binding sites within the system.

After the optimisation of the sample preparation, the UHPLC conditions (mobile phase gradient, column temperature and trapping flow rate, temperature and duration) were investigated to further increase sensitivity and limit carryover. A gradient with an initial fraction of organic modifier of 5% while keeping column temperature at 45°C resulted in the best signal. For trapping on the M-class Trap Symmetry C18 100Å 5µm 300µm x 50mm trapping column, a flow rate of 10 µL/min and a trapping volume of 30 µL were selected. Using the optimized method, the limit of detection in water was found in the 100 pM range. Finally, microdialysis samples were spiked with NmU at different concentrations to compare the sensitivity in water and in microdialysate. In microdialysis samples lower peak areas were consistently found, showing that further developments are required to measure NmU in *in vivo* samples.

P24- Workflow development for the characterization of neurofilament light chain (NfL) by LC-MS/MS for clinical applications

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Introduction: Neurofilament light chain is a 68kDa protein biomarker used in clinics to assess axonal degradation, as it is known to be increased in patients with neurological disease, in cerebrospinal fluid (CSF) and in blood. The degradation process of this protein is not fully understood, and most rapid detection methods rely on immunoassays, leading to cross-reactivity.

Objectives: The aim of our experiments was to develop a characterization method for NfL in a model using digested standards and CSF, with or without immunocapture, using mass spectrometry.

We focused first on developing and optimizing a sample preparation method that would deliver the best possible analysis performance.

Methods: A NanoACQUITY UPLC system coupled with a SYNAPT XS instrument operating in positive ion mode was used. All data were acquired using UDMSe acquisition mode and processed using Progenesis QI for proteomics and PEAKS DB.

To optimize the sample preparation, we assessed the best concentration ratio between NfL and trypsin, and the matrix composition for simulation models using standard without immunocapture. We then carried out immunoprecipitations using Dynabeads Protein G kit, adapting their data sheet, with on-beads digestion, followed by digestion prior to capture.

Results: The best performances were obtained with 10% ACN and 1% BSA spike matrix as well as high NfL standard concentration, from 1 up to 10 µg/ml. Several trypsin ratios showed good results, we selected 1:2 and 5:1 ratios of trypsin to NFL for our following manipulations. Further experiments directly on patient CSF enriched with high-concentration NfL are in progress.

This method shows definite potential for identifying NfL in a diagnostic and clinical setting. An assay method is conceivable and under development.

P25- Multiplexed Quantitative Proteomics Ac-IP Tag in Data-independent Acquisition Mode

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Introduction: Data-independent acquisition (DIA) has become increasingly important for quantitative proteomics. However, most current isotope labelling strategies such as TMT are not suitable for DIA, as they lead to more complex MS2 spectra or severe ratio distortion. As a result, DIA suffers from a lower throughput than data-dependent acquisition (DDA) due to a lower level of multiplexing.

Methods: We synthesized an isotopically labelled acetyl-isoleucine-proline (Ac-IP) tag for multiplexed quantification in DIA. Protein digestion is performed with LysC to ensure the presence of a Lys residue at the C-terminus of every peptide. Dimethylation is used to block the N-terminus and simultaneously improve ionization properties. The Ac-IP tags are coupled to the amino-group of Lys through NHS chemistry. Differentially labelled peptides have distinct precursor ions carrying the quantitative information but identical MS2 spectra, since the isotopically labelled Ac-Ile part leaves as a neutral loss upon collision-induced dissociation, while fragmentation of the peptide backbone generates regular fragment ions for identification. The Ac-IP labelled samples can be analyzed using general DIA LC-MS settings and the data obtained can be processed with established approaches. Relative quantification requires deconvolution of the isotope envelope of the respective precursor ions.

Results and conclusions: Suitability of the Ac-IP tag is demonstrated with a triplex-labelled (+0, +1 and +2) yeast proteome spiked with bovine serum albumin (BSA) that was mixed at 10 : 5 : 1 ratios resulting in measured ratios of 9.7 : 5.3 : 1.1. A 10-plex tag (+0 until +9) was synthesized next, and labelling and deconvolution are demonstrated on the peptide level. This tag promises to greatly expand the multiplexing possibilities for DIA-based proteomics.

P26- Exposing the Exposome: Global Plasma Profiling with LC-MS

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The term “exposome” describes all the environmental factors influencing our health. Environmental factors play a crucial role in many diseases such as cancer, diabetes and cardiovascular diseases. Since we can adapt most of our environmental exposures, understanding the exposome will be essential to evolve preventative medicine. Therefore, the Exposome-NL project aims to link specific exposures to metabolic pathways, changes in the gut microbiome and disease onset. Blood analysis using liquid chromatography coupled with mass spectrometry (LC-MS) can provide the information to link environmental exposures to biological adaptations. However, most studies into the exposome have only focused on one or a couple of compound classes while our environment is complex. Therefore, an analytical platform that can measure compounds from multiple classes relevant to exposome research could enhance our understanding of the exposome. Here we propose a global approach to analyze apolar exposure markers in plasma, such as PFAS, pesticides and drugs, using LC coupled with high-resolution mass spectrometry. A limited sample preparation procedure would be preferred, to get the most complete overview of the influence of our environmental exposures. However, this leads to a build-up of phospholipids and signal suppression of exposure markers. Therefore, using a hybrid SPE plate with zirconia-coated silica, phospholipids were depleted before LC-MS analysis of the plasma. The removal of phospholipids prevented the need for sample dilution and thereby improved the method’s sensitivity. The developed method will be applied to measure more than 3000 blood samples from multiple cohorts to generate a reference exposome dataset of the Dutch population within the Exposome-NL program. Together with other data acquired within the Exposome-NL project the database will be used to get an insight into the biological implications of the exposome.

P27- Comparison of sample preparation for determination of mycotoxins in cannabis and derived products using LC-FLD and LC-MS/MS.

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Due to the potential healthcare benefits of cannabis and hemp, the market for this plant and its products have increased dramatically in the last years. In several states cannabis is already legal for medicinal and/ or recreational use. Quality control before human consumption e.g., the determination of pesticide residues and mycotoxins in cannabis biomass and its derived products, is therefore mandatory. The number of regulated pesticides varies dependent on state/country/region. The U.S. Food & Drug Administration (FDA) sets a limit value of 20 ppb for mycotoxins in human food and animal feed. Similar but also lower values depending on the matrix are set by Commission Regulation (EU) 2023/915. Organizations like AOAC are developing method requirements for mycotoxin determination in cannabis matrices. AOAC SMPR® 2021.010 defines aflatoxins B1/B2, aflatoxins G1/G2 and ochratoxin A as analytes of interests and specifies limits of quantification and qualification for cannabis biomass and cannabis derived products. Real samples were mixed with a standard containing the mandatory aflatoxins (B1, B2, G1, G2), ochratoxin A and zearalenone. Sample preparation was carried out and mycotoxins were separated under RP-HPLC conditions.

Four different samples: hemp pellets, hemp seeds, commercially available hemp flour, and hemp oil were investigated using different sample preparation procedures: solid liquid extraction (SLE)/liquid-liquid extraction (LLE); a standard QuEChERS extraction with dispersive cleaning; SLE/LLE with following CrossTOX cleanup; SLE/LLE and solid phase extraction using immunoaffinity columns (IAC SPE). The results are investigated and evaluated in terms of time, costs per sample, solvent consumption, and achievement of limit values.

The most chosen detector for mycotoxin determination is the mass spectrometer (MS). The regulations are met easily but due to the complexity of an LC-MS system, the operation can be challenging. Therefore, fluorescence detection (FLD) is investigated as an alternative detection method. The results of LC-FLD and LC-MS/MS measurements are compared regarding the achievement of valid limit values as well as handling/user-friendliness of the detectors.

P28- Exploring chromatographic modes hyphenated to MS for improved characterization of phosphorothioated oligonucleotides.

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Oligonucleotides (ONs) are short strands of DNA or RNA that can be used for the treatment of diseases that are difficult to target using traditional small molecule drugs. ONs are produced via a repeating synthesis route, where each successive addition of a nucleotide has a success rate of approximately 99%. Consequently, a synthesized oligonucleotide product consisting of e.g., 20 bases has a purity of around 80%. For quality control, characterization of the synthesized drug substances is imperative.

There is an expectation that either two orthogonal denaturing methods, e.g., anion exchange chromatography (AEC) and ion-pairing reversed-phase liquid chromatography (IP-RPLC) with UV absorbance detection, or a single (IP-RPLC) LC-UV method hyphenated with mass spectrometry (MS) are utilized to assess ON purity. A prevalent method for pharmaceutical ON characterization is the Ionis method, which employs ion-pairing reversed-phase liquid chromatography (IP-RPLC) for separation, and is hyphenated to UV absorbance and single quad mass spectrometry (MS) detection for quantification and assignment of impurities. With this method, many impurities co-elute with the full-length product (FLP) and are distinguished by their mass only. Assuming equal ion suppression, the impurities can be relatively quantified against one common standard. Moreover, due to the used ion-pairing reagents (tributylamine acetate), the ON charge state distribution is narrow, increasing sensitivity. However, the method is typically less suited for quantifying impurities eluting before and after the FLP peak, and lacks the ability to differentiate deaminated impurities (1 Da difference). Additionally, the ion-pair reagent causes additional adduct formation, increasing the complexity of the mass spectra.

In the InnovATOR project, we aim to improve the separation and detection of ON and their respective impurities. Our current research involves the study of various LC selectivities that are compatible to MS. We aim to increase separation selectivity for e.g., the (n-1) shortmer(s), (n+1) longer(s), PS-PO impurities, deamination and/or PS diastereoisomers. Ultimately, these IP-RPLC and a complementary LC mode could be combined in a two-dimensional LC system hyphenated with MS, increasing the number of identifiable and quantifiable impurities. We explored IP-RPLC, hydrophilic interaction chromatography (HILIC), and mixed-mode chromatography, focusing on their ability to separate impurities from a fully phosphorothioated (PS) antisense ON. Promising results for HILIC-MS methods are presented, in particular demonstrating their aptness in giving insight into the diastereometric distribution of PS ON.

P29- A combined targeted-untargeted metabolomics workflow for identification and quantification of metabolites in complex samples

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Metabolites are chemically and structurally diverse compounds that cover a wide dynamic concentration range, which makes the metabolome challenging to characterize. Mass spectrometry can analyze this range of molecules within a complex biological sample. Data-dependent acquisition (DDA) is a commonly used analytical technique for untargeted metabolomics but does not provide accurate quantitation. Untargeted methods also use default parameters, such as collision energy, which means many compounds are sub-optimally analyzed. As the purpose of processing data is often to identify and quantify all metabolites within a sample accurately, high-quality MS/MS spectra are required to achieve these goals. The ZenoToF 7600 system is a uniquely designed instrument that enables high sensitivity and fast MS/MS acquisition speeds (133 Hz), providing significant gains in MS/MS sensitivity and improving MS/MS spectral quality. This novel workflow provides reproducible quantitation and low-level assay sensitivity by targeted data acquisition, as well as spectral matching with higher confidence and greater metabolite coverage than untargeted, DDA analyses. The system also offers electron-based fragmentation (electron-activated dissociation; EAD) that compliments traditional collision-induced dissociation (CID) based fragmentation The addition of high-energy, electron-activated dissociation (EAD) enables further characterization, increasing confidence in metabolite identification. A combination of these approaches, on the ZenoToF, improves data quality and confidence, increasing the reliability of biological interpretation.

P30- Mass Spectrometry imaging visualizes the different steroid hormones of growing follicles and developing corpora lutea in mature minipig ovaries

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The ovarian cycle involves the formation, growth, and ovulation of ovarian follicles and their transition to terminally differentiated structures called *corpora lutea*.[1-3] Steroidogenesis is the complex multienzyme process by which cholesterol is converted to biologically active steroid hormones. The ovarian steroidogenesis starts in the theca cells of the growing follicles, where cholesterol is converted to androgens and is completed by granulosa cells that convert androgens to estrogens. Following ovulation granulosa cells undergo a process of lutheinization which involves cell enlargement and increased progesterone production. These lutheinized granulosa cells eventually form the prominent progesterone-producing layer of the corpora lutea. The ovarian follicular cell proliferation and differentiation is affected by the circulation of different levels of steroid hormones (estrogens, progesterone, androgens…) in the follicular fluid and adjacent cells such as granulosa and theca cells.[4] Monitoring the distribution of this complex network of hormone steroids in ovaries will reveal details on the ovarian follicular growth as well as the maturation and development of corpora lutea, giving us better understanding of underlying processes and potential application in hormonal related safety endpoints of toxicity studies.

Recently, Mass Spectrometry Imaging (MSI) has been applied to monitor different steroid hormones in human adrenal gland tissue sections.[5] In our study, we monitored the tissue distribution of several hormone steroids on mature minipig ovaries to follow the ovarian follicular growth and development of corpora lutea. On-tissue derivatization of the steroid hormones using Girard's T reagent was performed prior to MALDI matrix spraying to increase their sensitivity.[5] The MALDI MSI experiments were carried out on a Bruker TimsTOF flex MALDI-2, taking advantage of the trapped ion mobility separation to differentiate isobaric species. Different steroid hormones, like e.g. estrogen, androgen, progestin, progesterone and their precursor cholesterol were identified and could potentially be linked to the maturation of follicles and subsequent development of corpora lutea.

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P31- Overcoming solvent effects at high injection volumes in PFAS analysis

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The analysis of per- and polyfluorinated alkyl substances (PFAS) often requires sample preparation techniques like solid phase extraction (SPE), especially in case of drinking water analysis with low detection limit requirements. The final samples ready for LC/MS/MS analysis are therefore usually dissolved in 80-100% organic solvents.[1] Additionally, recommended drinking water concentration limits are getting even lower, lately.[2] Injecting high sample volumes could improve sensitivity and therefore allow lower detection limits but this is limited by undesirable solvent effects caused by the high elution strength of the sample solvent in case of common reversed phase liquid chromatography.

Here we present how the use of feed injection, as an alternative injection principle to the common flow through injection, allows much higher injection volumes without negative impact on the peak shape, even when the sample is dissolved in 100% methanol. This is achieved by infusing the sample into the mobile phase stream with a special valve resulting in a dilution.[3] The use of a novel C18 reversed phase column designed to be compatible with a 100% aqueous mobile phase during the injection helps to maximize this effect.

Combining both technologies, we were able to achieve an increase of the peak intensities by a factor of 6 to 8 for most of the commonly analyzed PFAS and still a factor of more than 2 in case of short-chain PFAS, with samples dissolved in Methanol.[4]

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P32- Determination of Over 500 Pesticides in Cinnamon by EMR–GPD passthrough clean-up and a Novel Triple Quadrupole LC/MS system

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Multiresidue methods, capable of simultaneous analysis of over 500 pesticides by LC-MS/MS, is rapidly gaining popularity due to the cost, time, and labor efficiency. However, the large panel analysis poses more challenge for analyte identification when a pesticide residue is detected especially at low concentration levels and/or in highly complex matrices. An end-to-end workflow for the extraction and more efficient clean-up, screening, identification, and quantitation of over 500 globally important pesticides by a novel triple quadrupole LC/MS system (Agilent 6495D) was developed and validated. Cinnamon powder, which represents one of the most challenge matrices with high complexity and high pigment concentration, was selected to establish the workflow. The workflow addresses challenges of analyte identification and prevents false positive and/or false negative.

Organic cinnamon powder free of the targeted pesticides was prepared using extraction with QuEChERS AOAC extraction kit, followed by passthrough cleanup with Enhanced Matrix Removal– General Pigment Dry (EMR–GPD). The extracted samples were analyzed on a sensitivity-enhanced and intelligent triple quadrupole LC/MS system in both positive and negative electrospray ionization. This new mass spectrometer has enhanced sensitivity and due to the new ion funnel technology it is able to deliver high precision at sub-millisecond dwell times. Additionally, system intelligence is provided with early maintenance feedback (EMF), SWARM Autotunes and intelligent reflex workflows.

The developed workflow enables the analysis of over 500 relevant pesticides in highly complex cinnamon powder in a short analysis time (analyte elution in 12 minutes). The online sandwich injection program was applied to ensure excellent peak shapes of early eluting (more polar) analytes. The method was evaluated in terms of matrix removal; target recovery, reproducibility, and matrix effect; and matrix-matched calibration curve linearity and limits of quantitation (LOQs) in cinnamon. The SANTE guidelines (SANTE/11813/2017) for analytical quality control and method validation procedures were followed to meet the following identification criteria: retention time within ±0.1 minutes, ≥2 product ions, and ±30 % maximum relative tolerance for ion ratios.

In routine practice, especially when analyzing highly complex samples, preventing false positive (caused by matrix interference) or false negative results (caused by un-detection or incorrect ion ratio) is quite challenging. The novel and intelligent LC/TQ system along with passthrough cleanup for matrix interference removal provide high sensitivity, selectivity, and speed for the determination of a large number of pesticides at low concentration levels even in highly complex matrices, leading to improved identification confidence and potential false positive/false negative prevention. Therefore, fast decision can be made on accepting or rejecting the results.

P33- INVESTIGATION OF INFLAMMATION AND OXIDATIVE STRESS IN METABOLICALLY COMPROMISED OLDER COMMUNITY DWELLING PARTICIPANTS OF THE LEIDEN LONGEVITY STUDY COHORT

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INTRODUCTION The ageing population is facing an increase in late-life diseases. With the increasing human life expectancy, this becomes a global burden to current health care systems. One of the determinants of physiological vulnerability of older individuals is based on the decline of immune-metabolic health contributing to late-life disease such as diabetes, cardiovascular disease, hypertension, and obesity. Such decline is hypothesized to result from systemic inflammation, oxidative stress, and adipocyte dysfunction. Previously a score was constructed from 1H NMR-based metabolomics data in 44.000 individuals across all ages that predicts all-cause mortality and frailty among other endpoints (MetaboHealth score). This score is currently validated to indicate vulnerable older adults in the clinic, in population health and lifestyle intervention studies in the Vitality Oriented Innovations for the Lifecourse of the Ageing Society (VOILA) consortium.

METHOD Mass spectrometry-based metabolomics is a powerful tool to study the biochemistry behind inflammation and oxidative stress by analyzing pro- and anti-inflammatory metabolites. We have developed a targeted UHPLC-MS/MS method which enables profiling of these inflammatory markers in plasma of older adults. The study consisted of two groups of 20 older adults from the Leiden Longevity Study cohort with high and low MetaboHealth scores. EDTA plasma samples were collected from about 350 participants from the cohort. Metabolites were measured by LC-MS/MS after a liquid-liquid extraction. Internal standards were used to acquire relative metabolite concentrations. Quality control samples were replicated throughout the batch and used to assess data quality. Univariate and multivariate statistical analyses were performed to reveal differences and trends between the groups.

RESULTS AND DISCUSSION Metabolites from the classes oxylipin, endocannabinoids, and bile acids have significant differences between the high and low MetaboHealth scoring groups. In addition, ratios of precursor metabolite to derivative revealed differences in enzymatic activity between the groups. These results could improve the understanding of systemic inflammation and oxidative stress in metabolically compromised older adults.

CONCLUSION This study provides biochemical insight of inflammatory metabolites to facilitate further ageing research and to support potential lifestyle and therapeutic interventions in the VOILA consortium.

P34- Odour Characterisation of Poultry Red Mites by TD-GC-MS for Early Detection of Infestations in Laying Hen Farms

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The poultry sector is an important livestock sector in the Netherlands, with nearly 100 million chickens kept for meat or eggs in 2021. Most poultry farms are intensive farming operations at large scale, with sometimes hundreds of thousands of birds per farm. This makes the animals vulnerable to health problems. Infestation of laying hen poultry farms by the poultry red mite (*Dermanyssus gallinae*) is one such widespread problem that negatively impacts animal welfare and egg production, with an estimated financial impact of more than 230 million euros per year in Europe. These infestations often go unnoticed until large population sizes are reached. Detection of *D. gallinae* infestations in laying hen farms in an early stage is important for effective pest control and to minimize negative impacts. Impaired poultry health can lead to lower production efficiencies, increased greenhouse gas emissions, higher odour emissions and decreased chicken health and welfare.

Volatile organic compounds (VOCs) as specific biomarkers for diseases or infestations have been used for non-invasive monitoring of infestations in animals and crops. Collection of VOCs requires minimal sample preparation and allows for non-invasive, direct monitoring compared to biomarkers that must be extracted from tissues or fluids. The use of VOCs as biomarkers for infestation has not yet been reported for *D. gallinae*.

Here, samples of *D. gallinae* and litter were taken in commercial poultry farms and transferred to the laboratory in polystyrene (Sterilin™) containers. VOCs were subsequently collected from the headspace onto dual-bed sorbent tubes and analysed using thermal desorption - gas chromatography - mass spectrometry (TD-GC-MS). Furthermore, the VOC profiles of *D. gallinae* from different poultry farms were compared to find specific molecular profiles indicative of infestation. Principal component analysis (PCA) distinguished the VOC profiles from *D. gallinae* and litter samples collected in the stable. Characteristic VOC profiles were identified for *D. gallinae* across the investigated laying hen systems. Preliminary results indicate that *D. gallinae* presence is characterised by enhanced levels of aldehydes and nitrogen compounds. Further investigations are required to validate identified VOCs as biomarkers for monitoring of *D. gallinae* infestations.

Key words (max 5): poultry, Dermanyssus gallinae, volatile organic compounds, thermal desorption - gas chromatography - mass spectrometry (TD-GC-MS)

P35- Ion mobility spectrometry of polymer ions : impact of the monomer unit rigidity on the internal structure of polystyrene ions

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Some natural molecules show outstanding catalytic activity and specificity, for examples biomolecules and enzymes. The most important factor enabling their efficient catalytic activity and specificity is their unique three dimensional (3D) structural orientation, providing a highly specific local environment that enables very high reactivity and selectivity. Mimicking the precision chemistry of nature, single polymer chains intramolecularly collapsed into nanoparticles are promising candidates for biomimetic materials as well as for unravelling the folding processes of proteins. Therefore, we have previously taken inspiration from biomolecules as well as their catalytic activity and prepared metallo-folded single chain nanoparticles (SCNPs).[1] Here, metal centers enable the formation of SCNPs as well as act as a catalytic center.[2] SCNPs can exhibit variable reactivity depending on the number of catalytic centres and their structural embedding within the nanoparticle. A deep understanding of the relationship between structure and activity of individual SCNPs would thus be of utmost interest to target promising chain architectures.

Establishing the 3D structure of SCNPs with conventional methods remains challenging due to their intrinsic complexity and dynamics of the folding/unfolding processes. The current study combines ion mobility mass spectrometry with molecular dynamics[3] (MD) in order to characterize the folding and 3D structural orientation of polystyrene(PS)-based SCNP containing hydrophilic and hydrophobic end groups. Upon electrospray ionization, the PS-SCNPs mostly appear as doubly-charged cations (H^+ and Ag^+). Comparison of the experimental and theoretical collision cross sections suggests that the gaseous PS-SCNPs di-cations fold into eclipsed/egg-shaped structures rather than spherical forms, due to the rigidity of the polymer backbone. MD simulations give a fine atomistic description of the role of charged centers in the folding of the particles in the gas phase.

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P36- Comprehensive characterization of ADCs by intact protein analysis and an electron activated dissociation based peptide mapping

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This poster highlights streamlined intact protein analysis workflows to determine the average drug-to-antibody ratio (DAR) of trastuzumab emtansine (T-DM1) using liquid chromatography-mass spectrometry (LC-MS) and imaged capillary isoelectric focusing (icIEF)-UV/MS analyse. In addition, an electron activated dissociation (EAD)-based peptide mapping workflow was leveraged to provide confident identification of DM1-containing peptides and accurate localization of the payload in these peptides. EAD also generated DM1-specific fragments for the confirmation of the payload. Taken together, these workflows provide viable analytical solutions for the comprehensive characterization of antibody-drug conjugates (ADCs) with increasing variety and complexity.

P37- Development of a MALDI-Mass Spectrometry Imaging method to understand the necrotic mechanisms of snake envenomation.

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With an estimated 81,000 to 138,000 deaths per year, snakebite envenomation has been considered a neglected tropical disease by the UN since 2017. The aim of this method is to exploit the formidable capacity of MALDI mass spectrometry imaging (MALDI-IMS) to understand the mechanisms of tissue alteration and necrosis that can occur after envenomation.

Snake venom is a complex mixture of various toxins, mainly peptides and proteins, and enzymes. The toxins are highly selective, generally attacking the nervous and neuromuscular systems. Some enzymes can cause local tissue necrosis, which is still poorly described and has no real treatment. The cytotoxicity responsible for tissue alteration and necrosis is mainly caused by two families of protein toxins: PLA_2 (Phospholipases A_2) and SVMP (Snake Venom Metalloproteinases)

PLA_2 (10-20 kDa) cleave the cell membrane, inducing necrosis at the bite site. SVMP (20-100kDa) act, among other things, by hydrolyzing basement membrane proteins and altering cell adhesion to extracellular matrix.

The aim of this method is to use MALDI-IMS to visualize the action of (i) synthetic or purified toxins such as PLA_2 and SVMP, (ii) crude venoms and (iii) HPLC-separated venoms on murine tissues to induce tissue alteration or necrosis. The ultimate aim is to increase our knowledge of necrosis mechanisms, enabling us to better understand them and thus pave to way for new treatments.

P38- PTR-MS profiling of exhaled breath reveals mechanistic insights into cognitive performance in children

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The Central Nervous System (CNS) and gut microbiota are interconnected through the gut-brain axis, influencing digestive function, immune response, metabolism and neurological health. Diet significantly impacts this relationship, with certain foods promoting beneficial gut bacteria, which in turn affect the CNS. The goal of this exploratory study was to assess the relation between the metabolic profile reflected in exhaled breath, dietary quality for children with various diet habits and their abilities to perform cognitive tasks.

Children between 8 and 11 years old (N = 31) were recruited via flyers at schools and after-school care and subsequently invited for a visit to the Donders Institute for Brain, Cognition, and Behaviour. Parents were asked to fill a Food Frequency Questionnaire (FFQ) to assess the dietary quality using the Dutch Healthy Diet (DHD) index. During the visit, children performed a Flanker Task, a common test for assessing cognitive functioning where the output variables are the error rate and reaction time. Afterwards, duplicate breath samples were collected in 3-L Tedlar® bags and analyzed using proton transfer reaction – mass spectrometry (PTR-MS) for untargeted breath profiling. In addition, laser-based spectroscopy was used to determine the concentrations of ethylene and methane as these are relevant volatile organic compounds (VOCs) in relation to inflammation and the gut microbiome, respectively.

To find indicative metabolic signatures of cognitive performance, partial least squares (PLS) regression was used where breath volatile organic compounds (VOCs) and macronutrient intake served as independent variables and the error rate and reaction time as dependent variables. Selection of important features was done using the significant multivariate correlation (sMC) metric ($\alpha = 0.05$). The models found several VOCs linked to the gut microbiome (butyrate, methanol, ethanol, and methane) to be responsible for a decrease in reaction time (i.e. faster response). The VOCs that can be linked to inflammatory response showed to increase the error rate (i.e. worse cognitive functioning). These preliminary results indicate the use of breath analysis in children emerges as a valuable tool for monitoring dietary habits and the impact of diet on spatial cognition development.

P39- Analyzing Chemical and Morphological Attributes of Plant-Based Meats via MS Imaging for improved consumer acceptance

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Considering environmental concerns related to conventional mass meat production, this study utilizes cutting-edge Mass Spectrometry Imaging (MSI) techniques to comparatively analyze and study plant-based meat alternatives. We investigated the molecular profiles and their spatial distributions of these alternative proteins with Matrix-Assisted Laser Desorption/Ionization (MALDI)-MSI, as well as supportive techniques such as Desorption ElectroSpray Ionization (DESI)-MSI, Liquid Chromatography Mass Spectrometry (LC-MS). Various types of meat samples are procured, derived from plants and animals, in both cooked and uncooked forms. These samples have undergone MALDI-MSI for the primary analysis, with additional methods used to augment and confirm findings.

One of the pivotal aspects of the methodology includes the application of on-tissue tryptic digestion on selected thin sections of samples, enabling a detailed proteomics-focused analysis. This approach overcomes reveals the spatial distribution of proteins and peptides and offers critical insights into the composition and molecular profiles of different plant-based meat types. Peptide identities were investigated with LC-MS.

Advanced image registration and machine learning algorithms are applied to the MS and Raman imaging data to elucidate molecular variations, particularly focusing on sensory attributes like flavor with MS-MS exact mass measurements aid in their identification.

The study's significance lies in its potential to better understand consumer acceptance of sustainable meat alternatives by bridging the sensory gap between plant-based products and traditional meats. By combining environmental conservation, public health, and cultural adaptation, this research aims to contribute to a sustainable food system. Furthermore, the project underscores the innovative application of MSI in food science, a relatively unexplored domain, promising to set new standards in flavor analysis and food quality. The combination of a rapid, non-destructive (spatial) spectroscopic technique with MSI could facilitate real-time monitoring of sustainable meat alternatives in a process analytical technology framework, streamlining quality control and adulterant analysis to ensure product integrity and consumer safety.

P40- From the data onto your plate
Challenges while gathering yummy insights

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Sensory analysis can be defined as the measurement of both the flavor and several attributes using human senses. Sensory provides insights on the differences between products, however it does not reveal what is causing the differences. A Sensomics approach consists of combining the information provided by sensory with molecular data. Molecular data is obtained by profiling the chemical composition of a product, whereas sensory attributes are assessed following Quantitative Descriptive Analysis (QDA). Such a Sensomics approach results in challenges that are not uncommon in other profiling analysis using HR-MS, but which are more prominent due to the nature of the samples and products studied, the analytical measurement techniques and the sensory techniques.

This work focusses on the (un)targeted liquid chromatography platform that has been built in combination with high resolution mass spectrometry (LC-HR-MS) to characterize different food matrices. This platform is focused on the measurement and (semi-)quantitation of non-volatile compounds linked to flavor. Based on literature and in-house knowledge from flavorists, non-volatile compound classes related to flavor were selected. The strategy adopted to set up the platform was: a reverse phase chromatographic method (RPLC-HR-MS) to cover the non-polar compounds and two hydrophilic interaction chromatographic methods (HILIC-HR-MS) to cover the polar compounds.

The different challenges faced when covering a considerable wide range of compound classes with different polarities, data processing, (semi-)quantitation and compound annotation are tackled. The platform has been applied to create a flavor map of our process flavors portfolio. The performance of the platform on this study is discussed and several learnings are shared.

P41- Routine Pesticide Screening Solution: Data Independent Acquisition with a New LC/Q-TOF and Dedicated Screening Software

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Data independent acquisition (DIA) with high-resolution quadrupole time of flight (Q-TOF) facilitates complex pesticides analysis in various matrices with an ever-growing list of target compounds. Confident identification is made with a combination of high-resolution, extended dynamic range, stable accurate mass, and isotopic fidelity. Additionally, acquisition speeds with DIA support fragment ions for additional specificity. Non-targeted acquisition allows for retrospective analysis of emerging contaminants, not possible in the standard QQQ platform. The impact of the information rich data files can result in a large analysis time burden. However, the addition of novel screening software enables simple, fast, customizable, and thorough analysis of hundreds of suspects and targets in a succinct user interface.

Utilizing reverse phase chromatography on a high-speed binary pump, an All-Ions method was carried out with a novel LC/Q-TOF system (Agilent Revident). Broccoli matrix was extracted with QuEChERS sample preparation for pigmented fruits and vegetables and spiked with a pesticide mixture of over 200 compounds and 4 heavy labeled internal standards. A calibration curve was generated from 8 different concentrations ranging from 0.625 ng/ml to 100 ng/ml and each calibration level, matrix blank and solvent blank was injected in 6 replicates. The resulting data was analyzed using commercially available software and screened with the embedded screening software and utilizing a novel post-acquisition signal processing algorithm.

Over 200 compounds were identified in complex matrix in the calibration range. Analysis was completed in both positive and negative polarities allowing for maximum coverage of investigated compounds. The review of results was assisted by a commercially available MS/MS library, which was further customized through addition of retention times. The MS/MS information was imported into the screening software and included up to 8 qualifying fragments from the spectra. Utilizing effective heavy labeled internal standards, the majority of identified compounds showed good linearity and R^2 values above 0.99. The mass accuracy averages resulted in over 95% within a ±2 ppm window, independent of concentration (0.625 ng/ml to 100 ng/ml). At 5 ng/ml, over 85% of screened analytes had a relative standard deviation below 10% which increased to over 90% at 10 ng/ml. The culmination of results was analyzed simultaneously via the screening software. Here, as an example, at the 5 ng/ml calibration level of the 232 analytes screened for 183 of those were fully identified, 34 were selected for further review, and 15 not present. These classifications are assigned based on mass accuracy, fragment ion coelution score, isotope matching, retention time and number of verified ions.

P42- Advanced approaches for in-depth studies of toxin-antibody and toxin-ADDomer interactions.

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Snake envenomation constitutes a major health concern across Africa, Middle-east, Asia, and subtropical regions with rural populations primarily affected. Each year, between 80 and 138 thousand fatalities occur from snakebite envenomation and three times as many people with disabilities even after treatment. Snake venom is a complex mixture of various peptides and proteins called toxins. These toxins induced different biological effects, such as paralysis, necrosis, and also haemorrhage, leading to potent fatality. The only treatment available for envenomation is antivenom, which are serum containing Immunoglobulins G (IgGs) targeting venom toxins. These IgGs are purified from hyperimmunized horses or sheep blood. Even if antivenoms undeniably save lives, they also provoke drawbacks. Indeed, the purified serums contain toxin-specific IgGs but also IgGs from the immunogenic history of the animal. Unfortunately, these non-specific IgGs can trigger additional severe adverse effects for people already in vital emergency. In addition, antivenoms are heat sensitive and are stored far from the targeted population. To address public health concerns, the European ADDovenom project (2021-2024), funded by the European Commission (FET-Open), aims to develop a novel generation of antivenom based on ADDomers construct, ADDomers are thermally stable megadalton virus-like particles, produced at low cost and featuring 60 high-affinity binding sites.

In this work, we propose an innovative experimental protocol based on the use of magnetic beads to evaluate by mass spectrometry the efficacy of antivenoms. With this objective, magnetic beads are functionalized using antivenom IgGs then incubated with crude venoms. The supernatant, containing the non-recognized toxins, is collected and the toxins bound to the IgGs (and then to the beads) are eluted in acidic conditions (pH = 3). These two solutions (supernatant and eluate) are then analyzed for identifying and quantifying the immunorecognized toxins using LC-MS and shotgun bottom-up proteomics. This new method, based on magnetic beads and LC-MS(/MS) monitoring, makes it possible to use smaller quantities of solvents and valuable compounds such as venoms and antivenoms, which are of great medical importance.

P43- Automated workflow to study microsomal clearance and analysis of metabolites using collision-induced dissociation and electron-activated dissociation MS/MS data.

Bertram Nieland

Studies of *in vitro* metabolism of drugs in human and animal tissues help to predict the metabolic clearance rate of compounds and identify major metabolism pathways. For each study, metabolites identification is critical, end is often software-aided to ensure proper metrics are used for confident identification and prediction of the metabolism site. A software-aided methodology was developed to quantitatively study microsomal clearance and qualitatively identify the soft-spots for metabolism, aiding in the acceleration of the early drug discovery process. Datasets from collision-induced dissociation (CID) and electron-activated dissociation (EAD) were applied to predict the sites of metabolism.

P44- In depth-characterization of a mAb combined with a routine multiple-attribute methodology (MAM) using a novel fragmentation type

Alexandre Isabel

A complete qualitative and quantitative assessment is fundamental not only during quality control of any biotherapeutic product, but also during drug development. A streamlined approach is needed to obtain sequence information as well as type and localization of modifications. This qualitative analysis needs to be combined with a reproducible and accurate relative quantification of the quality attributes. Previous approaches were mainly based on peptide mapping with collision-induced dissociation (CID) for ID and a subsequent MAM leveraging the same data or MS only. Here, a novel fragmentation type based on ExD was investigated to combine information-richer fragmentation spectra and ID with an accurate MAM in one single injection.

P45- Specific determination of non-ionic surfactants in drinking-, waste- and surface water with high resolution mass spectrometry.

Dennis van den Heuvel

Shimadzu Benelux

Nonionic surfactants are surface-active compounds with hydrophobic and hydrophilic moieties. These surfactants do not ionize in aqueous solutions. Nonionic surfactants such as alkylphenol polyethoxylates (APEO), alcohol polyethoxylates (AEO) and alkanolamides are widely used in consumer products like, e.g., laundry detergents, cleaning and dishwashing agents, and personal care products. Nonionic surfactants are also widely used in cleaning agents formulated for the industrial and institutional sector. Surfactants released into the environment are biodegraded to short-chain ethoxylates, which are more toxic and more persistent than their parent compounds. Metabolites also exhibit estrogenic properties due to their structural similarity to estrogens. Therefore there is a considerable interest and public concern about the potential negative impacts of surfactants.

To 10 mL of drinking-, surface- or waste water is 10 mL acetonitrile added, followed by a filtration over a 0.45 µm membranefilter. From this mixture 5 µL is injected onto a C18 column (temperature 50 °C) and gradient analysis (0-0.5 min 25% B; 0.5-2.0 min linear gradient to 70%B; 2.0-3.0 min linear gradient to 100%B; 3.0-7.0 100%B; 7.0-8.0 linear gradient to 25%B) with high resolution mass spectrometry (NexeraX3/LCMS-9050, Shimadzu Corporation, Japan) was performed. A positive electrospray ionization scanning method (100 – 1000 Da, 10 Hz) was performed followed by automated data-analysis. For each surfactant a summation of XIC's with mass difference of 44 Da, corresponding to a single AEO or APEO are reported.

The addition of acetonitrile to the water sample prevents adsorbtion of the non-ionic surfactants to the membrane filter or debris in the water. Calibration curves were created from the automated summation of 16 XIC's for Tergitol NP-9 (APEO C9) and Tergitol TMN 10 (AEO C12). The group calibration curve from APEO C9 and AEO C12 (31.8 – 1000 µg/L) were used for semi-quantification of all short chain ethoxylates in the samples. For each surfactant the XIC was extracted and summarized according to the spectrum (regular mass distribution of 44 Da, corresponding to ethyleneoxide) and the total area was calculated. Linear regression was used for calculation and semi-quantification of the short-chain ethoxylates.. Both compounds showed good linearity (r2> 0.995). Method performance was evaluated with three different samples at different concentration levels for several surfactants resulting in positive identification. Further software method optimization, validation and comparison with existing methods is under development.

With this semi-automatic dataprocessing approach a costeffective and time saving systematic screening, identification and semi-quantification is developed.

P46- High-Throughput, Label-Free Cyclic Peptide Soft Spot Identification (SSID) by High-resolution Ion Mobility Mass Spectrometry

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Linear peptides therapeutics are often cyclized to increase half-life to improve efficacy and offer versatility in drug delivery. Improved pharmacokinetics (PK) opens the possibility for oral delivery of these therapeutics. The rate at which cyclized peptides are hydrolyzed by various proteases determines these PK properties. Identification of the first site of hydrolysis by biologically present enzymes, also known as the soft spot, is indicative of efficacy because once cyclic peptides are linearized, they are rapidly metabolized. If the soft spots of the therapeutic are identified, synthetic strategies can be employed to block sites susceptible to hydrolysis affording better PK properties. Additionally, peptide hydrolysis target location is enzymatically specific, so it is vital to understand not only where they cleave but also understand how the cleavage positions relate back to metabolism of the therapeutics.

Traditional mass spectrometry including MS/MS based identification of open forms often lack information necessary to make SSID assignments due to the presence of isomeric linear peptides from hydrolysis. Two popular methods to determine the SSID are computationally or by labeling techniques. Computational methods suffer from limitations such as accurately determining the SSID and lack the ability to determine SSID for mixtures of enzymes. Sample prep required for labeling cyclic can be lengthy and reaction efficiencies vary, burdening the screening of large peptides libraries in the discovery phase. Both methods are negatively impacted by the presence of unnatural amino acids.

Presented here is a label-free, fast chromatography method with a guard column for SSID of somatostatin. The cyclic peptide was incubated with trypsin or chymotrypsin to characterize protease specific soft spots. SSID sequences were confirmed either by mobility-aligned fragmentation (MAF) of diagnostic ions in an extended 10-minute reverse phase LC method or synthesizing the linear forms of the hydrolyzed peptides and comparing collision cross section (CCS) of the products. The CCS of the hydrolyzed peptides were less than 0.1% relative standard deviation (RSD), regardless of the enzyme or length of chromatographic method employed, providing a robust MS1-based identification tool capable of resolving isomers, as seen with the chymotrypsin-somatostatin digest. Additionally, the expected linear forms of oxytocin were synthesized, and CCS determined for two proximal cleavage sites and confirmed by MAF, demonstrating HRIM's ability to resolve fragments in 1 minute. CCS-based SSID can be utilized to evaluate the specific enzymes responsible for hydrolysis in mixtures and eventually in biological systems with complex matrices where current modeling and fragmentation systems are poor predicters of SSID.

P47- High-throughput intact protein analysis using Acoustic Ejection Mass Spectrometry (AEMS) on a quadrupole time of flight mass spectrometer

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Proteins play a crucial role in almost every biochemical process. The analysis of intact protein molecules via traditional mass spectrometry allows for the characterization of these proteins. For example, accurate molecular weight determination is essential to understand their role in the biophysical landscape. The need for high-throughput intact protein analysis has increased in the pharmaceutical/biopharmaceutical industries. The most common way to analyze these proteins is by peptide mapping or a "bottom-up" experiment, in which a protein is digested with an enzyme (such as trypsin), and the resulting peptides are analyzed by LC-MS to determine site-specific post-translational modifications. Throughput currently limits peptide mapping LC-MS analysis, as minutes or hours are needed to achieve the necessary characterization. An alternative approach is to analyze the intact mass of the protein first and compare the data against a known sequence, therefore allowing faster characterization and analysis time, while preserving high sensitivity and mass accuracy.

This presentation describes the use of an Echo® MS+ system with ZenoTOF 7600 system for the analysis of intact proteins. The Echo® MS+ system with ZenoTOF 7600 system uses an alternative approach to rapidly analyze proteins and solve some challenges faced by traditional techniques. Samples are placed into well plates held by the system and acoustic energy is applied to the bottom of the well plate, causing a droplet of the sample to be ejected. The Open Port Interface (OPI) is located above the well plate and captures the ejected droplet for dilution and transport by a carrier solvent (~400 µL/min) of the diluted sample to an ESI for ionization and delivery of diluted sample ions to a high-resolution mass spectrometer for analysis.

This study explores important parameters (for example, carrier solvent composition, chemical additives, and varying matrices) that affect data quality of high-resolution AEMS on a high-throughput scale for intact protein analysis. Modal proteins (for example, ubiquitin, lysozyme, streptavidin, c-reactive protein, and NISTmAb IgG1k) were selected to benchmark the system in various concentrations and buffers with a Beckman Coulter® Biomek i7 liquid handler to create assay-ready plates. Preliminary AEMS data demonstrate the rapid analysis of compounds, as a 384-well plate can be analyzed in 10-30 minutes (1-5 seconds/sample).

P48- Scrutinizing genome and proteoform heterogeneity of rAAVs by novel liquid chromatography - mass spectrometry approaches

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In the last years, recombinant adeno-associated viruses (rAAVs) were introduced in the biopharmaceutical market as delivery vehicles for gene therapies. AAV-based biotherapeutic are composed of AAVs packed with single-stranded DNA (ssDNA) containing a gene of interest that is delivered to the targeted tissue. However, the packing efficiency is not always complete leading to empty AAVs or AAVs carrying truncated genome. Moreover, the proteinaceous virus capsid is composed by three types of viral proteins (VP1, VP2, VP3) displaying different post-translational modifications (PTMs) that can potentially influence the efficacy of the drug. Thus, the development of novel analytical methods is required to scrutinize genome and proteoform heterogeneity.

Here, we investigated the structural complexity of different AAV serotypes. We chromatographically separated full and empty AAVs by a strong anion-exchange (SAX) liquid chromatography (LC) approach involving a pH gradient of volatile buffers compatible with mass spectrometry (MS). The method was directly coupled with MS or integrated with a second chromatographic dimension using a two-dimensional LC system. This approach allowed for the collection of SAX-LC fractions in a loop and the disassembling of the capsid in an automated fashion by the active-solvent modulation (ASM). The fractions could then be analyzed in the second dimension using reversed phase (RP) LC. For genome investigation, ion pairing (IP) RP-LC with UV detection was employed and proved to be useful to assess ssDNA integrity.

Using these approaches, both AAV VP PTMs and packed genome integrity could be assessed. The analysis automation and the minimal samples preparation required for the proposed methods make them suitable to be potentially implemented in a pharmaceutical quality control environment.

P49- Molecular profiling of glioblastoma patient-derived single cells using combined MSI and MALDI IHC

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MALDI-IHC is a novel technique that allows targeting of multiple mass tagged molecules of interest, using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI), with no risk of overlapping antibody signals, in contrary to standard immunohistochemistry (IHC). Additionally, MSI instruments are improving at an unprecedented rate, allowing for routine measurements with a resolution down to single cells. Here, patient-derived cells from glioblastoma tumour samples are imaged, first with high-resolution MSI to provide a lipid profile, followed by MALDI-IHC highlighting cell-specific markers. Furthermore, we investigated the effect of the initial MSI measurement and the required sample preparation on the subsequent MALDI-IHC measurement.

Glioblastoma patient-derived cell lines were grown on poly-L-lysine coated ITO slides. The cells were sublimated with 2,5-DHB before MALDI MSI measurement on a Bruker rapifleX MALDI Tissuetyper with a pixel size of 10x10 µm. After MSI, the matrix was removed and cells were stained with photocleavable mass-tagged antibodies, targeting 14 brain tissue markers, and analysed with MALDI-IHC. MALDI-IHC images were acquired with a pixel size of 5x5 µm for high spatial resolution images. To investigate the multimodal capacities of MALDI-IHC, three conditions were compared: 1) MALDI-IHC with no prior treatment; 2) MALDI-IHC after MALDI MSI sample preparation (sublimation and matrix removal); 3) MALDI-IHC after MALDI MSI measurement and matrix removal.

Initial results show that 12 out of 14 MALDI-IHC probes were successfully detected on a single cell level after an initial MSI measurement was conducted. As expected, the intensity of the signal from the cells, previously measured by MSI, was lower as compared to cells that had undergone no prior treatment. On the other hand, preliminary data indicate that the required sample prep has minimal effect on MALDI-IHC signal after removal of matrix. Detection of several of the MALDI-IHC markers indicate that multiple neuronal cell types are present in the cell culture, including astrocytes, macrophages, and neuronal cells. We aim to confirm this using the golden standard of fluorescent imaging, by first stripping the cells of the MALDI-IHC antibodies and subsequently stain with cell-specific antibodies, potentially providing three modalities measured per cell. Furthermore, RNA sequencing data from the plated cells as available and can give further insight into the cell-type ratio, present on the slide. This work has the potential to further the field of single cell molecular profiling and therapy testing. The use of cell-type specific markers in MALDI-IHC would enable the creation of a cell recognition model in a heterogeneous sample or tissue. The presented cell samples contain multiple cell types derived from the tumour environment, giving a unique insight into the dynamics of how the cell-to-cell interaction drives intratumour heterogeneity. Here, we show that multiple modalities on single cells create opportunities in connecting molecular profiles, cell types and cell-cell interactions.

P50- Unraveling antisense oligonucleotide complexity in brain tissue using a multi-omics mass spectrometry imaging approach

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In the dynamic field of drug discovery and development, a comprehensive understanding of drug absorption, distribution, metabolism, excretion and toxicity (ADMET) is crucial. Within the landscape of therapeutic innovation, antisense oligonucleotides (ASOs) are emerging candidates for treating a diverse range of diseases. However, an optimal workflow to study the biodistribution of therapeutic ASOs and its metabolites as well as drug ADMET effects is lacking. ASOs are chemically modified oligonucleotides, generally 10 to 30 nucleotides in length (6-10 kDa) that are designed to bind specific regions of RNA. Following binding to the target RNA, ASOs modulate the function of RNA by different mechanisms leading to altered protein expression. In this study, we present a novel workflow that enables the detection and spatial visualization of ASOs within brain tissue, utilizing state-of-the-art mass spectrometry imaging (MSI). On consecutive tissue sections, we explored the effects of the ASOs on the lipidome, proteome, and metabolome. This study will shed light on the comprehensive molecular landscape of ASO-mediated interventions and its potential as therapeutic treatment.

In this study, we investigated a subset of therapeutic ASOs that target specific RNAs in the central nervous system. A method was optimized to visualize ASOs in tissue samples, focusing on brain, kidney, and liver tissues. Fresh frozen tissues were sectioned on ITO glass slides, and various wash protocols were tested to enhance ASO detection in brain tissue using Matrix-Assisted Laser Desorption/Ionization (MALDI-) MSI. On a consecutive slide, the effect of ASO administration was analysed by imaging the lipid and peptide profile. Next, a method to visualize neurotransmitters in brain tissue was optimized using a reactive matrix. The addition of a deuterated neurotransmitter mix prior matrix application provided the visualization and quantification of neurotransmitters. We successfully visualized the ASO backbone in brain tissue for the first time using MALDI-MSI. The ASO backbone was also visualized in kidney and liver tissue as control. While ASO administration did not alter the brain's lipid profile, alterations in peptide distribution were observed in spatial proteomics data.

This study highlights a novel approach for ASO visualization in tissues and provides insights into ASO's biological effects, particularly in brain tissue, paving the way for further research on ASO biodistribution and its impact on various tissue components. Future analysis include the dissection of regions of interest (using the MSI data as guide), followed by ASO quantification using LC-fluorescence. Altered peptides in the MSI data will be identified by performing LC-MS/MS.

P51- Clinical antibody analysis beyond peptide approaches: Illuminating autoimmune responses by Fc-proteoform profiling

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Autoantibodies and their post-translational modifications (PTMs) are insightful biomarkers of autoimmune diseases providing diagnostic and prognostic clues, thereby informing clinical decisions. Yet, current studies and methodologies are mainly focused on IgG1 glycosylation, which represents only a subset of the IgG proteome. Quite the contrary, amino acid sequence variations, multiple glycosylation sites, and additional PTMs cumulate in structural and functional complexity of the immunologically decisive Fc-domain. While this interplay of coinciding features is not amenable to conventional bottom-up approaches, it may be essential to understand the role of antibody-proteoforms and to fully unravel their biomarker potential.

Here, we present a holistic analytical approach based on the isolation and analysis of the intact Fc-subunit to decipher (auto)antibody Fc-proteoforms and demonstrate its capabilities for the prototypic autoimmune disease rheumatoid arthritis (RA). To this end, Fc-subunits were obtained from anti-citrullinated protein antibodies (ACPA) via antigen-specific immunocapturing and total IgG via Fc-specific capturing. Analytical characterization of Fc-subunits required the development of a nanoscale reversed-phase HPLC approach coupled to mass spectrometry via dopant-enriched nanoESI that allowed separation of IgG allotypes and subclasses, while providing the necessary sensitivity to assess low abundant antigen-specific (auto)antibodies. By introducing the Skyline software to intact protein analysis, identification and quantification of Fc-proteoforms could be streamlined during data evaluation. Assessment of paired plasma and synovial fluid samples in a cohort of RA patients revealed a clear molecular distinction of ACPA compared to total IgG besides plasma- and synovial fluid-dependent differences. Prominent changes in glycosylation included high fucosylation in ACPA both from plasma and synovial fluid and lower galactosylation in ACPA from synovial fluid. Monitoring of hitherto neglected IgG features such as allotype ratios, C-terminal truncations, disulfide variations, as well as doubly- and non-glycosylated Fc-subunits, expanded the current view of (auto)antibody complexity. Integration of this wealth of Fc-proteoforms by multivariate statistic showed a separation of patients that differed in disease activity and led to the identification of disease-associated proteoforms. Taken together, the developed methodology provided comprehensive allotype- and subclass-specific Fc-proteoform profiles expanding on state-of-the-art peptide approaches. Profiling of (auto)antibodies from RA patients uncovered disease-associated proteoforms that may represent prospective biomarkers or allow monitoring disease activity, thus calling for implementation of such methodologies in autoimmunity and beyond.

P52- A proteomic platform to determine system-wide effects of pharmalogical inhibition of endothelial inflammation states.

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BACKGROUND
Hemostasis and inflammation are tightly intertwined processes in the vasculature in which vascular endothelial cells (ECs) play a central role. Previously we described unique endothelial inflammation states induced by TNFα (through NFKB activation) and IFNγ (through JAK/STAT activation) and a synergetic response by combined addition. The design of intervention strategies at the crossroads of thrombo-inflammation requires detailed insight in the mechanistic processes that underly the interplay of these states.

AIM
Understanding the molecular interplay and inhibition of endothelial inflammatory responses.

METHODS
We developed a high-throughput endothelial proteomic screening platform using an Evosep LC system coupled to a TimsTOF HT mass spectrometer to dissect system wide effects of seven NFKB and JAK/STAT-pathway inhibitors at three concentrations in TNFα, IFNγ and TNFα + IFNγ inflamed endothelial colony forming cells (ECFCs).

RESULTS
We analyzed 288 samples in five days, quantifying an average of 6,188 unique proteins per sample. Out of seven, 3 inhibitors resulted in differentiating the endothelial proteomes. Inhibitor Itacitinib (JAK1i) completely inhibited IFNγ responses (e.g. STAT1, HLAs, CXCLs) while TPCA-1 (IKK2/STAT3i) inhibited TNFα-induced responses (e.g. ICAM1, NFKB2) and affected the IFNγ response. Neither inhibitor was able to completely reduce the TNFα + IFNγ inflammation response, indicating both pathways separately induce endothelial inflammation. Finally, STAT6 inhibitor AS1517499, affected the EC steady-state, inducing a dormant state in which all inflammation responses were completely ablated.

CONCLUSION
Our integrated analysis reveals tightly controlled dynamic regulation of inflammatory processes in ECs. Moreover we highlight the feasibility of dissecting pharmaceutical-inhibition of EC inflammation states from a system-wide perspective, indicating mechanisms beyond classical induction of transcription/translation underlying the regulation of thrombo-inflammation.

P53- Improved HPLC-MS identification of short peptides in complex food samples using sequence-based retention time predictions.

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There are several challenges associated with proteins in foods. Of particular concern is the hydrolysis of proteins into peptides. Peptides are an important group of compounds contributing to the desired, as well as the undesired taste of a food product. Their taste impressions can include aspects of sweetness, bitterness, savoury, umami and many other impressions depending on the amino acids present as well as their sequence. Identification of especially small peptides (less than 6 amino acids in length) is challenging. In this contribution analytical methods based on chromatography, mass spectrometry and in silico predictions are discussed that allow us to accurately identify small peptides in typical foods.

Large sets of small peptides were generated using 'swopped-sequence' synthesis and by hydrolysis of protein standards. Peptides were separated by reversed phase chromatography and peptide MS2 data was acquired on an orbitrap system using Data-Dependent Acquisition (DDA). The identified peptide sequences and their experimental retention times were used to train and validate a Support Vector Regression model to predict retention times.

Our predictive model used, next to common peptide descriptors (as e.g. length, MW, logP etc.), a set of amino acid indices that showed excellent performance in a sequence dependent model. We achieved a strong correlation between measured and predicted retention times, also for small homologous peptide structures. Our study reveals that particularly peptide sequences play an important role in the LC retention behavior of short peptides. The identification of small peptides can be improved by accurate prediction of their retention time using our presented model.

P54- Polyethylene microplastics in zebrafish, what can we learn with MSI?

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Year after year, the quantity of pollutants in our environment continues to rise. Among these, microplastics (MPs) with sizes between 100 nm and 5 mm are continuously found in soil and water environments. Within aquatic ecosystems, filter-feeding organisms are unable to discriminate MPs from food particles, but fish may intentionally ingest MPs by mistaking them for prey. In both cases, MPs can accumulate in tissues with subsequent implications for human health. In the present study, we developed a zebrafish (*Danio rerio*) model to investigate the acute effects of MPs in fish with mass spectrometry imaging (MSI). *Daphnia magna* specimens were exposed for 24h to fluorescent polyethylene MPs (20 and 50 µm). The exposed daphnias were fed to the zebrafish (9 daphnia/fish) to study the MPs exposure via foodborne. Fish were sampled after 2 and 12h. Fluorescent microscopy and matrix-assisted laser desorption/ ionization (MALDI) imaging were used to study the distribution and metabolic effects in exposed daphnia and zebrafish. All specimens were embedded in a mixture of 1% carboxyl methyl cellulose and 9% porcine gelatin. Snap-frozen samples were cryosectioned and sprayed with N-(1-naphthyl) ethylenediamine dihydrochloride (NEDC) matrix 7 mg/ml in 70% MeOH before the analysis on a timsTOF fleX (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). All measured tissue sections were stained with hematoxylin and eosin (H&E) to evaluate morphological damage induced by polyethylene MPs. Data visualization and analysis were carried out using FlexImaging and SciLS Lab software (Bruker Daltonics). The short-term exposure to polyethylene MPs resulted in a significant accumulation of microplastics in the gut of *D. magna*. Fluorescent images of daphnia show that some polyethylene MPs were also found in the antenna muscle. The metabolic profiles of daphnia and zebrafish were investigated to evaluate polyethylene MPs toxicity. Ion images at different *m/z* values highlight the different organs of daphnia and zebrafish. The MSI data reveal metabolic differences between the exposed and control daphnia specimens. The results of this work show the potential of multimodal approaches based on MALDI-MSI to study challenging pollutants, such as MPs, and their interactions with tissues. Our results also provide new insights into the mechanisms of action of MPs in metabolic processes in *D. magna* as well as zebrafish.

P55- Profiling of induced $mTOR_{FRB}$:FKBP12 ternary complexes with collision-induced dissociation and ion mobility-mass spectrometry

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Ternary complexes induced by molecular glues (MGs) and proteolysis-targeting chimeras (PROTACs) have recently attracted enormous interest. In particular, the formation of ternary complexes of target proteins with E3 ligases for targeted protein degradation (TPD) is a prominent feature in the use of MGs and PROTACs, but not their only mode of action. MGs are used, for example, as immunosuppressants, highlighted by the FDA-approved drug rapamycin, which binds the 12 kDa FK506-binding protein (FKBP12). This binary complex then hijacks the FKBP-rapamycin binding (FRB) domain of the mammalian target of rapamycin (mTOR), forming a ternary complex and inhibiting its activity. Our collaborators have recently developed several next generation drug candidates that form a ternary complex with FRB and FKBP12, possibly through a different interaction mechanism and by forming a divergent spatial orientation of the complex, as indicated by X-ray crystallography. The conformational architecture of ternary complexes is of crucial importance, for instance for target protein inhibition or for an appropriate steric orientation for ubiquitination in TPD approaches.

Structural biology techniques like X-ray crystallography, nuclear magnetic resonance, and electron microscopy can provide high-resolution structural information about ternary complexes, but have significant drawbacks, such as time-consuming sample preparation, high protein consumption, or difficulty capturing conformational dynamics. The current work focusses on the characterization of ternary and binary complex formation using native mass spectrometry (MS), collision-induced dissociation (CID), and ion mobility -MS (IM-MS) with collision-induced unfolding (CIU).

We identified ternary complexes of the proteins FRB and FKBP12 with rapamycin and with two next-generation MGs with a dissimilar chemical structure. We also found that, even without MG, a binary protein-protein complex was formed, albeit with low affinity. Total complex intensities and CID stability correlated to known binding affinities. Conformational analysis of the ternary complexes was performed using IM-MS, whereby the collision cross sections (CCSs) calculated from the measured drift time were compared with computed CCS values from crystal structures. Building on these studies, we obtained CIU fingerprints which showed that the ligand-induced ternary complexes adopt different structural transition states during unfolding, which allows conclusions to be drawn about the nature of the interactions. Here we observed differences depending on the affinity and interaction mode.

Based on the variety of MS techniques we used for these interaction studies, we were able to validate and complement available structural and biophysical data on the ternary complexes of interest and show that this workflow can provide strong scientific results for characterizing and validating ligand-induced complex formation.

P56- Mixed-phase weak anion-exchange/reversed phase chromatography for separation and mass spectrometric analysis of nucleotide sugars

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We developed a method for fast and reliable detection of nucleotide sugars without the reliance on ion-pair or Hilic chromatography. The achieved obsolescence of ion-pair reagents is of special interest to the mass spectrometry community as they are known to cause significant pollution to instrumentation and warrant regular cleaning to maintain peak performance.

Our method uses a mixed phase weak anion-exchange/reversed-phase chromatography in combination with MRM-MS/MS for separation of 17 nucleotide sugars standards, of which several have been found in human cell lines. We monitor up to 8 transitions per nucleotide sugar to distinguish most isobaric nucleotide sugars by their transition ion ratios. The usage of multiple transitions improves confirmation of a nucleotide sugars identity as well as provides useful information for the elucidation of novel nucleotide sugars.

This method has already successfully been applied to metabolite extracts of patient cell lines with known defects in the biosynthesis of nucleotide sugars, derivates of naturally occurring nucleotide sugars as well as identifying novel nucleotide sugars.

P57- Glycosphingolipid Profiling in Neuroblastoma Organoids with Ion Mobility-Mass Spectrometry

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Glycosphingolipids consist of complex oligosaccharides that are conjugated to sphingolipids. Changes in glycosylation are frequently observed in disease, and are commonly associated with cancer. One example is neuroblastoma, a cancer that develops in immature nerve cells and that is predominantly found in children aged 5 years or younger. Glycosphingolipid GD2 are used as a target for monoclonal antibody therapy against neuroblastoma, but the presence of GD2 in healthy neural tissue causes severe pain as a side effect for this treatment strategy. Therefore, GD2 with O-acetylation on its sialic acids was proposed as a more specific and safe target. We previously developed an ion mobility-mass spectrometry (IM-MS) method using a reference library of standards with isomeric O-acetylated sialosides (1). This method employed ion mobility to measure ion-neutral collisional cross-section values that identify the O-acetyl position on glycans from proteins and tissues. Here, we present procedures to extract glycosphingolipids from cells for glycan analysis with IM-MS. This method aims to validate proposed treatment targets and identify novel potential targets for neuroblastoma treatment.

Glycosphingolipids were isolated from neuroblastoma organoids derived from different patients with extraction procedures that were tolerated by the base-labile O-acetyls. Enzymatic cleavage was used to detach the sphingolipid from the glycan and the free glycan was provided with a chargeable label. The glycans were analyzed by hydrophilic interaction chromatography IM-MS. In-source collision induced dissociation was employed to sequence exact glycan structures by fragment analysis with IM-MS.

First, standards of O-acetylated glycosphingolipids were subjected to the glycan isolation procedures that are to be used for the isolation of glycosphingolipids from organoids. This was done to evaluate the degree of migration and hydrolysis of the O-acetyls during the isolation of glycosphingolipids from organoids. Glycosphingolipids from four neuroblastoma organoid samples were examined for the presence of GD2 and its O-acetylated derivative. The O-acetyl position was readily determined, but expression across the different organoids was variable. As a result, the scope of analysis was expanded to other abundant glycosphingolipid structures. With in-source fragmentation, diagnostic fragments of glycosphingolipid glycans could be identified by referring to collisional cross-section values found from glycosphingolipid standards. This revealed the differences and similarities between the glycosylation expressed by the organoids, providing information about upregulated and downregulated glycan synthesis pathways. These results demonstrate that ion mobility-mass spectrometry can be applied to analyze glycosphingolipid glycosylation in biologically relevant samples. On top of that, this method can be applied for discovery of new biomarkers or therapeutic targets.

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P58- Characterizing Intact Proteins and Complexes by Online Nanoflow Size-Exclusion Chromatography – Native Mass Spectrometry

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Native mass spectrometry (nMS) allows the analysis of intact proteins and non-covalent complexes. Hyphenating nMS with native separation methods enables a detailed characterization of protein chemical and oligomeric composition. However, native separation approaches, such as ion-exchange chromatography (IEC) or hydrophobic interaction chromatography (HIC), rely on the interaction between analytes and stationary phase in a non-neutral environment or under high salt concentration, which has risks of damaging structural integrity and functional states of proteins. In contrast, size exclusion chromatography (SEC) is a non-retentive separation technique based on the hydrodynamic radius of analytes and provides physiological-like conditions during the separation. Although coupling SEC to nMS has been reported, so far this has been realized at analytical flows (*e.g.* 200 µL/min) or capillary flow rates (*e.g.* 10 µL/min). Under these conditions, the relatively harsh desolvation conditions needed to desolvate protein ions during electrospray ionization can result in structure alteration, compromising the analysis of labile non-covalent protein complexes.

In our research, we developed a novel method using nanoflow size exclusion chromatography – native MS (nanoSEC-nMS) to characterize intact proteins and complexes. Our strategy allows direct coupling of SEC to MS at nanoliter flow rates (0.5 µL/min). Under this condition, sensitivity is enhanced, and ionization is achieved without assistance from heated gas flow and source temperature, enabling us to ionize liable proteins and complexes. Moreover, the use of up to 400 mM salts in this system helps reduce interactions with the stationary phase and preserve the native structure of proteins during the separation and ESI ionization.

Developing nanoflow SEC – nMS presents several challenges. SEC separations rely on analyte dispersion, and in the separation process no chromatographic focusing takes place. Therefore, separations can be highly affected by the presence of system dead volumes and the volumes used during injection. Furthermore, capillary SEC columns in the nanoflow-rate regime are not commercially available. In our work, we prepared our own SEC columns in 200 µm ID capillaries and optimized resin-slurry conditions. Moreover, we tested two approaches to enhance the SEC separation efficiency by mitigating the impact of the injection step. In particular, we used a nano-injection port or preconcentration prior analysis using trap columns with mixed anion and cation exchange packing materials. Next, to achieve stable ESI at high concentrations of volatile salt, we tested three types of nano emitters, among which the hydrophobic-coated emitter performed the best. Our setup probed to be 10 times higher sensitivity than what we reported for microflow analysis. In the presentation, we will showcase examples of the separation in oligomeric forms of proteins. We believe our strategy will provide a great opportunity in native mass spectrometry such as structural biology investigation.

P59- Harnessing Chemical Reaction Networks through closed loop experimentation.

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Nature contains many systems that show remarkable abilities to process information, such as biochemical reaction networks, cells and the brain. Many molecular and DNA-based systems have been designed to unlock the information processing potential in chemistry. However these systems are generally very hard to scale up.

Here we harness the complexity of the Formose Reaction Network (FRN), a prebiotically plausible reaction network that produces a complex mixture of sugars from recursive sugar forming reactions. We have previously demonstrated the environmental and history dependence for the composition of the FRN[1,2]. This allows us to use the FRN as a physical reservoir computer[3].

For this purposed we have created an interface that couples the output of a syringe pump driven continuous stirred tank reactor (CSTR) to the inlet of the ESI-source of our ion mobility-mass spectrometry instrument, through the use of a dilution line and a back-pressure regulator. This allows us to monitor the output of our out-of-equilibrium FRN in real-time, yielding semi-quantitative information for > 100 species individually.

To take advantage of the information processing of our chemical computer, we have developed a python code that can automatically analyse data as it is measured, perform a variety of computations and submit new reaction inputs. We demonstrate how this closed-loop system can autonomously predict chaotic systems, or optimize reaction conditions.

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P60- Expanding the applicability of multidimensional LC-MS for automated bottom-up analysis of biopharmaceuticals by integrating in-solution on-line digestion

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One of the fastest growing and largest class of biotherapeutics are monoclonal antibodies (mAbs). The interest in this type of therapeutic proteins is attributed to their high specificity for their targeting antigen and thereby the option to treat several diseases. MAbs and new antibody formats are complex glycoproteins, exhibiting a wide range of heterogeneities. As these heterogeneities can affect the pharmacodynamic and pharmacokinetic properties of the protein, it is important to develop a very good understanding of the biotherapeutic and its modifications. Characterization of mAbs is commonly performed by bottom-up approaches, involving sample preparation and peptide analysis by liquid chromatography-mass spectrometry (LC-MS). Sample preparation for conventional bottom-up approaches is a very time-consuming process and can increase the risk of inducing artificial modifications as many off-line steps (denaturation, reduction, alkylation and digestion) have to be performed. Other drawbacks are long incubation times (several hours) and low efficiency of tryptic digestion. To overcome these issues, we developed previously a multidimensional LC (mD-LC) set-up for fast analysis of formulated conventional mAbs and newer antibody formats. We used immobilized enzyme reactors (IMERs) for a fast and efficient online digestion. A downside of these IMERs is that only a limited number of enzymes are available as well as the low-pressure resistance of which can lead to problems using them in a mD setup.

In the current presentation we will show a new setup which omits the need of using an IMER for the online digestion but instead uses the digestion enzyme in solution. We will show that the developed approach has a high efficiency and is reproducible and permits digestion with several proteases including Trypsin, Chymotrypsin, LysC, Thermolysine and ProNase. In most cases the same digestion conditions (buffer/temperature) can be used with minor adjustments making this an ideal platform to switch rapidly between different proteases. Additionally, we integrated our approach in a 4-dimensional LC-MS system with different first dimension separation modes (ion exchange vs. size exclusion) and different formulated mAbs samples (standard mAb vs. bispecific mAb) which in all cases showed a very high digestion efficiency. Additionally, the develop approach is faster than compared to our previous approach by omitting the need to trap the protein and perform an on-column reduction but instead doing it in-solution. We believe that this platform will be a great addition for pharmaceutical company's toolbox as it allows fast characterization of early research and development products. Additionally, due to the possibility of using different 1D separation modes it can as well be very useful to characterize unknown peaks appearing during routine QC assessment.

Abstracts Flash presentation and Poster (FP)

FP61 - Deciphering Aggregation; Studying hIAPP aggregates with nanoESI-TIMS-TOF Mass Spectrometry

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The misfolding and aggregation of proteins has become a central focus in the quest to unravel the molecular mechanisms that underly degenerative diseases. In type 2 diabetes, aggregates and deposits of the human islet amyloid polypeptide (hIAPP), i.e., a 37-residue polypeptide hormone, is hallmarking the disease and contribute to β-cell death and dysfunctions leading to aggravation of the disease. In similarity to the well-studied neurodegenerative diseases, also for type 2 diabetes holds that the early oligomerization of hIAPP is the primary pathological factor. hIAPP monomers aggregate into insoluble amyloids fibrils, that are caused by a misfolding into β-sheets oligomers at an early pathological stage. However, the exact mechanism that probes those interactions and formations of oligomers to fibrils, remains poorly understood. Investigating the aggregation behavior of hIAPP is therefore crucial for understanding the pathogenesis in type 2 diabetes.

Ion mobility-mass spectrometry (IM-MS) is a powerful tool for studying the early-stages of protein aggregation, because it enables the identification, in their native state, of dynamic and transitory structures in the aggregation process, ranging from dimer to oligomers. IM-MS is usually combined with a soft ionization method. Here, we use nanoESI, as with nanoESI lower flow rates can be achieved, which enhance ionization efficiency and reduce sample consumption. Additionally, nanoESI offers improved reproducibility in ion formation from solvents with high surface tension, such as aqueous solutions containing high concentration of volatile buffers. Together with the lower capillary voltages, this allows for conserving the fragile oligomers under the native conditions of the targeted protein.

The complete 37-residue polypeptide of hIAPP, with emphasis on the peptide fragments of the core aggregating region, i.e, region 22-27 (NFGAIL) and 22-29 (NFGAILSS) are being analyzed. The oligomers formed are studied under different experimental conditions of pH and salt concentration. Our preliminary results reveal distinct mass spectra and mobilograms for the (singly charged) protonated monomeric, and doubly and singly charged dimeric species, as well as conformational changes and structural rearrangements in the IM-MS spectra over an extended period of time. Correlating these structural insights with biological assays, such as Thioflavin T (ThT) fluorescence imaging, getting a broader overview of the β-sheet formation and aggregation process within time we aim to elucidate the toxic species responsible for hIAPP aggregation.

FP62- Dissecting the heterogeneity of C1-Inhibitor by integrating mass spectrometric approaches

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C1-Inhibitor (C1-INH), a member of the serine protease inhibitors or shortly serpins, is a plasma glycoprotein that plays a crucial role in controlling the contact activation and the complement systems. Deficiency in C1-INH has been linked to the genetic disease hereditary angioedema (HAE), and replacement therapy is available using both plasma-derived and recombinant technologies for treatment purposes. C1-INH is a heavily glycosylated protein, containing 6 N- and 26 reported O-glycosylation sites. The critical importance of studying the glycosylation of C1-INH lies in the less-described impact on its functionality, subject to significant variations depending on the source or production process. Yet, its structural complexity has limited the understanding of C1-INH glycosylation.

Our research aims to characterize these glycans and unravel the structural and functional heterogeneity of C1-INH. For this, different mass spectrometric approaches will be established. The glycosylation will be studied at different protein levels (released glycan analysis, glycoproteomics and intact analysis), which allows us to resolve the protein's complexity in detail. Also, the presence of additional post-translational modifications or genetic variants will be assessed upon glycan removal. Our methods involve integrating a dopant gas to enhance ionization of highly glycosylated molecules to achieve high-quality mass spectra. The developed methods will be used to analyze C1-INH from different biological sources and production systems. Preliminary results reveal distinct glycosylation patterns for the different biological sources, providing valuable insights into the heterogeneity of C1-INH.

FP63- Reducing charge states and extending the molecular weight range in the ESI-MS analysis of polyesters using microflow size exclusion chromatography

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Polyesters have become a popular choice for the development of environmentally friendly materials due to their natural degradability. However, the performance of these polymers is significantly influenced by their microstructures. Hence, there is a growing interest in analyzing these microstructures.

In this regard, size exclusion chromatography-electrospray ionization-mass spectrometry (SEC-ESI-MS) is a powerful technique that enables the analysis of both the chemical-composition distribution and functionality-types distribution based on their exact mass. However, it can be challenging to ionize high molecular weight polymers or those with hydrophobic structures due to their weak ionizability and low affinity for cations. As most polymers, including polyesters, are hydrophobic, there is a strong need to improve the ionization efficiency for analyzing polymers with a higher molecular weight or those having more hydrophobic structures.

Supercharging agents have been used to enhance the ionization efficiency of high molecular-weight polymers. However, these techniques often result in complicated mass spectra, which can make it challenging to characterize the microstructures. To avoid the complexity of mass spectra, various charge reduction techniques have been explored to expand the detectable range of molecular weights without excessive supercharging. One of the charge reduction methods reported is the use of quaternary ammonium salts, but this method has not been applied to polyesters that can be hydrolyzed in base conditions. In this research, we present a new charge reduction method for polyesters using a micro-flow method coupled with the cesium iodide (CsI) additive. The micro-flow method coupling CsI expanded the detectable limit of molecular weights without using base additives. Our study also revealed that this method worked better for polyesters with hydrophobic end groups than a conventional-flow method.

FP64- The Infrapatellar fat pad proteome and secretome reveal new biomarkers for knee osteoarthritis

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Introduction: Knee Osteoarthritis (KOA) is a multifactorial and highly prevalent disease. However, molecular mechanisms involved in its pathogenesis, onset, and progression remain elusive. KOA can affect several articular tissues, including the infrapatellar fat pad (IPFP). Recent research has showed that prominent inflammation of the IPFP could worsen joint damage during KOA. In this work, our aim was to study the proteome and secretome of human IPFP and associated molecular mechanisms in an *in vitro* explant-based model for OA disease.

Methods and results: IPFP from KOA (n= 12) and cartilage defect (CD) (n=13) patients were cultured with IL-1β, TNF-α or IL-1β + TNF-α, at two different concentrations (10 and 100 ng/mL) for 48 h. Then, we analyzed the proteins released from explants (secretome) as well as the explant proteome using high-throughput label free proteomics in a Q exactive mass spectrometer with data independent acquisition. Data analysis was performed with Perseus software. METASCAPE and Reactome were employed for pathway analysis. ELISA for prostaglandin E2 (PGE2) was used to validate the inflammation after cytokine treatment. The explant viability was also monitored by using LDH assay and LIVE/DEAD staining. Secretome analysis revealed inflammation in IPFP explants from KOA and CD patients through the PGE2 release and the upregulation of chemokines and pro-inflammatory cytokines. In contrast, we observed a down-regulation of proteins related with intermediate filament organization. Similarly, explant proteome showed the upregulation of pathways related to cytokine signaling in immune system, Interleukin-4, Interleukin-13, and Interleukin-10 signaling, except in KOA explants treated with TNF-α which displayed signaling pathways linked to matrix remodeling and axon guidance.

Conclusion: IPFP may exert a protective role during inflammation induced by IL-1β or IL-1β + TNF-α through the upregulation of IL-10. On the other hand, TNF-α may promote tissue remodeling and activation of pathways associated to pain. Our developed IPFP explant model coupled to high-throughput proteomics allows the discovery of biomarkers and therapeutic targets for KOA.

FP65- May post-translational succination be involved in cardiac arrhythmia? A joint experimental and theoretical study combining CIU and molecular dynamics approaches

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During the Krebs cycle, the fumarate is converted into malate by the addition of a molecule of water catalyzed by fumarate hydratase. But in some diseases, there is a germline mutation of the fumarase gene resulting in an enzymatic deficiency and therefore an accumulation of fumarate [1]. This molecule can undergo a Michael addition with proteins that present free thiol moiety (free cysteines), and this physiologically irreversible reaction is called succination. 2-succinocystein molecules are known to be involved in some pathologies such as diabetes or cardiomyopathies when they are overexpressed [2]. An immunoassay highlighting the reaction products of succination is available. However, no information about the spontaneity and the kinetics of these reactions can be deducted and their efficiency remains relatively controversial in the literature [3]. In this study, an MS-based approach was selected as a potential alternative to the current assay. Indeed, using this technique could increase the knowledge about succination by determining the reaction spontaneity and the maximal number of moieties that can be grafted into the target. During this study, the targeted protein is SUMO1 (Small Ubiquitin like MOdifier 1). SUMO1 is used in the stabilization of some target proteins, including SERCA2, a protein involved in Ca^{2+} regulation during cardiac contraction in healthy cardiac myocytes [4]. The objective of this work is to determine if succination may modify the structure of SUMO1 and therefore impact the cardiac contraction process. For this study, experimental and theoretical investigations are considered. For the experimental investigations, Ion Mobility Spectrometry Mass Spectrometry (IMS-MS) is used to determine the Collisional Cross Section (CCS) of protein ions. By measuring the CCS before and after succination, the impact of this side reaction on the 3D structure can be evaluated. However, IMS-derived CCS values alone often yield insufficient information to define the structures of proteins in detail. Therefore, to visualize the 3D structure modifications, molecular dynamics have been realized. Recently, to deepen our investigation, Collision Induced Unfolding (CIU) was implemented in our laboratory [5]. The principal aspect of this technique assesses stability changes before and after succination. This is characterized by the CIU_{50}, the inflection point of the fitted sigmoid. And in this case, if the succination affects stability, this point would undergo a shift in its position.

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FP66- Structural characterization of mobility-selected ions with infrared ion spectroscopy

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Mass spectrometry (MS) encounters challenges in fully characterizing the molecular structure of detected compounds. To overcome this bottleneck, MS is frequently combined with infrared ion spectroscopy (IRIS) to characterize the structure of gas-phase ions. However, IRIS alone fails to capture isomer-selective infrared spectra in mixtures. With trapped ion mobility spectrometry (TIMS), isomers can be separated so that IRIS spectra can be recorded for individual isomers, thereby extending the selectivity of MS and IRIS.

This combination was realized on a prototype TIMS-enabled Bruker SolariX XR 7T FT-ICR MS coupled to the beamline of the free-electron laser FELIX, where optical access is provided to the ions in the ICR cell through a backside window. The instrument features an ESI/MALDI dual source, enabling the analysis of complex mixtures and combination of spectroscopic characterization with MALDI imaging. Additionally, the instrument is equipped with a prototype TIMS unit, which enables us to measure the mobility of ions and to derive their collision cross section. This can be used to enhance the identification of unknown mass peaks, but the TIMS unit can also be used as a filter, allowing us to record IRMPD spectra of mobility-selected (isobaric) ions to deconvolute the spectral contributions from different isomeric species in a sample.

Samples containing the antibiotic ciprofloxacin were generated via both ESI and MALDI. By utilizing the TIMS filter, we investigate its competing protonation sites. Previous studies have shown that ESI produces two protonation isomers (protomers) of ciprofloxacin that can be distinguished with ion mobility. We have used our TIMS device to separate the two protomers and record IRIS spectra for the individual protomers. Hence, we can definitively assign their structures using infrared ion spectroscopy and quantum-chemical calculations. We have also generated the protonated ciprofloxacin with MALDI ionization, which is shown to yield only one of the two protomers. This allows us to analyze the intriguing difference between ESI and MALDI ionization in more detail.

FP67- Charge Variants Separation of Intact Monoclonal Antibodies at pH 5 using Nanoflow Sheath liquid CZE-MS

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Charge heterogeneity constitutes the main mAb variant and their acidic (e.g., deamidation) and basic species (e.g., lysine truncation) and is necessary to determine for quality perspectives. In this study, we developed a low-flow sheath liquid capillary zone electrophoresis (CZE) - mass spectrometry (MS) method for the in-depth characterization of the charge heterogeneity of mAbs. A static neutral (hydroxypropyl) methylcellulose-based coating was used to avoid protein adsorption and suppress the electroosmotic flow to increase the separation performance. Antibodies with a pI range of 7.4.-9.4, different degrees of heterogeneity and variations in IgG subclasses were analyzed intact at 50 mM acetic acid (pH 5). The use of acetic acid adjusted with ammonia did not cause ionization problems by using the nanoCEasy interface that was developed by Schlecht *et al*. due to the low flow. [1] Thanks to the selectivity of this method, low-abundant species, such as C-terminal lysine (9% relative abundance to main variant) could be separated from the main peak. Other PTMs, such as conversion pyroglutamate, deamidation, glycation, glycosylation, oxidation, etc. were also characterized. This highlights the potential of this CZE-MS method that can be a useful tool for in-depth mAb charge variant characterization.

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FP68- Comparison of CCS values of isobaric and asymmetric dimers of PFCA to assess the gas-phase conformation of PFCA dimers.

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Per- and polyfluoroalkyl substances (PFASs) are emerging pollutants of great concern, with over 5,000 compounds currently reported. Hyphenated techniques such as ion mobility spectrometry (IMS) coupled to liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) hold promise for non-targeted screening of these substances. Among the many advantages of IMS, a descriptor related to molecular shape is obtained through the collision cross-section (CCS), which can be calculated from the measured ion mobility and provides an additional identification point. However, when trapped IMS (TIMS) is coupled to LC-MS to analyze legacy perfluoroalkyl carboxylic acids (PFCAs), multiple mobility peaks are observed at the mass-to-charge ratio *(m/z)* of a single deprotonated ion ($[M-H]^-$), preventing the determination of an unambiguous CCS as an identifier. We determined that one of the unexpected peaks was due to a homodimeric PFCA ion ($[2M-H]^-$) that existed prior to ion mobility separation and could dissociated after mobility separation into the corresponding deprotonated ion ($[M-H]^-$). As CCS-*m/z* trendlines could be obtained for the multiple monomeric PFCA homologues and their corresponding homodimeric ions, a plausible structural conformation was hypothesized. All the PFCA monomers detected shared the same linear relationship between CCS and *m/z*, suggesting that the addition of CF_2 units induces a growth of the ion in a cylindric shape with a constant diameter (Haler et al., JASMS 2022 33(2):273-283). For the homodimeric ions, the CCS-*m/z* trendline deviated from linearity and was best fitted with a power regression model. This suggests that the proton-bound PFCA homodimer ($[2M-H]^-$) more likely adopts a V-shape with the proton bridging the carboxylate extremities, rather than a cylindric shape. To support this hypothesis, we performed IMS-MS measurements of asymmetric, but isobaric (i.e., sharing the same *m/z* ratio and number of CF_2 units) proton-bound PFCA dimers. If PFCA dimers do indeed adopt a V-shape, the CCS values of these asymmetric dimers can be expected to differ. To this end, several mixtures of two PFCA homologues capable of forming isobaric dimers, e.g., C_4+C_{14} and C_8+C_{10}, were analyzed in direct injection (i.e., without prior LC separation), to promote the formation of the corresponding heterodimers (e.g., C_4-C_{14}). The results show that the more asymmetric the dimer is, the higher is its CCS value. Consequently, the CCS value is influenced by the longer fluorinated chain of the two coordinated monomers, suggesting that the two chains have not folded over each other, which tends to confirm the V-shape hypothesis. To further support this, theoretical calculations will be performed to determine whether it is possible to predict the observed CCS trend. Finally, the influence of monocharged cations other than the proton (H^+) on the CCS trendlines of the PFCA heterodimers was investigated to gain insight into their influence on the overall shape of these dimers. For this purpose, four alkali metal cations (Li^+, Na^+, K^+, and Cs^+) were added to the injected PFCA solutions to promote the formation of cation-bound dimers and the resulting solutions were analyzed by IMS.

FP69- Towards reliable single cell proteomics data generation and analysis

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Single cell proteomics (SCP) is an emerging technique advancing our understanding of cellular heterogeneity by allowing the analysis of proteins within individual cells. Although single cell proteomics has proven to be a valuable technique, accurate data handling and interpretation is challenging as broadly accepted community guidelines and standardized methods are still lacking. To ensure reliable and reproducible results, contamination controls and validated data analysis methods are crucial.

In this study, we aimed to explore different validation parameters by analyzing the proteome of single cells derived of the human carcinoma cell line N2102Ep. Cells were sorted and processed for proteomic measurements by the CellenONE system in a 96-well plate. Isolation parameters were set to a cell diameter range of 16-26.5µm and a maximum elongation factor of 1.68. Next to single cells (*N=36)*, additional samples were taken along containing 2 (*N=9*), 4 (*N=9*), 10 (*N=9*), or 50 (*N=3*) cells. In addition, controls were taken along to investigate potential contamination. For this purpose '0 cells' were dispensed (*N=16*). All samples were analyzed on the nanoElute2 LC system coupled to the timsTOF SCP mass spectrometer and acquired data was analyzed using Spectronaut V18.

First, it was investigated which analysis method in Spectronaut was providing the most reliable results. Namely, the single cells were either analyzed separately (non-boosting) or together with the samples containing higher cell numbers (boosting). Analyzing the single cells separately (n=36) led to the identification of around 2000 protein groups per single cell and boosting resulted in an increased amount of identified proteins (~2500). However, this increase was accompanied by an increased variation in identifications between single cells (standard deviation increased from 118 to 500, respectively). The amount of protein groups identified with only one peptide increased from 9% in the non-boosted data to 34% in the boosted data, suggesting a less reliable protein identification in the boosted data analysis method. In line with this, filtering the data for proteins identified with at least two peptides and at least one protein specific peptide resulted in a similar number of protein IDs (around 1600 protein groups) for both analysis methods, where the non-boosted set resulted in lower variation. Next, it was investigated how much proteins could be identified in the blanks (0 cells). Strikingly, in this analysis, the software identified up to 600 proteins of which 39% of the identified proteins were based on a single peptide, suggesting a high false discovery rate. Additionally, it was investigated whether the cell diameter was correlated to the number of identified protein IDs. However, as little variation was found in the diameter of the cells that were isolated (23 µm ± 2) no direct correlation was found and further research is needed that includes a wider variety in diameter of cells.

In conclusion, as SCP is gaining momentum, more research should be performed in order to obtain standardized data analysis methods which could serve the SCP community. Especially, how data curation should be performed to exclude false discoveries as well as the importance of including appropriate controls to account for contamination during the workflow.

FP70- Identification of synthetic cathinone positional isomers using electron-activated dissociation and chemometrics

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Synthetic cathinones (SCs) are a main category of new psychoactive substances (NPS), posing a serious threat to public health. Typically, many SCs are closely related and present in isomeric forms. Differentiation of isomeric SCs is essential for both legislative and public safety reasons since minor differences in their molecular structures will change their legal status and toxicological profiles. A new workflow for SC isomer differentiation has been developed and tested using liquid chromatography - high-resolution tandem mass spectrometry (LC-HRMS^2). In this new analytical approach, electron activated dissociation (EAD) was used as fragmentation method for MS^2 in combination with chemometric analysis. This work demonstrates that EAD product ions and their characteristic ion ratios can be used to distinguish between the ring-substituted isomers of methylmethcathinones (MMCs), methylethcathinones (MECs), and chlorolmethcathinone (CMCs). The kinetic energy in the EAD cell was investigated at three levels (i.e., 15 eV, 18 eV and 20 eV) for each group of SCs. The resulting EAD mass spectral data was analysed to train and test chemometric approaches via merging EAD mass spectral data of the mentioned three levels of kinetic energy for each SC positional isomer. Through the chemometric analysis of the EAD mass spectral data of three sets of ring-substituted SCs using t-distributed stochastic neighbour embedding (t-SNE) and Random Forest (RF) algorithms, SC isomeric classes could confidently be distinguished. The developed approaches were validated via assigning the isomers present in blind authentic street samples, demonstrating its discriminative potential in distinguishing positional isomers of each SC class. Via the RF model developed, the combined classification accuracy for the 26 blind authentic street samples was 92% (72/78), thereof 15 eV of kinetic energy showed greater classification accuracy (i.e., 100% (26/26)) for these street samples than the accuracy of 96% (25/26) from the 18 eV data and 81% (21/26) from the 20 eV data. These results highlight the impact of electron kinetic energy on the classification accuracy and provided an impetus to investigate improvements in RF classification using a subset of the original data set focusing on only the 15 eV data. Through the another RF model developed solely based the data acquired at 15 eV kinetic energy, classification accuracy of 100% (26/26) for the same street samples analysed was achieved. These results show the value of the combination of EAD and chemometric approaches for SC isomeric identification and as such also show promise to isomeric identification of other classes of NPS.

FP71- Collecting and analysing pig mucosal scraping samples for mass spectrometry-based proteomics: investigating variation factors

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Proteomics has emerged as a valuable tool in gut-related research in pigs (*Sus scrofa*). However, detailed protocols for sample collection and preparation, particularly concerning mucosal scraping samples, are often insufficiently described. We suspect that several factors, such as inter- and intra-technician variability in the collection of samples, individual variability among pigs, or batch effects arising from sample processing and time of analysis, might lead to potential sources of variability in proteomic analyses.

To address these concerns, we conducted a reproducibility study involving the collection of mucosal samples from 12 piglets fed two distinct diets (n=6 per diet). These piglets were weaned at four weeks of age and subsequently fed *ad libitum* until euthanization at seven weeks. Mucosal samples were obtained from a 60 cm segment collected from the middle of the jejunum, which was then divided into six equal segments. Scrapings were collected by two samplers, who each handled three of the six segments per pig. Both samplers were given the exact same instructions for performing the mucosal scrapings: each segment was scraped twice using a microscope slide, using the same amount of pressure as much as possible every time. Samples were then randomized and processed in two separate groups on different days to be able to examine batch effects. Protein extraction was carried out using Midi S-Traps (ProtiFi), a sample processing technology using SDS, suspension trapping and trypsin digestion. The peptide extracts were analysed via LC-MS/MS utilizing an Ultimate 3000 RSLCnano system directly linked to an Orbitrap Fusion Lumos mass spectrometer (Thermo), employing a DIA approach. Protein identification and relative quantification were achieved through MaxQuant, and statistical analysis was conducted using RStudio. Our findings revealed a notable batch effect, indicating the influence of time of analysis on the proteomic results. Additionally, variability between individual pigs was observed, though the extent of this variability varied among animals. Interestingly, no significant differences were detected between samples collected by the two samplers, suggesting consistency in sample collection technique.

In conclusion, this study underscores the importance of standardized protocols for sample collection and processing in proteomic studies, particularly in the context of gut-related research in pigs. Addressing sources of variability such as batch effects and inter-individual differences is crucial for ensuring the reliability and reproducibility of proteomic analyses in this context.

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