MSBM

Fundamentals & Applications in Mass Spectrometry

XVIII

5 - 10 July 2026
Centre for Advanced Academic Studies (CAAS)
Dubrovnik, Croatia

Welcome to the 18th edition of the "Mass Spectrometry in Biotechnology and Medicine" (MSBM) Summer school. The program will focus on teaching and discussing current best practices and knowledge in mass spectrometry to advanced graduate students, post-docs and scientists in the area of biochemistry, biology, molecular biology, biotechnology and medicine. You can follow MSBM on various social media platforms such as https://www.linkedin.com/groups/3414688/ with photos from past events here https://www.instagram.com/msbmionlover/ and at https://msbm.org.

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ACKNOWLEDGEMENTS

The members of the MSBM Organizing Committee: Dr. Laura Bindila (Mainz, Germany), Prof. Garry L. Corthals (Amsterdam, The Netherlands), Prof. David R. Goodlett (Victoria, BC Canada), Prof. Ron M. A. Heeren (Maastricht, The Netherlands), Dr. David P. A. Kilgour (Nottingham, UK), Dr. Harry Taylor (Nottingham, UK), and Dr. Ljiljana Paša-Tolić (Richland,WA USA) thank their home institutions for support. Finally, and importantly, we thank Ms. Željana Mikovčić (Rijeka, Croatia) for general assistance in organizing MSBM XVIII and Mr. Graham R. Goodlett for administrative assistance (Victoria, BC Canada).

SPONSORS

Corporate Support

Institutional and University Support

PROGRAM

Program NOTES:

*As part of our Sunday night gathering, we will have a group photo.

**Each day breakfast will be served at the CAAS café, but only for those staying in the CAAS dormitory or an apartment. All other food and drink is for everyone.

***On Wednesday we will hand out tickets to take a boat to Lokrum island. Sack lunches will be handed out prior to departing CAAS. Boat to Lokrum departs from the Southern harbor found by walking along the promenade from Pile gate to the clock tower and under it to the harbor. Departures are on the half hour. Ticket needed only for transit to Lokrum. You may return whenever you like, but you may not spend the night on the haunted island. After you have had a decent swim and worn yourselves out, the faculty cordially challenge the students to lose a game of volleyball. We'll gather on the ball fields on the South-western side past the monastery to play against those students brave or naïve enough to have a go against the well trained, well-oiled and uber athletic faculty.

Day	Start time	Duration (mins)	End time	Session code	Topic/title (Draft)	Lecturer (Draft)
Sunday	1500	120	1700		Registration and Poster Set-up at CAAS	
Sunday	1700	15	1715		Opening Address from MSBM	Goodlett
Sunday	1715	30	1745		Plenary - Open Access and Artificial Intelligence in Scientific Publishing	Trevorrow
Sunday	1745	75	1900		Posters and drinks in Poster hall(s)	Heeren
Sunday	1900	60	2000		Opening Gathering in CAAS courtyard with food and drinks	
Monday	900	15	915	Admin time	Discussion of workshops, participant presentations, exam	Taylor
Monday	915	45	1000	Didactic 1	Fundamentals inc Isotopes	Taylor
Monday	1000	45	1045	Didactic 2	MALDI and TOF Analysers	Sellami
Monday	1045	30	1115	Coffee		
Monday	1115	45	1200	Didactic 3	ESI and Nanospray ionisation	Kelly
Monday	1200	120	1400	Lunch		
Monday	1400	45	1445	Didactic 4	Introduction to Chromatography for MS	Spelling
Monday	1445	75	1600	Didactic 5	Quads and Traps	Kilgour
Monday	1600	30	1630	Coffee		
Monday	1630	30	1700	Sponsor 1	Shimadzu - MALDI	Sellami
Monday	1700	30	1730	Didactic 6	Introduction to IMS	Daly
Monday	1730	90	1900	Swim break	Only those who can swim, no life guards 🩲	
Monday	1900	60	2000	Evening workshop	In the courtyard - with pizza and drinks	All Faculty
Tuesday	900	45	945	Didactic 7	Tandem MS Basics of how to break up molecules - CID, ExD, xPD etc.	Lekha
Tuesday	945	45	1030	Didactic 8	Hybrid instruments: Q>QqQ>QToF, 4 std modes of LC-MS/MS	Heeren
Tuesday	1030	30	1100	Coffee		
Tuesday	1100	60	1200	Didactic 9	Bottom up proteomics - and intro to manual sequencing	Taylor
Tuesday	1200	120	1400	Lunch		
Tuesday	1400	30	1430	Workshop 1	Protein Sequencing challenge - exam question help	Taylor, Goodlett
Tuesday	1430	45	1515	Didactic 10	FT Mass Analysers and Systems: ICR	Tsybin
Tuesday	1515	45	1600	Didactic 11	FT Mass Analysers and Systems: Orbitraps	Tsybin
Tuesday	1600	30	1630	Coffee		
Tuesday	1630	60	1730	Didactic 12	Informatics Fundamentals – Introduction to automating your data processing	Samanipour
Tuesday	1730	75	1845	Swim break	Only those who can swim, no life guards 🩲	
Tuesday	1845	75	2000	Evening workshop	In the courtyard	All Faculty

Wednesday	900	30	930	Didactic 13	Intro to Metabolomics	Lekha
Wednesday	930	30	1000	Didactic 14	Introduction to Ambient Ionization	Kelly
Wednesday	1000	30	1030	Didactic 15	Intro to Lipidomics	Bindila
Wednesday	1030	30	1100	Coffee		
Wednesday	1100	90	1230	Workshop 2	i) FTMS data simulation & ii) Data processing workshop	Tsybin, Colonius
Wednesday	1230	330	1800	Excursion	Juxtaposition of the Paradise Island and the devilish staff volleyball team	

Thursday	900	30	930	Didactic 16	Introduction to MS imaging - or intro to databases - or intro to stats?	Heeren
Thursday	930	60	1030	Seminar 1	Careers - "The skill sets that industry values"	Industrial faculty
Thursday	1030	30	1100	Coffee		
Thursday	1100	60	1200	Workshop 3	LC Method development, Extra data workshop time	Spelling
Thursday	1200	120	1400	Lunch		
Thursday	1400	30	1430	Sponsor 2 – Bruker	Bruker Sponsor Lecture	Mitulovic
Thursday	1430	90	1600	Workshop 4	Informatics workshop - Final title to follow	Samanipour, Colonius
Thursday	1600	30	1630	Coffee		
Thursday	1630	30	1700	Sponsor 2 – MSVision	MSVision Sponsor lecture / Focused look at some specially modifed instruments	Daly
Thursday	1700	30	1730	Didactic 18	Advanced tandem MS - DDA, DIA etc	Corthals
Thursday	1730	75	1845	Swim break	Only those who can swim, no life guards 🕶	
Thursday	1845	75	2000	Evening workshop	In the courtyard	All Faculty

Friday	900	30	930	Didactic 19	Intro to intact/native and Top down protein analysis	Sellami
Friday	930	30	1000	Didactic 20	Biopharma applications	Tsybin
Friday	1000	30	1030	Didactic 21	Protein footprinting	Taylor
Friday	1030	30	1100	Coffee		
Friday	1100	60	1200	Student presentations	5 x 10 mins each for the Poster winners	Pasa-Tolic
Friday	1200	120	1400	Lunch		
Friday	1400	30	1430	Didactic 22	Imaging applications	Heeren
Friday	1430	90	1600	Workshop 5	Clinical studies and applications (Workshop element)	Bindila
Friday	1600	90	1730	Coffee and Swim Break		
Friday	1730	30	1800	Sponsor 3 - Thermo	Thermo	Colonius
Friday	1800	60	1900	Evening workshop	In the courtyard	All Faculty
Friday	1900	30	1930	Admin break	Time to get into your glad rags / pleather onsie / wookie outfit / formal lab coat etc	
Friday	1930	120	2130	Dinner	Banquet dinner	

KEY

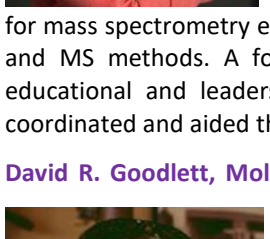
Lecture	Social Networking	Workshop	Refreshments	Evening Workshop

ORGANIZING COMMITTEE BIOGRAPHIES

Laura Bindila, University Medical Centre Mainz, Germany is Head of Lipidomics/Mass Spectrometry Facility at the Institute for Physiological Chemistry, University Medical Centre Mainz, where her scientific interest is unravelling the lipid signals involved in various neurobiological processes, and more generally in physiological and pathophysiological states. She is Principal Investigator at the Mainz Research School for Translational Biomedicine and member of the Research Centre for Translational Neuroscience, of the University of Mainz. She has previously worked as a scientist at Luxembourg Clinical Proteomics, and as postdoc and PhD student at the University of Münster where she has focused on glycoconjugates and (glyco)proteomics in cancer research and rare diseases under the mentorship of Prof Peter-Katalinic, who developed the concept MSBM holding the first one in 2001. She serves as advisory and editorial board member for several peer-review journals and published more than 70 peer-reviewed articles. See: https://www.unimedizin-mainz.de/lipidomics-unit/lipidomics-core-facility.html?L=1.

Garry L. Corthals, University of Amsterdam, The Netherlands is Professor and Chair of the Analytical Chemistry Group at the University of Amsterdam and a guest professor at both the Amsterdam UMC and M4I. He obtained his PhD at Macquarie University in 1997, focusing on the separation of biological molecules. He began his MS career in Seattle with Prof. R. Aebersold's group, combining biological mass spectrometry, protein science, and biology. In 1999, he moved to Sydney as a group leader in MS at the Garvan Institute, developing new proteome analysis methods for medical and clinical research. His academic research interests still include clinical mass spectrometry, MSI and clinical imaging, and AI methods for patient classification. His interests also lie in rapid MS methods and advancements in ambient ionization, including 'Science for Art,' environmental, and forensics analysis. Prof. Corthals has been a passionate advocate for mass spectrometry education since 1997, contributing to education, training, and promotion of proteomics and MS methods. A founder of the European Proteomics Association (EUPA), he has also held various educational and leadership roles. Within HUPO he served as head of education, and regionally he has coordinated and aided the development of national and international research facilities.

David R. Goodlett, Molecular Life Sciences, University of Victoria, Canada is a Professor of Molecular Life Sciences at the University of Victoria (UVIC) where he holds the Don and Eleanor Rix BC Leadership Chair in Biomedical and Environmental Proteomics (since 2020). Prior to this he was Professor at the Universities of Maryland (2013-2020) and Washington (2004-2012) as well as first Director of Proteomics at the Institute for Systems Biology (2000-2003). At UVIC he is also Director of the 30+ year old Proteomic Centre, which is known for expertise in targeted metabolomic and proteomic assays. After postdoctoral work with Richard D. Smith at Pacific Northwest National Laboratory (1991-1993) on fundamentals of electrospray ionization and native MS, he spent five years in the pharmaceutical industry. Prior to that he received his Ph.D. from North Carolina State University (1986-1991) with Richard B. van Breemen (now at Oregon State) and Frank B. Armstrong (deceased) using mass spectrometry to characterize proteins and peptides. He was a Finland Distinguished Professor at the University of Turku (2012-2016); see https://www.youtube.com/watch?v=jfOOMNJivvY. He was also Visiting Professor at the University of Gdansk's International Centre for Cancer Vaccine Science (2017-2022) where he helped establish a proteomics laboratory. He has been an Editor at Rapid Communications in Mass Spectrometry since 2005 and since 2007 he has helped organize MSBM. Recently, he gave a public lecture at Herman's Jazz Club on his research in microbial pathogenicity https://www.youtube.com/watch?v=y6vVaJVFDgM&t=309s. *You can find out more at goodlettlab.org and https://www.instagram.com/goodlettlabb/*.



Ron M. A. Heeren, Maastricht MultiModal Molecular Imaging Institute (M4I), University of Maastricht, The Netherlands, is a distinguished professor and Limburg Chair at Maastricht University (since 2014). He is the founder and scientific director of M4I, the Maastricht MultiModal Molecular Imaging institute on the Brightlands Maastricht Health campus. Prof. Heeren obtained a Ph.D. degree in technical physics in 1992 at the University of Amsterdam on plasma-surface interactions. He started to work on molecular imaging instrumentation and its application as a research group leader at FOM-AMOLF, Amsterdam. In 2001, he became professor at the chemistry faculty of Utrecht University lecturing on the physical aspects of biomolecular mass spectrometry. He was awarded the prestigious 2019 Physics Valorization prize by

the Dutch organization for scientific Research, NWO and the 2020 Thomson medal of the international mass spectrometry foundation. In 2021 he was elected as a member of the Royal Dutch Academy of Sciences, KNAW. His academic research interests are mass spectrometry based personalized medicine, translational molecular imaging and "omics" research, high-throughput bioinformatics and the development and validation of innovative molecular analytical imaging techniques across the scientific disciplines. *For more information: www.maastrichtuniversity.nl/r.heeren*

David P. A. Kilgour, Vibrat-Ion Ltd, UK is currently Head of Research for Vibrat-Ion Ltd, developing the Beacon ion source range and AutoVectis, a suite of data processing software for mass spectrometry. During a varied career, he has also been a Principal Scientist for the UK government, a scientific advisor to NATO, and an academic in both the UK and US. He has worked on prototype quadrupole, ion trap, ToF and Orbitrap and FT-ICR systems and has developed modelling, control, acquisition and data processing systems for all of them - both hardware and software. He has also undertaken research in a variety of different ion sources: two-stage laser desorption and ionisation, counterflow ionisation, piezoelectric droplet-on-demand ionisation and cold cathode ionisation. Along the way he has busted a few myths about mass spectrometry - for example, be warned, you have to have a really good reason before you shrink your mass analyzer; and internal protein fragments, used carelessly, may only solve a problem that you already know the answer to. He has been providing professional training in mass spectrometry and Scientific Leadership for >15 years, and runs the @AutoVectis YouTube channel that hosts videos explaining many different mass spectrometry and analytical chemistry concepts.

For more information: http://www.vibrat-ion.com & http://www.kilgourlab.com

Ljiljana Paša-Tolić, Pacific Northwest National Laboratory, USA is a Staff Scientist at the Pacific Northwest National Laboratory (PNNL) and the Capability Steward for Mass Spectrometry at the Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility operated by PNNL for the U.S. Department of Energy (DOE). Her research centers on developing sophisticated analytical methods with emphasis on Fourier transform (FT) mass spectrometry (MS) and micro-separations, as well as the application of these techniques to accurately quantify changes in protein abundance, identity and activity during cell development or in response to stimuli. Prior to accepting a postdoctoral fellowship at PNNL in 1995, she served as a Visiting Associate in Research at the National High Magnetic Field Laboratory in Prof. Alan G. Marshall's laboratory, where she designed the world's first 9.4T FTICR spectrometer and developed techniques for characterizing large biopolymers with high sensitivity. As a result of this expertise, her first assignment at PNNL was to bring the 11.5T FTICR to fruition for the EMSL Project. Since that time, Dr. Paša-Tolić have continuously been involved in EMSL's user program, and over the years have worked with numerous collaborators and outside users of MS instrumentation, developed core instrumentation and methods to enable previously intractable MS applications, and managed the operation of FTICR mass spectrometers for proteomics-related research. Furthermore, she was a key contributor in the pioneering efforts of PNNL's high throughput accurate mass and time (AMT) tag strategy for ultra-sensitive proteomics. *For more information: https://www.pnnl.gov/science/staff/staff_info.asp?staff_num=5883*

Željana Mikovčić, University of Rijeka, Croatia works as the head of the office for professional administrative affairs, ensuring efficiency and organization within the office. Additionally, she is actively involved in the administrative implementation of projects, coordinating all phases of a project. In addition to her University position, she has worked as the primary administrator for MSBM for the last decade. You can find out more about her at https://www.linkedin.com/in/zeljana-mikovcic-65656332/

Harry Taylor, School of Chemistry, University of Nottingham, UK leads the project “Photene”, a business unit within the University of Nottingham which uses advanced proteomics to perform protein footprinting for clients in the pharmaceutical, therapeutic and agrochemical industries. Dr Taylor obtained his PhD at Nottingham Trent University in 2022, focusing on the development of novel acoustic ambient ionization sources for mass spectrometry. After a short period continuing this work as a postdoctoral research fellow, he joined the University of Nottingham as a Business Science Fellow. In addition, Harry is responsible for the chromatography and mass spectrometry analysis performed by the School of Chemistry for businesses. *You can read more*

INVITED FACULTY BIOGRAPHIES

Dr. Immo Colonius, Thermo Fisher Scientific, Germany. He received his PhD in Computer Science from the University of Bremen, where he worked in the Cognitive Systems (CoSy) group as part of the Transregional Collaborative Research Center on Spatial Cognition, focusing on logic-based reasoning, process recognition, and planning for autonomous agents in dynamic environments. Immo then moved into the mass spectrometry industry, joining Thermo Fisher Scientific in Bremen as a software developer for instrument software. At the moment he's the tech lead of Astral Software. He has since contributed to the development of several generations of Orbitrap-based mass spectrometers, including the Orbitrap Astral and Orbitrap Astral Zoom platforms, and is co-author on multiple peer-reviewed publications describing their architecture and performance in high-throughput proteomics

Dr. Steve Daly, MSVision, The Netherlands. Dr Steve Daly is a research and design scientist at MSVision, based in. He specializes in the modification of mass spectrometry systems – to give them new and novel capabilities. He especially likes adding lasers!

Prof. Ryan Kelly, Brigham Young University, USA. Ryan Kelly is a professor in the Department of Chemistry and Biochemistry at Brigham Young University. His recent research efforts have focused on single-cell and spatial proteomics, which have involved tailoring sample preparation, separations, ionization and mass spectrometry acquisition for low-input samples. His group's efforts have enabled thousands of proteins to be rapidly profiled from single cells. He is now working to dramatically lower the cost of proteomics, with the ultimate goal of profiling any biological sample for just ~$10.

Dr. Goran Mitulović, Bruker, Austria. is Senior Application Scientist at Bruker and a lecturer at the Medical University of Vienna and the University of Applied Sciences in Wiener Neustadt. He obtained his PhD at the University of Vienna in 2001, focusing on developing methods and hardware for separating biological molecules. He began his professional career in Amsterdam with LC Packings (developer and manufacturer of the UltiMate nano LC, the PepMap nano separation columns, etc.). In 2004, he moved back Vienna to the Research Institute of Molecular Pathology in the group of Karl Mechtler. In 2009, he moved to the Medical University of Vienna with Christopher Gener. In 2011, he started the Proteomics Core Facility at the Medical University of Vienna, which he headed until 2021, when he joined Bruker. His research interests include nano LC, stationary phases for proteomics analyses, clinical mass spectrometry, and high throughput clinical proteomics methods.

Prof. Saer Samanipour, University of Amsterdam, The Netherlands. In the Department of Chemistry at the University of Amsterdam he is a Principal Investigator at the Van 't Hoff Institute for Molecular Sciences (HIMS). His research focuses on developing and applying high-resolution mass spectrometry and data analysis tools to study complex mixtures of organic compounds in environmental and biological systems. Before joining UvA, he held postdoctoral positions at Northeastern University (USA) and the Helmholtz Centre for Environmental Research (Germany) and earned his PhD in Environmental Chemistry from the University of Queensland (Australia). 2026 will be Dr, Samanipour's first visit to MSBM, and he will be a valuable addition to the faculty team.

Dr. Lyna Sellami, Shimadzu Lyna Sellami has a pharmaceutical educational background, she obtained her PhD in Proteomics applied to human pathologies at Aix-Marseille University (France). Her research aimed to gain insight into the oxidative stress process using mainly MALDI mass spectrometry; she also developed and patented an analytical method for the characterization of oxidative post-translational modifications. She worked afterwards an application scientist in MALDI mass spectrometry at Shimadzu in the UK then at Ascend diagnostics where she adapted several methods for clinical microbial identification. She currently works in the center of innovation and product Support of Shimadzu European Headquarters in Germany.

Prof. Lekha Sleno, Université du Québec à Montréal, Canada. She received her PhD in 2006 from Dalhousie University with Dietrich Volmer at NRC in Halifax, NS, Canada on the elucidation of small molecules using high resolution and tandem mass spectrometry. She then completed two post-doctoral stays, at the University of Geneva in pharmaceutical analytical mass spectrometry and the University of Toronto in proteomics, before starting her independent research career in the chemistry department at Université du Québec à Montréal in 2008. She is currently a Full Professor and Strategic Research Chair in Bioanalytical Chemistry. Her group's interests include developing novel analytical workflows for biomarker discovery and exposomics research using mass spectrometry. She is currently co-director of the CERMO-FC, a multi-institutional research network on orphan diseases (Centre d'Excellence de Recherche sur les Maladies Orphelines – Fondation Courtois) and is the director of the**ir** metabolomics and proteomics platform

Mr. Victor Spelling, AstraZeneca, Sweden. He is a separation scientist at AstraZeneca in Gothenburg and holds a master's degree in pharmaceutical engineering from Uppsala University. He began his career at AstraZeneca in 2019, working to extend the use of supercritical fluid chromatography for the analysis of amino acids and peptides. He now works in the Separation Science Laboratory, primarily supporting early drug discovery for small molecules. His day-to-day responsibilities include chiral and achiral separations at both analytical and preparative scale, using reversed-phase, normal-phase, and supercritical fluid chromatography. The purifications are carried out in milligram to kilogram scale. Recent projects have focused on evaporative light scattering and charged aerosol detectors, including signal characterization and modelling.

Mr. Paul Trevorrow, Wiley, UK. He is Associate Director of Publishing Development at Wiley, working across Life Sciences journals with a particular focus on strengthening editorial workflows, supporting editors, and improving author experience. Paul collaborates widely with societies, editorial boards, and research communities, and enjoys bringing people together to solve practical challenges in publishing.

Dr. Yury Tsybin, Spectroswiss, Switzerland. He received his PhD degree in ion physics in 2004 from Uppsala University. For the next 2 years Yury was a postdoctoral research associate with Prof. Alan G. Marshall at the National High Magnetic Field Laboratory in the USA. From 2006 to 2014 Yury was an assistant professor of physical and bioanalytical chemistry at the Ecole Polytechnique Fédérale de Lausanne (EPFL) in Switzerland where he established and headed the Biomolecular Mass Spectrometry Laboratory and served as a Director of the Mass Spectrometry Service Facility. In 2014 he founded an EPFL spin-off company, Spectroswiss Sàrl, which he has directed since then. Dr Tsybin's research focus involves FTMS fundamentals, ion motion and ion detection, electron injection systems for ion fragmentation in the gas phase, data acquisition and signal processing. Application interests are in structural analysis of monoclonal antibodies and their mixtures for improving drug discovery and quality control technologies; middle-down and top-down proteomics development. Yury is a former member of the MSBM Organising Committee, serving from 2008 to 2017.

POSTER ABSTRACTS

Kelly Barko of University of Pittsburgh

Sex Differences in the Proteome of Major Depressive Disorder

Major depressive disorder (MDD) is a leading cause of disability characterized by sex differences in prevalence, symptom presentation, and corticolimbic dysfunction. Previous studies implicate dendritic spine alterations and neuroimmune disruption in MDD; however, underlying molecular mechanisms remain unclear. To identify MDD-associated proteomic signatures, we performed mass spectrometry on postmortem anterior cingulate cortex tissue from MDD subjects and unaffected comparisons (UC; n=30/sex/group). We identified 162 proteins altered in MDD compared to UC ($q<0.1$), enriched for neuronal pathways including dendritic spine organization and synaptic transmission. Sex-stratified analyses revealed distinct MDD-associated proteomic alterations in females and males ($q<0.1$). In females with MDD, upregulated proteins were enriched for immune-related pathways, including type I interferon response. Conversely, males with MDD exhibited downregulation of proteins involved in synapse-related pathways. These findings suggest that sex-specific mechanisms may differentially contribute to neuroimmune regulation of dendritic spine pathology in MDD.

Co-authors: Shelby Ruiz, Lambertus Klei, Bernie Devlin, Thien Pham, George C. Tseng, Akayla Lewin, Jordan Gilardi, Matthew MacDonald, and Marianne Seney

Henriett Barta of University of Graz

Novel pyrazolo(terpenyl)-curcuminoid hybrid molecules are dual inhibitors of ferroptosis and inflammation

Ferroptosis is a regulated cell death program characterized by iron-dependent lipid peroxidation and implicated in degenerative diseases. Starting from curcumin, a secondary metabolite from Curcuma longa with antioxidant, anti-inflammatory, and anti-ferroptotic properties, we recently developed a series of rotationally restricted pyrazolo-curcuminoids. In this study, we discovered these pyrazolo curcuminoids and additional terpenyl-substituted derivatives as dual inhibitors of ferroptosis and inflammation. In M1 macrophages, they suppressed 5-lipoxygenase-derived pro-inflammatory lipid mediator biosynthesis while enhancing the production of the pro-resolving mediator protectin D1 and/or isomers in M2 macrophages. The compounds displayed up to 20-fold higher anti-ferroptotic activity than curcumin in human and murine cell lines. Notably, the anti-ferroptotic activity of pyrazolo(terpenyl)-curcuminoids was independent of NRF2 activation, enhanced antioxidant capacity, and iron chelation, indicating a non-canonical mechanism distinct from curcumin. Transcriptomic profiling revealed modulation of previously unrecognized gene candidates linked to ferroptosis regulation. These findings identify pyrazolo(terpenyl)-curcuminoids as promising leads against ferroptosis-driven necroinflammatory disorders.

Co-authors: Minh Bui Hoang, Rao Zhigang, Claudia Kostner, Alberto Minassi, Andreas Koeberle, Solveigh Koeberle

Anja Bošnjaković of Institute for Anthropological Research

Graphlet-Guided Lipidomics Network Analysis Suggests Membrane Lipid Remodeling in Vemurafenib-Resistant BRAFV600E Colorectal Cancer Cells

BRAFV600E-mutant metastatic colorectal cancer is a clinically aggressive subgroup with limited benefit from vemurafenib monotherapy because of rapid development of resistance, and associated lipid-metabolic adaptations remain incompletely defined. We analyzed lipidomics data from parental and vemurafenib-resistant RKO cells under control and treated conditions using an integrated workflow combining interpretable machine learning, BioPAN reaction prediction, and graphlet-based network comparison. Machine-learning models prioritized resistance-linked and treatment-interaction lipid features enriched for PC, PI, and LPC species. BioPAN reaction/gene ranking highlighted PLA2 phospholipase and LPCAT reacylation axes as the strongest candidate mechanisms, with additional support for DGAT2, MBOAT, and PEMT-related remodeling. Graphlet degree distribution analysis showed low structural agreement between active and suppressed RT_vs_PT reaction networks (GDD agreement = 0.35), indicating distinct local reaction topologies. Active reactions were PLA2-centered, whereas suppressed reactions were dominated by LPCAT1-4 and DGAT2. Together, these results support PC/LPC-centered membrane remodeling and lipid-storage rewiring as candidate features of acquired vemurafenib resistance.

Co-authors: Bošnjaković Anja, Šunić Iva, Karlović Nina, Meščić Macan Andrijana, Žepić Ines, N. Rechberger Gerald, Züllig Thomas, Lovrić Mario, Sedić Mirela, Bočkor Luka.
1.Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb,
2. Institut für Molekulare Biowissenschaften, Karl-Franzens- Universität Graz, Graz,

Nadine Bothen of Max Planck Institute for Chemistry

When Pollen Meets Pollution: Oxidative Protein Modifications Linking Endogenous Chemistry and Urban Air to Allergen Health Impacts

My work combines high-resolution mass spectrometry and biophysical approaches to characterize oxidative post-translational modifications of proteins, using an Orbitrap Exploris 240 coupled with a Vanquish LC-system. I apply bottom-up LC-MS/MS peptide mapping to localize and quantify the extent of site-selective tyrosine nitration, and to identify covalent dityrosine cross-links. Intact-protein MS is used complementary to track modification abundance at the whole-protein level and size-exclusion chromatography to resolve oligomeric species. Molecular dynamics simulations provide structural context for the observed site selectivity. I apply this workflow to the grass pollen allergen Phl p 5a exposed to peroxynitrite and various ratios of ozone and nitrogen dioxide, linking endogenous oxidative chemistry and exogenous air pollution to potential health impacts. I aim to apply based on my laboratory studies targeted MS to air filter samples to determine which modifications occur under real atmospheric conditions.

Keila Cavalcante of University of São Paulo (IQSC-USP)

Foodomics Investigation by LC–qTOF–MS of Tryptic Digestion of β-Lactoglobulin after UV-C Processing in a Deep Eutectic Solvent

β-Lactoglobulin (β-LG), the major whey protein, is a source of bioactive peptides but exhibits limited susceptibility to enzymatic hydrolysis due to its compact structure stabilized by disulfide bonds. UV-C irradiation can modify protein conformation and enhance enzymatic accessibility; however, solvent environments may influence these effects. This study evaluated the impact of UV-C processing of β-LG in a hydrated deep eutectic solvent (DES) on subsequent tryptic hydrolysis under conventional aqueous conditions.β-LG variants (A/B) (2 mg mL^{-1}) were prepared in a choline chloride:glycerol DES (1:2 molar ratio, 40% w/w water) and irradiated at 266 ± 4 nm for 2 h. Phosphate-buffered saline (PBS) samples irradiated under identical conditions were used as controls. After treatment, tryptic digestion was performed at pH 7.8 and 37 °C for 120 min. Proteolysis kinetics were monitored by LC–qTOF–MS, with residual β-LG quantified using external calibration and angiotensin I as internal standard. Peptide formation was characterized by broadband collision-induced dissociation mass spectrometry (bbCID–MS).LC–MS analysis revealed variant-dependent digestion behavior. β-LG A showed faster hydrolysis than β-LG B, with no significant effect of prior UV-C processing in the DES. In contrast, DES-treated β-LG B exhibited reduced proteolysis compared with the UV-C-irradiated PBS control. After 120 min, approximately 60% of β-LG B remained intact in DES-treated samples, compared with 40% in PBS controls. Peptidomic profiling further showed lower formation of digestion products, including a threefold reduction in peptide f(142–148). These findings demonstrate that DES-mediated UV-C processing influences β-LG hydrolysis in a variant-dependent manner and highlight the value of LC–qTOF–MS-based foodomics for investigating protein digestibility and peptide release.

Co-authors: Pedro Costa Ferreira and Daniel Rodrigues Cardoso

Akash Singh Chauhan of M4i, Maastricht University

Optimizing mass spectrometry imaging of N-glycans in FFPE lung cancer samples

Glycans are complex carbohydrate structures involved in key biological processes. Alterations in N-glycosylation are strongly associated with cancer and provide valuable information in tumor characterization and biomarker discovery. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) enables untargeted, spatially resolved analysis of biomolecules, including N-glycans, directly from tissue sections. We therefore follow the standard protocol published by X Lu et al. (2023) where formalin-fixed paraffin-embedded (FFPE) tissue sections (5 µm) are mounted on ITO-coated slides, followed by dewaxing and rehydration using sequential xylene and graded ethanol washes. Antigen retrieval is then performed using low pH buffers in a pressure cooker. N-glycans are enzymatically released by spray application of PNGase F followed by an incubation at 37 ºC in humid chamber. Subsequently, CHCA matrix is applied using an HTX M3+ sprayer before the MSI experiment. In this study, we optimized this workflow by varying critical sample preparation steps, including deparaffinization (more xylene washes), antigen retrieval (different buffers), the incubation of the sample with PNGase F in different environments, the number of applied matrix, and different MS instrumentation with the aim to maximize the number and intensity of N-glycan signals while preserving tissue morphology. The optimization led to the detection of ~100 glycan species with enhanced signal intensity on a MALDI timsTOF flex, highlighting its potential for applications in cancer glycomics and clinical biomarker discovery.

Courtney Clough of University of Alaska Fairbanks

Thermal stability and endogeneity of amelogenin and immune proteins in human dental tissues: Implications for biomolecular archaeology and forensic anthropology

Palaeoproteomics enables the study of past health and demographics by leveraging information from proteins preserved in ancient skeletal tissues. This project investigates preservation thresholds and endogeneity of structural and serum immune proteins recovered from ancient dental tissues, using progressive thermal exposure to simulate diagenetic processes in modern dental substrates. Four modern human third molars were quartered and heated under controlled conditions. Enamel and/or dentine samples were then analyzed using nano-liquid chromatography orbitrap tandem mass spectrometry (nano-LC-MS/MS). Raw spectrometry data were processed using PEAKS X-pro. Our analysis indicates that many dental proteins survive up to 350℃, but are denatured below detectable limits by 450℃. Importantly, patterns of thermal decay support the hypothesis that serum immune proteins are endogenous to human enamel and dentine. These results encourage future studies in biomolecular archaeology and forensic anthropology to consider immune proteins preserved in dental tissues as sources of individual and population health data.
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Sara Dobbin of Ulster University

Automated High-Throughput Analysis of Vitamin D Metabolites in Human Serum and Plasma by LC-APCI-MS/MS

A validated method for the quantification of vitamin D metabolites was developed to support the assessment of vitamin D status and the evaluation of targeted interventions across different stages of the life course. Accurate measurement of 25-hydroxyvitamin D_3 [25(OH)D_3] (m/z 401.100 → 383.400 and 365.300) and 25-hydroxyvitamin D_2 [25(OH)D_2] (m/z 413.200 → 395.300 and 159.000) in human plasma requires a robust, reliable, and high-throughput analytical workflow. To improve efficiency, analytical consistency, and reproducibility, sample preparation was automated using a Gerstel Multipurpose Sampler (MPS), integrating internal standard addition (25-Hydroxyvitamin D3-d6), protein precipitation, centrifugation, and miniaturised solid-phase extraction (SPE). Low-volume SPE elution enabled analyte preconcentration prior to offline LC-APCI-MS/MS analysis. The automated workflow eliminated the need for liquid–liquid extraction and PTAD derivatisation, substantially reducing sample preparation time. Cleaner extracts improved chromatographic performance and reduced instrument contamination. This streamlined platform provides a reproducible, sensitive, and high-throughput approach for vitamin D metabolite analysis in serum and plasma samples.

Thi Phuong Thanh DUONG of TU Wien, Vienna, Austria

"Two-Step-One-Pot" Thiol-Selective Enrichment Strategy for In-depth Redox Proteome Profiling

Cysteine oxidation is a central mechanism of redox signalling. However, the comprehensive characterization of these modifications remains limited by the sensitivity and proteome coverage of current mass spectrometry-based redox proteomics workflows. To address these challenges, we developed "Two-Step-One-Pot" cysteine enrichment strategy based on sequential isotopic labelling of reduced and oxidized cysteine residues using thiol-specific click chemistry probes. This approach enables differential cysteine labelling within a single sample, eliminating the need of separating light and heavy tagged replicates and requiring only minimal starting sample amounts. Concomitantly, systematic optimization of labelling and enrichment resulted in improved identification of cysteine-containing peptides. Application of the workflow to redox proteome profiling of cells showed increased sensitivity and improved protein sequence coverage compared to our previously published approaches. In summary, we present a novel platform for quantitative redox proteomics which enables in-depth profiling of cysteine-mediated redox regulation.

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Lisa Geißler of University Hospital Essen

Integrated urine and plasma proteomics identifies molecular signatures of pyelonephritis severity

Pyelonephritis is a severe bacterial infection of the kidney associated with high morbidity and increasing therapeutic challenges due to antibiotic resistance. Here, we investigated the urinary and plasma proteomes of human pyelonephritis cohort (n=53). Urinary proteomic profiling enabled patient stratification according disease severity. Milder pyelonephritis was associated with increased complement activation, elevated antimicrobial peptides (AMPs) level, and leukocyturia, whereas severe pyelonephritis showed signatures of kidney injury and profibrotic remodelling. To achieve deep plasma proteome coverage, we applied an adapted Mag-Net plasma-enrichment workflow, which reduce the suppressive effects of highly abundant plasma proteins such as albumin and enabled the identification of more than 2,000 plasma proteins across the pyelonephritis cohort. The plasma proteome revealed increased abundance of complement protein abundance in severe pyelonephritis, while milder pyelonephritis showed higher endopeptidase activity. These findings highlight the potential of integrated urine and plasma proteomics for disease stratification and precision management of patients with pyelonephritis.

Co-authors: Stephanie Tautges, Hannah Voss, Angelo Novak, Devon Siemes, Florian Wagenlehner, Daniel R. Engel, Olga Shevchuk

Esma Günaydın of Wageningen University

Comprehensive Chemical Characterization of Cowpea Seeds by Targeted, Non-targeted and Imaging Mass Spectrometry

The increasing use of alternative protein sources such as cowpea (*Vigna unguiculata*) raises important questions regarding their safety and nutritional quality. These matrices may simultaneously contain harmful contaminants, such as mycotoxins, pesticide residues and antinutrients, as well as beneficial bioactive compounds, including phenolic compounds.
This project aims to perform a comprehensive chemical characterization of cowpea seeds by combining targeted and non-targeted LC-MS-based approaches. Targeted analysis will focus on known contaminants, including selected mycotoxins and pesticides, while non-targeted metabolomics will be used to explore the broader chemical space, including antinutrients and bioactive compounds. In addition, mass spectrometry imaging (MSI) will be applied to determine the spatial distribution of selected compounds within different anatomical parts of the seed, such as the seed coat, cotyledon and embryo. This spatially resolved analysis may provide novel insights into the co-localization of contaminants and bioactive compounds. The generated data will support improved risk-benefit assessment of alternative protein sources and contribute to the development of more comprehensive food safety evaluation strategies.

Juho Heininen of University of Helsinki

Absolute metabolite quantitation in single cells with LC-MS using isobaric labeling and a carrier

We developed a multiplexed nanoLC-HRMS workflow for absolute quantification of amine metabolites in single cells and applied it to 108 individual HEK-293 and ovarian adenocarcinoma cells (TYK-nu and TYK-nu.CP-r). Cells were isolated using CellenONE, prepared in low-microliter volumes, spiked with internal standards, labeled with TMT10plex, and combined with an abundant carrier channel. Targeted tPRM enabled absolute amino acid quantification, while DDA provided complementary untargeted metabolomics. Intracellular concentrations were calculated by integrating quantified metabolite amounts with single-cell size measurements obtained during dispensing. The method was validated for 10 amino acids, achieving low-attomole detection limits, linear responses from approximately 30 amol to 120 fmol, and correlation coefficients above 0.99. Repeatability was 4.9% at 3 fmol and 18.3% at 0.3 fmol. Millimolar intracellular concentrations were obtained, revealing amino acid differences between cell types. This workflow enables sensitive, multiplexed absolute metabolite quantification in single cells.

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Evgenia Herbold of University of Tübingen

Developability Evaluation of Single-Domain Antibody Chelator Conjugates for Diagnostic Radiotracers

Single-domain antibodies (sdAbs) are emerging as promising therapeutic and diagnostic molecules due to their small size, favorable biophysical properties, and suitability for molecular imaging applications. However, their unique structural characteristics require dedicated developability assessment strategies. This poster summarizes the key findings of our recently published work on the developability assessment of sdAbs and their conjugates. The study combined sdAb generation and screening with comprehensive physicochemical and functional characterization. Analytical approaches included mass spectrometry, nano differential scanning fluorimetry (nanoDSF), dynamic light scattering (DLS), biolayer interferometry (BLI), and size-exclusion chromatography (SEC) shift assays. Together, these methods enabled the assessment of molecular integrity, stability, and target binding properties. The presented workflow provides a structured framework for candidate selection, formulation development, and analytical method optimization for sdAb-based therapeutic and diagnostic applications.

Co-authors: Philipp D.Kaiser, Simon Straß, Sandra Maier, Bjoern Traenkle, and Anne Zeck

Aleksandra Hilliard of Florida State University and National High Magnetic Field Laboratory

Molecular Profiles of Interfacially Active Compounds in Lignocellulosic Pyrolysis Bio-Oils Revealed by Off-line LC, Online LC-MS, and FT-ICR MS

Fast pyrolysis of biomass produces renewable, high-energy fuel precursors referred to as bio-oils. However, large-scale utilization is hindered by complex composition and poor physicochemical stability, which leads to deterioration over time. A primary challenge is the presence of water, as bio-oils are rich in polar compounds that promote formation of water–oil emulsions. These emulsions increase viscosity and delay phase separation, and compromise oil homogeneity, which hinder storage, transport, and refinery upgrading. A relatively small fraction of polar molecules contributes to interfacial behavior and emulsion stability. Thus, isolation and characterization of interfacially active bio-oil species is essential to improve processing and inform targeted demulsification strategies. Ions from the bio-oil and separated fractions were generated via (-) ESI using 21 Tesla FT-ICR MS through both direct infusion and online LC-MS for comparison. The result of these comparisons highlighted the complexity of the bio-oil samples that can be revealed through chromatographic separations.

Co-authors: Olivia C. Moss, Ryan P. Rodgers, Kristina Hakansson, and Martha L. Aguilera

Emily Hughes of National High Magnetic Field Laboratory

Ganglioside isomer differentiation by trapped ion mobility spectrometry combined with anion-electron reactions

Gangliosides are involved in various biochemical processes in the central nervous system, including immune recognition and cellular communication. GD1A and GD1B are disialoganglioside isomers that differ in glycosidic linkage of one sialic acid. GD1A/B have been studied by negative ion mode liquid chromatography-collision induced dissociation tandem mass spectrometry (LC-CID MS/MS). While LC separation is slow, trapped ion mobility spectrometry (TIMS) has been shown to separate ganglioside isomers in milliseconds. Also, CID MS/MS of sialylated ganglioside anions shows poor differentiation between GD1A and B. Electron detachment dissociation (EDD) is an alternative tandem MS activation technique that can provide rich structural information, including cross-ring fragments, from anionic carbohydrate-containing molecules. Here, we demonstrate coupling of TIMS with EDD for ganglioside isomer analysis.

Co-authors: Steven A. DeFiglia, Christopher A. Wootton, Kristina Hakansson

Eleonora Perak Junaković of Croatian Veterinary Institute

Towards MALDI-TOF MS Library Development: Optimization of Sample Preparation for Environmental Filamentous Fungi

Identification of filamentous fungi by MALDI-TOF MS remains challenging due to limited reference library coverage and biological factors. This study compared four sample preparation protocols for environmental isolates of Cladosporium tenuissimum, Penicillium polonicum, and Fusarium temperatum: direct on-target procedure, ethanol–formic acid-acetonitrile extraction using loop- or cotton swab-collected biomass, and bead-assisted protein extraction. Two isolates per species were analyzed, generating a total of 144 MALDI-TOF MS spectra. Spectra were evaluated based on spectral quality, shared dominant peaks, and inter-isolate similarity. Sample preparation had a pronounced effect on MALDI-TOF MS spectral fingerprints. Full extraction using loop yielded the highest inter-isolate similarity for C. tenuissimum and F. temperatum, whereas cotton swab full extraction was optimal for P. polonicum. The number of dominant shared peaks between isolates ranged from 7 to 76, indicating method-dependent differences in fingerprint conservation among protocols. These findings highlight the importance of species-specific optimization for MALDI-TOF MS library development.

Co-authors: Mislav Unger, Irena Reil, Tina Lešić, Marijana Sokolović, Manuela Zadravec

Benjamin Kluwe of Thermo Fisher Scientific (Brno s.r.o.)

Orbitrap Astral Zoom Mass Spectrometer Accelerates Discovery via Enhanced Speed, Sensitivity, Resolving Power and Dynamic Range

The drive to ever higher-throughput quantitative methods demands mass spectrometers selectively process ever greater numbers of co-eluting analyte ions. The Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer, via parallel operation of its accurate Thermo Scientific™ Orbitrap and fast and sensitive Thermo Scientific™ Astral (ASymmetic TRAck Lossless) analyzers, has proven itself a leading platform to meet this challenge. Here we present a new Orbitrap Astral instrument, the, Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometer (Figure 1) with multiple enhancements integrating novel technologies and multiplexed modes of operation that together defeat prior limitations on speed and duty cycle. Orbitrap dynamic range has been enhanced via multiplexing of the mass range into multiple separate injection stages, while a novel multi-pass Astral operation doubles resolving power, enabling fast analysis of Thermo Scientific™ TMTpro™ 32plex labelled peptides.

Co-authors: Hamish Stewart, Johannes Petzoldt, Bernd Hagedorn, Eduard Denisov, Matthew J. Garland, Amelia Peterson, Christopher Rathje, Dmitry Grinfeld, Eugen Damoc, Martin Zeller, Tabiwang Arrey, Anna Pashkova, Julia Kraegenbring, Bernard Delanghe, Arne Kreutzmann, Immo Colonius, Arne Stefes, Ankit Dwivedi, Daniel Mourad, Max Hoek, Yannick Mueller, Till Zickmantel, Philipp Cochems, Robert Ostermann, Maximilian Ochmann, Marek Niemiec, Peter Krueger, Alexander Wagner, Sebastian Kanngiesser, Michael Wiedemeyer, Carla Flosky, Florian Bonn, Anastassios Giannakopulos, Daniel Hermanson, Vlad Zabrouskov, Alexander Makarov and Christian Hock.

Maria Kolonas of University of Parma

Molecular characterization and evaluation of the allergenic potential of duckweed (Lemna minor and Lemna gibba) as novel food

This study investigated how alternative extraction processes, in comparison with conventional ones, impact protein profiles, nutritional value, and allergenic potential of two duckweed species: Lemna minor (LM) and Lemna gibba (LG). The samples analysed for both species were: i) whole plant material (CTRL); ii) conventional extract (C); iii) innovative extract (USUF). Protein content was estimated with Kjeldahl method, while the amino acid (AA) profile was determined by UHPLC-ESI/MS. Protein profiles were characterized via SDS-PAGE and confirmed with High-Resolution Mass Spectrometry (HR-MS). Allergenicity assessment was based on in silico analysis and in vitro immunoblotting using sera from allergic individuals. AA profiles showed that LM contained higher AA contents in both the extracts. All samples met FAO/WHO EAA requirements for children and adults. SDS-PAGE analysis indicated that USUF preserved better protein integrity, whereas C method caused significant protein denaturation. In silico allergenicity assessment highlighted homologies with celery and spinach proteins, known food allergens, suggesting the possibility of cross-reactivity. Finally, in vitro tests confirmed the immunoreactivity of the identified proteins, whose presence was confirmed by HR-MS. The results highlight duckweed potential as an efficient alternative protein source, but also the need for further studies on allergenicity given the observed cross-reactivity.

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Leonarda Lazarin of Ruđer Bošković Institute

Degradation and persistence of lipids from Chaetoceros affinis: Implications for Marine Carbon Sequestration

Diatoms contribute substantially to oceanic carbon sequestration by fixing CO_2 into organic matter and promoting biomass export to the deep ocean through rapidly sinking silica frustules. This process enhances the biological carbon pump and supports long-term carbon storage. Here, we investigate the 14-day degradation of particulate lipids associated with the marine diatom Chaetoceros affinis, cultured under nutrient-replete conditions at 18 °C. The genus Chaetoceros includes some of the most diverse and abundant marine diatoms, while C. affinis is widely distributed across the world's oceans. Because lipids are important carbon-rich biomolecules with potential relevance for long-term carbon preservation, we developed and optimized a HILIC-LC-MS/MS method to characterize lipid composition and transformation. The method separates lipids according to polar headgroup composition, enabling lipid-class quantification. We aim to assess lipid degradation driven by photooxidation and phycosphere-associated bacteria, and determine the fraction resistant to degradation.

Co-authors: Robert Horn, Jelena Godrijan, Blaženka Gašparović

Gillian Leach of University of Victoria

Agnostic Identification of Peptide Modifications from MS1 Data using Mass Defect

Complex mixture analysis remains a significant challenge in mass spectrometry (MS). Kendrick mass defect (KMD) is a powerful data-transformation technique used to sort chemically related ions. However, standard monomer-based KMD calculations confine its applications to polymeric organic compounds with limited use on biological samples where repeating units vary. We developed a novel Discovery Mass Defect (DMD) algorithm that rapidly searches for chemical motifs, transcending prior need to input a monomeric unit for KMD analysis. Once discovered, chemical units are employed to identify modified/unmodified ion pairs. As a proof-of-concept, a mass list from an LC-MS/MS analysis of acetylated BSA peptides produced by trypsin digest was input. The DMD algorithm correctly identified acetylation as the primary modification. This sample-agnostic method facilitates discovery of low-abundance or unexpected species that may be missed by conventional database-driven tandem MS-centric proteomic workflows. Based on our preliminary data, the concept is applicable to de novo characterization of any molecular class.

Co-authors: Abanoub Mikhael, Darryl Hardie, Derek Smith, Helena Pětrošova, David R. Goodlett

Linglan Li of Maastricht University

Extending High-Mass Performance in Orbitrap-Based CDMS using FTMS Booster-Enhanced Acquisition and Isotope Depletio

Orbitrap-based Fourier transform mass spectrometry (FTMS), particularly using the Q Exactive Ultra-High Mass Range platform, enables high-resolution analysis of large biomolecular assemblies. However, increasing molecular weight results in broadened isotopic distributions, reduced signal-to-noise ratio, and increased spectral complexity, limiting accurate mass determination for heterogeneous systems. Here, we present a strategy combining FTMS Booster-enhanced charge detection mass spectrometry (CDMS) with isotope depletion to extend high-resolution analysis into the high-mass regime.
Three protein standards (29–66 kDa; carbonic anhydrase, streptavidin, and bovine serum albumin) were analyzed on a modified Q Exactive UHMR Orbitrap coupled to an FTMS Booster X2. Survey scans identified suitable charge states, followed by single-charge-state isolation and long-transient CDMS acquisition. Data were processed using Peak-by-Peak software with signal filtering and calibration. FTMS Booster-enabled CDMS improved spectral resolution across all proteins compared to standard acquisition. Isotopic resolution was observed for lower-mass proteins, while larger systems showed reduced resolution and statistical limitations. Ongoing isotope depletion experiments are expected to further reduce spectral complexity and enhance isotopic resolution for high-mass proteins.

Co-authors: Linglan Li, Sneha S. Hakke, Anton N. Kozhinov, Konstantin O. Nagornov, Yury O. Tsybin, Anjusha Mathew and Ron M.A. Heeren

Pei-Chen Lin of National Taiwan Normal University

Integrated SWATH-MS Platform for In-Depth Profiling of Microplastics-Induced Hepatic Molecular Alterations

Microplastics (MPs) are pervasive environmental contaminants, yet their impact on hepatic molecular networks remains incompletely characterized. In this study, we implemented an integrated analytical platform combining SWATH-MS proteomics and dual-mode (Amide–F5) LC–MS metabolomics to systematically profile hepatic alterations following PS-MP exposure. By leveraging an orthogonal two-dimensional and dual-mode separation strategy, we achieved enhanced coverage depth quantification of 2479 proteins and 976 metabolites. Pathway convergence analysis revealed alterations in oxidative phosphorylation and amino acid metabolism, delineating a coherent hepatic molecular signature. The synergy between high-depth proteomic profiling and synchronized metabolomic characterization facilitated the detection of coordinated remodeling across mitochondrial respiratory chain complexes. Our results demonstrate that this integrated approach provides comprehensive data for bioenergetic stress induced by MPs, establishing a robust analytical platform for assessing the potential biological risks of environmental stressors in complex biological systems.

*Co-authors: Kuan-Lu Wu, Sung-Fang Chen**

Caroline Lööf of Chalmers University of Technology

Chemical Signatures of the Prostate Tumor Ecosystem Revealed by Mass Spectrometry Imaging

The tumor microenvironment comprises complex interactions between malignant and stromal cells that drive cancer progression, and characterizing its biochemical landscape remains challenging. Mass spectrometry imaging (MSI) enables label-free mapping of molecules directly in tissue, providing spatially resolved insights into tumor heterogeneity. Human prostate tissue sections (tumor and adjacent normal) were analyzed using Matrix-Assisted Laser Desorption/Ionization-Fourier Transform Ion Cyclotron Resonance MSI. Principal component analysis and receiver operating characteristic analysis demonstrated clear separation between the regions, with lipids contributing strongly to the distinction and several features showing high discriminatory power. These results demonstrate that high-resolution mass spectrometry imaging can reveal spatially resolved biochemical signatures of tumor biology. The work also supports broader efforts to improve cancer diagnostics through accessible, non-invasive sampling strategies such as hair analysis, with the aim of earlier detection and more sustainable healthcare.

Co-authors: Kenneth J. Pienta, Emma U. Hammarlund and Per Malmberg

Kate McMurray of University of Victoria

Ultra-Sensitive NanoLC-MS Assay Enables Amino Acid Quantitation in Low-Input Cancer and Immune Cell Populations

Amino acids play diverse roles in cancer and immune cells, acting as carbon/nitrogen sources for tumor proliferation, regulators of redox balance, and T-cell function modulators. It is therefore important to analyze these cell populations separately, but these subsets can yield limited cell numbers, often below the 1,000,000 cells required for conventional LC-MS metabolomics. We developed an ultra-sensitive amino acid quantitation assay using nanoLC-MS, employing a custom column configuration to balance sensitivity, isomer resolution, precision, and throughput. The assay demonstrated high-sensitivity detection in the low femtomole range, excellent linearity (R2 > 0.995) across > 250-fold concentrations for each analyte, and improved sensitivity by an average of 100-fold compared to UPLC. Across cancer and immune cell lines, 95% of analytes remained precisely quantifiable (CV < 15%) in 10,000 cells with 67% quantifiable in only 1,000 cells. This breakthrough enables amino acid quantitation in low-input samples and the study of cell-type-specific metabolic adaptations previously inaccessible to standard methods.
Co-authors: Darryl Hardie, Jessica Morgan, Jun Han, Julian J Lum, David R Goodlett

Karolina Mermer of Selvita S.A.

Selecting the right lipophilicity assay in drug discovery: A comparison of shake-flask UV-VIS, shake-flask LC-MS/MS and ChromlogD

Lipophilicity, commonly characterised by the distribution coefficient (logD) at physiological pH, is a key determinant of membrane permeability, plasma protein binding, tissue distribution and overall pharmacokinetic behaviour of drug candidates. While the shake-flask (SF) assay remains the reference method, its throughput and applicability are limited, leading to increasing reliance on chromatographic and mass spectrometry (MS)-base approaches in early drug discovery. The analytical detection strategy increasingly impacts data quality, with MS-based techniques offering enhanced sensitivity and selectivity for structurally diverse compounds. This study compares three complementary approaches for lipophilicity determination: SF coupled with UV-VIS detection, SF coupled with LC-MS/MS detection, and chromatographic logD (ChromlogD) method supported by MS confirmation. A set of reference drugs covering diverse physicochemical properties and ionisation classes was selected. Initial results demonstrate strong overall agreement between UV-VIS and LC-MS/MS measurements across a broad logD range, alongside compounds-dependent differences reflecting the influence of detection strategy. This study evaluates how MS-based detection enhances specificity and dynamic range, while addressing ionisation and matrix related biases in logD determination. The comparison of orthogonal approaches to lipophilicity determination may provide valuable insight into the relationship between experimentally derived logD values. The advantages and limitations of UV-VIS, LC-MS/MS and chromatographic methodologies are discussed in the context of drug discovery workflows.

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Salazar Paloma Munguía of University of Exeter

A Distinct Semiquinone Conformation in ErCry4a Supports a Signaling-Competent Magnetoreceptor State

In the migratory bird European robin, cryptochrome 4a (ErCry4a) has been proposed as the primary magnetoreceptor, where magnetic field sensitivity is thought to arise through the radical pair mechanism. However, a major unresolved question is how the quantum information encoded in radical pair spin dynamics is converted into a biological signal. If signal transduction occurs through protein conformational changes, a signaling-competent state must adopt a distinct structure capable of interacting with downstream signaling partners. The semiquinone state has long been considered the most likely candidate for this role; however, whether it exhibits a conformation distinct from the other states of the photocycle has remained unknown. Here, we used hydrogen–deuterium exchange mass spectrometry (HDX-MS) to characterize structural dynamics across different redox states of ErCry4a. Our results reveal that the semiquinone state displays a unique conformational signature, providing direct evidence that it adopts a distinct structural ensemble and supporting its proposed role as the biologically active signaling state involved in magnetoreception.

Co-authors: Monika Kish, Suchitra Pradhan, Jessica L. Ramsay, Paloma Munguía Salazar, Jonathan Phillips, Daniel R. Kattnig (paper en arXiv https://arxiv.org/abs/2604.19579)

Kinga Ozga-Wybranowska of The John Paul II Catholic University of Lublin.

Development and Validation of an LC-MS/MS Method for the Determination of Kynurenine Pathway Metabolites in Biological Samples from Gastric Cancer Patients

Kynurenine pathway metabolites play an important role in the regulation of immune processes and are involved in the pathogenesis of numerous diseases, including cancer. An increasing number of studies indicate their potential utility as diagnostic and prognostic biomarkers. However, the simultaneous analysis of multiple kynurenine pathway metabolites in biological samples remains analytically challenging due to their low physiological concentrations, high polarity, and limited stability. This study presents preliminary results concerning the development and validation of an LC-MS/MS method for the determination of a panel of kynurenine pathway metabolites in biological samples obtained from patients with gastric cancer using high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).[

Co-authors: Ilona Sadok

Ema Pavičić of Faculty of Food Technology Osijek

Effects of sample preparation and acquisition parameters on CD19+ lymphocytes' MS imaging

Single-cell MALDI-TOF MS imaging (MSI) requires optimised sample preparation and acquisition parameters to maximise sensitivity. This study evaluated the effects of washing buffer, matrix deposition time, and acquisition settings on the discrimination of human CD19+ lymphocytes from the medium. The sample preparation procedure included a washing step for CD19+ lymphocytes using either phosphate-buffered saline (PBS) or CellPack (Sysmex, Japan). CHCA matrix was applied onto the ITO-coated slides by sublimation for 2.5 or 5 minutes. MSI data were acquired using an iMScope TRIO instrument with 10 µm lateral resolution. Two acquisition modes, 10 Hz/10 shots and 20 Hz/20 shots, were evaluated, yielding eight experimental conditions. Principal component analysis of recorded single-cell spectra was used to assess the separation between cell and medium regions of interest (ROIs). The optimal performance was achieved using PBS washing, 2.5 minutes matrix deposition, and acquisition at 20 Hz/20 shots. Results indicate condition-dependent differences in ROI clustering.

Co-authors: Ivana Marković, Željko Debeljak

Bastiaan Poetsma of HFML-FELIX

Structure determination of mass selected ions: from multiple photon to cryogenic ion spectroscopy at an IR-FEL facility

The vibrational modes of molecules are unique identifiers of their structure, which can be probed using IR spectroscopy. At HFML-FELIX, broadband and intense infrared Free-Electron Lasers are coupled with various tandem mass spectrometers for gas-phase ion spectroscopy. Direct absorption spectroscopy is not possible due to the low density of charged particles in the gas-phase. Therefore, action-spectroscopic methods are used. Here, we present the application of InfraRed Multiple Photon Dissociation (IR-MPD) to measure the IR spectra of cationic cobalt clusters (CoxSx; x=2,3,4,6) in a room temperature FT-ICR mass spectrometer. However, this technique requires high internal energies resulting in broadened spectral lines and non-linear effects. To achieve higher spectral resolution it is necessary to use more refined methods such as IR predissociation of tagged complexes or leak-out spectroscopy. Here, we outline our plans for a new cryogenic ion trap combining ion mobility, tandem mass spectrometry and IR spectroscopy.

Co-authors: Sandra Brünken, Joost M. Bakker, Jos Oomens

Svatava Polsterova of Thermo Fisher Scientific

A novel Ion Processor Device for High-Throughput Analysis in a High-Resolution Mass Analyzer

Ion accumulation devices are routinely used to shape ion packets prior to pulsed extraction into an analyzer. A prominent example is the C-Trap coupled to a Thermo Scientific™ Orbitrap™ analyzer. Compared to conventional orthogonal extractors found in Q-ToF instruments, these benefit from vastly higher duty cycle but require a compromise between buffer gas pressure to capture and thermalize ions and minimized fragmentation and scatter during extraction. A novel ion accumulation and extraction device formed of two pressure regions for parallel accumulation/fragmentation and extraction is introduced. It has a unified, phase-locked RF pseudopotential channel for seamless transfer of pre-cooled analyte ions to overcome common limitations of ion transfer energy, cooling time, device size and buffer gas pressure. Axial accumulation and movement are performed via DC electrodes, allowing fast movement/processing at reduced pressures.

Co-authors: Hamish Stewart, Eduard Denisov, Alexander Wagner, Alexander Kholomeev, Dmitry Grinfeld, Alexander Makarov and Christian Hock

Alexander Schaefer of Thermo Fisher Scientific (Bremen)
High-Throughput Analysis with Parallelized Pre-Accumulation

Pre-accumulation adds a parallel ion-processing step to Thermo Scientific™ Orbitrap™ Excedion™ Pro and Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometers by trapping ions in a curved quadrupole ion guide between isolation stages. This enables ion collection during downstream processing, increasing effective injection time, duty cycle, and sensitivity without reducing acquisition speed. Performance was evaluated using FlexMix™ calibration solution and high-throughput DIA proteomics of HeLa or HEK digests. On Orbitrap Excedion Pro, pre-accumulation more than doubled sensitivity at >40 Hz Orbitrap operation, enabled >70 Hz repetition rates, and increased duty cycle to >95%. In short-gradient DIA, identification gains were strongest at low sample loads and complemented Phi-SDM signal processing. On Orbitrap Astral Zoom, pre-accumulation increased detected ions by 44% during 270 Hz DIA, maintained ~80% MS/MS duty cycle, and delivered >8100 protein groups from 200 ng HEK at 300 samples per day, representing a 1.7-fold throughput increase versus Orbitrap Astral.

Co-authors: Hamish Stewart, Bernd Hagedorn, Eduard Denisov, Yannick Mueller, Johannes Petzoldt, Tabiwang Arrey, Eugen Damoc, Julia Kraegenbring, Kyle L. Fort, Erik Couzijn, Jan-Peter Hauschild, Florian Harking, Ulises H. Guzman, Jesper V. Olsen and Christian Hock

Agnieszka Słodkowska of The John Paul II Catholic University of Lublin
Effect of Copper Ions on Biomimetic Bacterial Membranes: A ToF-SIMS Study

The development of antibacterial biomaterials is of particular importance for medical devices susceptible to bacterial colonization and biofilm formation. In this study, the influence of copper ions, known for their antimicrobial properties, on a biomimetic bacterial membrane model was investigated. Monolayers of sodium salt of 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG), representing a model bacterial membrane, were transferred onto nitinol (NiTi) wires using the Langmuir-Blodgett technique. The transfer process was carried out from aqueous subphases containing copper ions at different concentrations. The deposited films were subsequently characterized using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) to evaluate the chemical composition, molecular organization, and distribution of membrane components on the NiTi surface. Particular attention was paid to the interactions between copper ions and DPPG molecules, as well as their influence on membrane integrity. The obtained results provide insight into the molecular mechanisms underlying copper-induced modifications of bacterial membrane models and may contribute to the design of advanced antibacterial coatings for biomedical applications.
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Chloe Spencer of University of Nottingham

Carbene Footprinting: Mapping Protein Interaction Sites

Identifying and understanding protein-protein and protein-ligand interactions is crucial to the development of drug candidates, biotherapeutics and agrochemicals. Therefore, Carbene Footprinting was developed to map these interactions. We use a diazirine-based label, which upon irradiation with near-UV light undergoes photolysis to yield a carbene, which irreversibly reacts with the target protein within nanoseconds. The reaction between the label and the accessible protein surface causes an increase in mass, which can be observed by nanoLC-MS/MS analysis via a bottom-up proteomic workflow. Herein, we describe a case study that uses Carbene Footprinting to show whether VRT-043198 binds to Caspase-1 via a non-covalent interaction with C285A mutant Caspase-1. Masking of the protein was observed around the binding site of VRT-043198 and wild-type Caspase-1, indicating that despite the substitution of the catalytic cysteine for alanine (C285A), a non-covalent interaction occurred. This study highlights the effectiveness of Carbene Footprinting as a technique to map protein-ligand.

Co-authors: Dr Harry Taylor, Professor Neil Oldham

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Automation and Optimization of SP3-based Sample Preparation and Phosphopeptide Enrichment for High-Throughput Phosphoproteomics Studies

This project will develop and optimize automated SP3-based protein sample preparation and phosphopeptide enrichment workflows on Agilent Bravo and KingFisher magnetic-particle robots. We will determine optimal starting volumes and protein inputs for both platforms and benchmark automated protocols against manual preparations. Protocols will be adapted to flexible throughput (12–96 samples per run) and validated across diverse biological sample types. Additionally, we will create automated phosphopeptide enrichment on the Bravo, optimized for low-input samples (1–100 µg). Outcomes include robust, standardized, facility-wide SOPs, performance metrics, and validated workflows enabling scalable high-throughput phosphoproteomics of organoids and other complex samples currently limited by time and resources. These optimized, automated methods will increase reproducibility, reduce hands-on time, and expand the facility's capacity for comprehensive phosphoproteomic studies. We will quantify recovery, reproducibility, and phosphopeptide coverage, and provide detailed SOPs, troubleshooting guidance, and training materials to ensure broad adoption across the facility and future studies.

Mohd Umar Tabrez of University of Turku

Proteomic profiling of Extracellular Vesicles associate with disease activity in Systemic Lupus Erythematosus

Extracellular vesicles (EVs) are emerging as a key regulators of immune system and have been implicated in autoimmune diseases, including systemic lupus erythematosus (SLE). We aimed to evaluate their relationship with disease activity, and characterize EV protein cargo.Serum EVs were isolated by ultracentrifugation from 14 patients with SLE and 10 controls, characterized and validated by nanoparticle tracking analysis (NTA) and immunoblotting. EV protein was profiled using data independent acquisition (DIA)-LC MS/MS. Patients with SLE showed significantly higher circulating EV concentrations than controls ($p = 0.0176$), primarily driven by patients with high disease activity. Proteomic analysis identified 954 proteins. High disease activity was associated with higher proteomic alterations and enrichment of innate immune, neutrophil, platelet, and homeostasis pathways, as well as immune cell and tissue signatures. In conclusion, Circulating EV abundance and protein cargo closely reflect SLE disease activity, supporting EVs as potential biomarkers for SLE.

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Extending Protein Sequence Coverage in Orbitrap-Based Top-Down Proteomics Using FTMS Booster-Enhanced Acquisition and Isotope Depletion

Introduction. Native top-down mass spectrometry (nTDMS) on Orbitrap platforms provides a powerful view into intact protein biology. It maintains native folding, non-covalent stoichiometry, and post-translational modifications (PTMs) all at once, without losing sequence information. However, sequence coverage remains a key limiting factor, especially as protein mass increases. We address these challenges through the combination of isotope depletion [1] and FTMS Booster-extended transient acquisition [2]. These methods are expected to enhance sequence coverage for larger protein molecules. Methods. We systematically benchmarked coverage performance using three protein standards spanning a mass range of approximately 29–66 kDa: carbonic anhydrase II (~29 kDa), streptavidin (~53 kDa, as the intact homotetramer), and bovine serum albumin (BSA, ~66 kDa). This range was selected to observe the transition from the mass domain, where top-down coverage is feasible under standard conditions, to where it becomes more challenging. All proteins were buffer-exchanged into a native MS-compatible buffer and analyzed on a modified Q Exactive UHMR (Ultra High Mass Range) Orbitrap mass spectrometer coupled with FTMS Booster X2, using nano-electrospray ionization (nESI). Spectra were acquired at a resolving power of 200,000 (defined at m/z of 400), and top-down MS/MS was conducted using higherenergy collisional dissociation (HCD) with normalized collision energy (CE) varied from 20 to 200 eV to determine the optimal conditions for each protein. AutoVectis was used to generate sequence coverage maps. Preliminary Results. Top-down HCD fragmentation under standard acquisition conditions confirms the expected relationship between precursor mass and sequence coverage. The streptavidin monomer (~13 kDa) achieved 90% coverage, while carbonic anhydrase II (~29 kDa) reached 86%, and BSA (~66 kDa) yielded 66%. As protein size increases, isotopic peaks broaden, and the quality of fragment ion signals decreases, complicating confident fragment ion assignment and confirming the baseline of our project. The next step involves repeating these experiments using FTMS Booster-enhanced acquisition and isotope-depleted samples to directly measure the improvements in coverage contributed by each method.

References

[1] Gallagher, K. J. et al. J. Am. Soc. Mass Spectrom. 31, 700–710 (2020).
[2] M. S. Silva and C. Cordeiro, Analytical Science Advances 6, no. 2 (2025):
[3] Popovic, Z., et al. J Am Soc Mass Spectrom 2023, 34, 137–144.
[4] Polák, M., et al. Analysis. Anal Chem 2024, 96, 1478–1487.
[5] Denisov, E., et al. Int J Mass Spectrom 2021, 116607.

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Marijke Vermuyten of SGS - Institute for Applied Chromatochraphy
Multi-Compound Analysis in Environmental Matrices Using LC-MS/MS

My work as a senior analyst at SGS within the Institute of Applied Chromatography focuses on environmental analysis using LC-MS/MS. Both targeted and non-targeted approaches are applied to a wide range of compounds such as: PFAS, pesticides, detergents, active pharmaceutical ingredients and small organic compounds. The primary matrices investigated are environmental samples such as wastewater, surface water, ground water, soil and industrial waste materials. In smaller quantities, matrices intended for human and animal consumption are also investigated. For targeted methods we mainly use Triple Quads from Waters, for non-targeted workflows we use high-resolution instrumentation from Sciex and Agilent. At the Institute of Applied Chromatography we aim to provide our customers with solutions for routine and non-routine contaminant problems in complex matrices. With our broad experience and a wide range of complementary analytical techniques, such as GC-FID, GC-MS, HRGC, LC-UV, LC-MS/MS and ICP, tailored analytical solutions can be delivered to meet diverse customer needs.

Petra Votavova of Thermo Fisher Scientific (Brno, Czech Republic)
Enabling up to 270Hz Acquisition Seed on the Orbitrap Astral Zoom MS

High-throughput experiments demand shorter gradients to analyze more complex samples in a short amount of time. The Thermo Scientific Orbitrap Astral MS acquires up to 200 MS/MS scans per second, allowing for sufficient points across the LC peak even at a throughput of 180 samples per day. To further increase this number while maintaining quantitative accuracy, an even higher acquisition rate is highly desired. The Astral analyzer is hereby mostly limited by the switching times of ion optics and the transfer of ions through the instrument. We present a new version of the Orbitrap Astral MS, the Thermo Scientific Orbitrap Astral Zoom mass spectrometer with optimized ion filter and quadrupole components, as well as faster ion transfer to enable higher scan speeds for data independent acquisition (DIA) and data-dependent acquisition (DDA) applications. Additional pre-accumulation in the Bent Trap is utilized to improve the overall duty cycle of the instrument.

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Mass spectrometry on intact biomolecules: a pivotal tool for studying proteoforms and macromolecular complexes

Intact protein mass spectrometry (MS) and Native MS are complementary approaches to peptide-level proteomic workflows, adding invaluable structural insights.
Intact protein MS allows gas-phase interrogation of an intact protein in its denatured state. This enables in-depth characterization and differentiation of proteoforms. For instance, it is uniquely suited for profiling monoclonal antibodies. When coupled with middle-up workflows (IdeS digestion, reduction, deglycosylation), Intact protein MS successfully maps differential glycosylation patterns and identifies chain-pairing variants. Beyond looking at single biomolecules, Native MS has emerged as a pivotal tool in structural biology. It preserves intra- and inter-molecular non-covalent bonds, allowing the study of macromolecular complexes. Native MS's broad scope encompasses oligomerisation state of a complex, ligand competition, binding dynamics and can even study membrane macromolecular assemblies. It has proven valuable for investigating NME1, an ATP-sensitive AcCoA carrier, demonstrating that CoA binding and ATP-driven histidine phosphorylation are mutually exclusive events. Native MS can capture weak, transient, or heterogeneous interactions, thereby complementing cryo-EM. Ultimately, Intact-level MS techniques provide essential information about proteoform heterogeneity and biomolecule interactions. They constitute a powerful toolset for validating in silico prediction models such as AlphaFold and confirming the stoichiometry of assemblies.

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Polypeptide Size Affects Tyrosine-O-Sulfation Retention in Electron-Based Dissociation

Tyrosine-O-sulfation is an important post-translational modification involved in protein–protein interactions, but its analysis by mass spectrometry remains challenging due to extensive SO_3 loss during activation. We systematically investigated the effect of polypeptide size on sulfate retention using bovine fibrinogen as a model system. Reduced, alkylated fibrinogen was digested with trypsin or Arg-C for bottom-up analysis, while PNGase F-deglycosylated fibrinogen chains were analyzed by top-down LC-MS/MS. Peptides and proteins were subjected to CID, HCD, ETD, and proton transfer reaction (PTR) on an Orbitrap Eclipse instrument. Sulfopeptides ranging from 1.6–3.1 kDa showed complete SO_3 loss in CID/HCD and partial sulfate retention in ETD, with retention increasing with peptide size. In contrast, intact ~50 kDa fibrinogen β and γB chains exhibited complete sulfate retention during ETD and partial retention during CID. Optimization of ETD/PTR conditions increased sequence coverage from 1% to 28% without detectable SO_3 loss. These results demonstrate a size dependence of sulfate retention and provide a for improved localization of tyrosine-O-sulfation in proteins.

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HPLC-ESI-IT-MS^n Characterization of Flavonoids and Naphthopyranones in Paepalanthus chiquitensis and Their Mutagenic Effects

A rapid and sensitive HPLC-ESI-IT-MS^n method was developed for the characterization of secondary metabolites in Paepalanthus chiquitensis Herzog. Using high-performance liquid chromatography coupled with electrospray ionization multistage ion-trap mass spectrometry in negative-ion mode, 24 compounds were identified and characterized in methanolic extracts of capitulae and scapes, including 18 flavonoids (flavonol and flavanonol derivatives) and six naphthopyranones. The method enabled efficient metabolite profiling without the need for prior isolation of reference compounds, providing valuable support for chemotaxonomic studies and chromatographic fingerprinting. Mutagenic activity of the methanolic extracts was evaluated using the Salmonella/microsome assay. Both capitulae and scape extracts showed mutagenic effects against Salmonella typhimurium strain TA97a, suggesting the presence of compounds capable of inducing frameshift mutations. The observed activity was associated with the occurrence of naphthopyranones and quercetin derivatives in the extracts.

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Interspecies Variations in the Metabolism of Altenuene and Tentoxin in Primary Hepatocytes

Alternaria fungi produce structurally diverse mycotoxins, including altenuene (ALT) and tentoxin (TEN), which have been detected in various food products. Although both toxins are considered non-genotoxic, studies have reported adverse effects, including ALT-induced cytotoxicity and TEN-associated hepatotoxicity. However, their toxicodynamics and particularly their metabolism remains poorly understood, with existing studies providing only limited toxicokinetic data.
This study, conducted within the European Partnership for the Assessment of Risks from Chemicals (PARC), investigated the metabolism of ALT and TEN in primary rat and human hepatocytes. Cells were incubated with 10 µM ALT or TEN for up to 4 hours, followed by LC-MS/MS analysis to quantify the parent compounds and semi-quantify known and predicted metabolites. Cell culture experiments were performed at the French Agency for Food, Environmental and Occupational Health & Safety, while analytical method development and measurements were carried out at the University of Vienna.
Rat hepatocytes exhibited faster metabolism than human hepatocytes. TEN was primarily metabolized via phase I reactions, whereas ALT predominantly underwent phase II conjugation, particularly glucuronidation and sulfation. These findings improve the understanding of ALT and TEN toxicokinetics and provide valuable data for future physiologically based toxicokinetic (PBTK) modeling and toxicological assessment of their metabolites.

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