DGMS
Deutsche Gesellschaft für Massenspektrometrie

SFSM
Société Française de Spectrométrie de Masse

## European Mass Spectrometry Conference

# EMSC 2018

Saarbrücken • Germany • 11-15 March

# Program - Abstracts


*35ème Journées Françaises de Spectrométrie de Masse*
*51. Jahrestagung der Deutschen Gesellschaft für Massenspektrometrie*

## Welcome to Saarbrücken

## European Mass Spectrometry Conference EMSC2018

On behalf of the French and German mass spectrometry societies - La Société Française de Spectrométrie de Masse (SFSM) and the Deutsche Gesellschaft für Massenspektrometrie (DGMS) - we have the great pleasure to invite you to the European Mass Spectrometry Conference (EMSC), taking place between 11 to 15 of March 2018. **The EMSC incorporates the 35$^{th}$ SFSM and the 51$^{st}$DGMS annual mass spectrometry conferences**. For the first time, the SFSM and DGMS are organizing a joint conference on mass spectrometry and its applications. This conference is open to any MS scientist worldwide, with a focus on mass spectrometrists from Europe, to attend this joint French/German initiative. We hope that other national MS societies from Europe will continue this format in the future, to foster a regular European MS exchange.

This meeting will be held from Sunday evening, 11 March, through Thursday after lunch, 15 March 2018 in Saarbrücken (Germany), which is right on the border to France. Saarbrücken is located in the heart of Europe, easily accessible from almost everywhere in Europe. There will be several short courses on Sunday before the conference officially opens on 11 March.

We will have 12 plenary lectures, 18 sessions of contributed talks, accompanied by poster presentations, and an instrument vendor exhibition in the field of mass spectrometry and its applications. This is a unique opportunity to build new or reinforce existing scientific exchanges and collaborations, and to create new links at the European and international level. The conference equally welcomes scientists from academia, government, industrial and non-profit research organizations, instrument vendors as well as the industry and the private sector, working on fundamental research, instrumentation, sample preparation, data processing and applied aspects of mass spectrometry.

We hope to see you in Saarbrücken at the EMSC 2018.

Sincerely,

Frédéric Aubriet, Vincent Carré and Dietrich Volmer, Conference Chairs

on behalf of the organizing committee

## Local organizing committee - Scientific committee

**Marc Angotti** Luxembourg Institute of Science and Technology, Luxembourg

**Frédéric Aubriet** LCP–A2MC, Université de Lorraine, France

**Vincent Carré** LCP–A2MC, Université de Lorraine, France

**Patrick Chaimbault** LCP–A2MC, Université de Lorraine, France

**Cédric Guignard** Luxembourg Institute of Science and Technology, Luxembourg

**Carsten Hopf** ABIMAS, Mannheim University of Applied Sciences, Germany

**Emmanuelle Leize-Wagner** LSIMS, Université de Strasbourg, France

**Hans H. Maurer** Experimental & Clinical Toxicology Department, Saarland University, Germany

**Markus R. Meyer** Experimental & Clinical Toxicology Department, Saarland University, Germany

**Gereon Niedner-Schatteburg** Fachbereich Chemie, Technische Universität Kaiserslautern, Germany

**Cédric Paris** LIBio, Université de Lorraine, France,

**Dietrich Volmer** Institute of Bioanalytical Chemistry, Saarland University, Germany

# Miscellaneous information

## Registration desk

Registration desk opening hours

March 11: 13h00 – 19h00
March 12: 8h00 – 12h00
March 13: 8h00 – 12h00
March 14: 8h00 – 12h00

On-site registration is open Sunday 11 and Monday 12.

## Certificate of attendance

Certificates are available, only upon request, at the registration desk.

## Luggage storage service on Thurday 15

A luggage storage service is ensured from 8:00 AM to 1:30 PM.

## Oral presentations

Presentations must be uploaded on the computers provided by EMSC2018, at least 30 minutes before the beginning of each session. Slide format may be 16:9 (preferred) or 4:3.

## Poster presentations

For each session (see the program), the posters must be displayed before 8:30 AM and removed before 7:00 PM, at latest. Push pins will be provided.

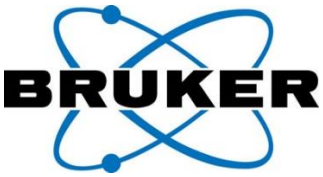
## Gala Dinner

Bus departure at 17:30 sharp. Bring your badge. Take also warm clothes for the visit will start at 18:00, followed by the dinner.

For specific dietary only (without nuts, without gluten, without meat, vegan, vegetarian) please give the information to the registration desk before Monday 12 at noon. The menu is without pork.

## Sponsors and partners

# Sponsor Platinium






# Sponsor Silver









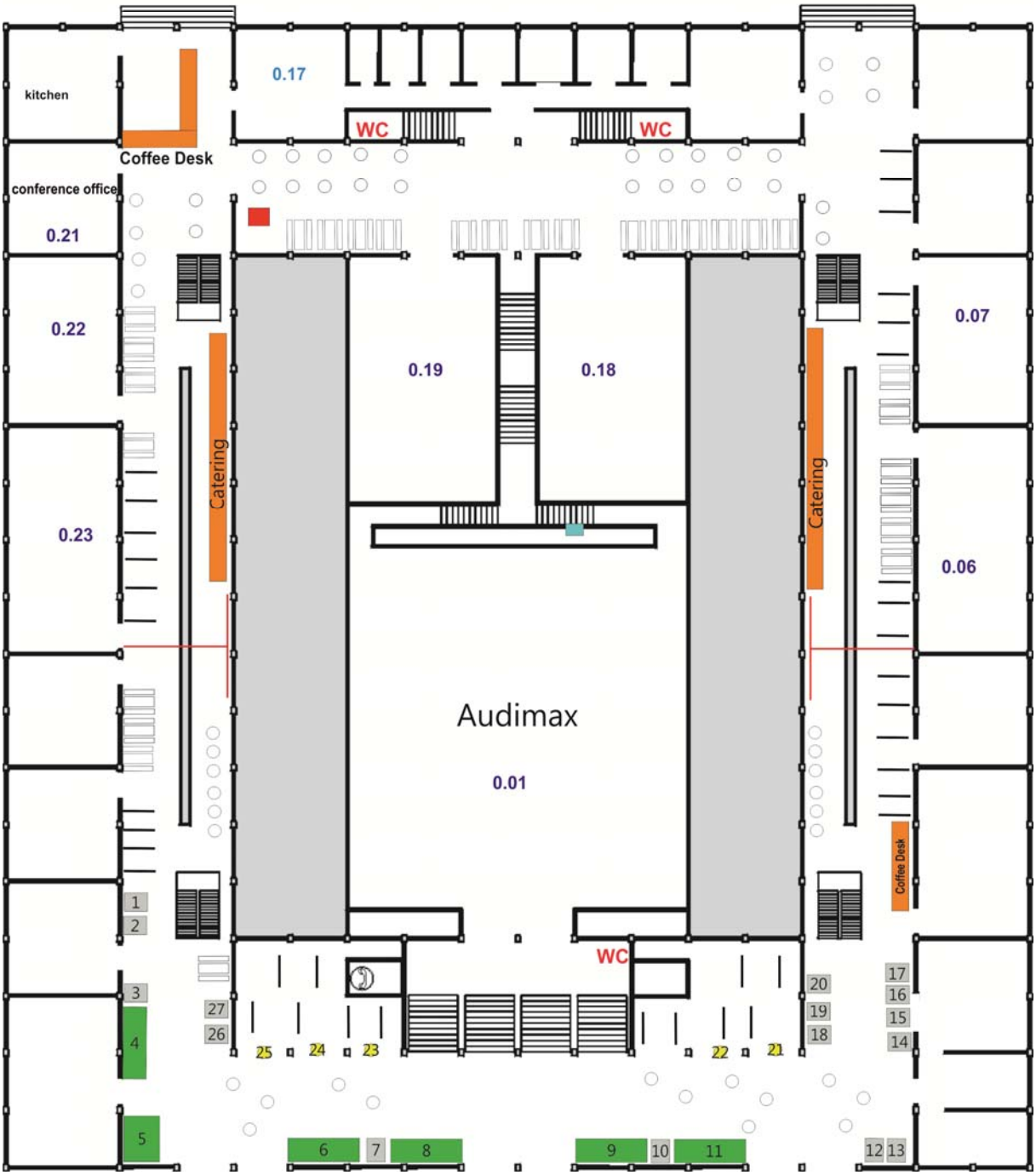


**Partners:** IonTOF, KR Analytical Ldt., Pfeiffer Vaccuum

## Institutional Sponsors

## Media Partners

# EMSC 2018
## 11-15 March 2018

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SFSM
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1st European Mass Spectrometry Conference
EMSC 2018
Saarbrücken • Germany • March11-15

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| 1 | Prolab Instruments GmbH |
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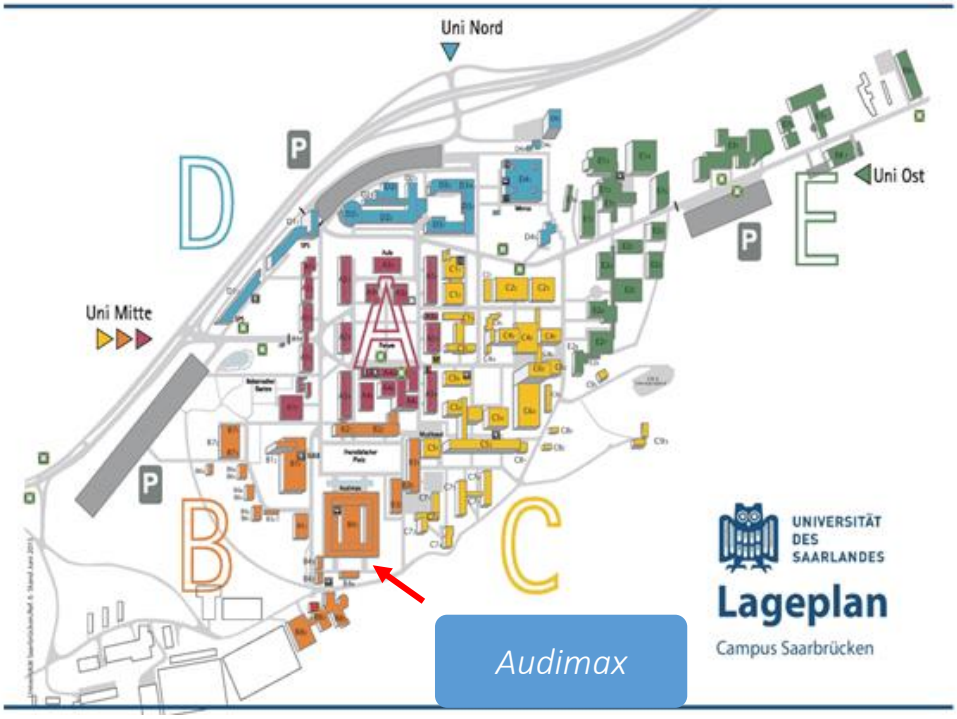
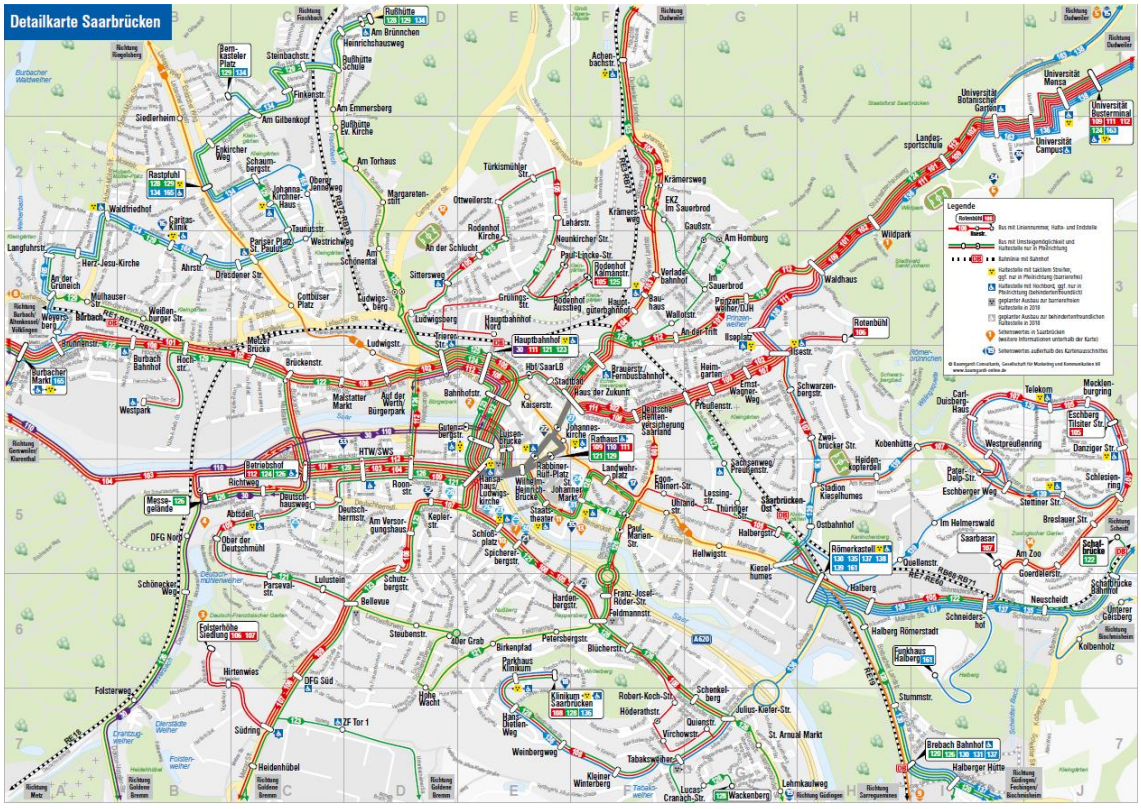
## Saarbrücken campus




## Bus services to Saarbrücken campus

To get from Saarbrücken central station to the university campus, take either bus number 102 (to 'Dudweiler-Dudoplatz') or bus numbers 112 or 124 (to 'Universität'). These services run every 30 minutes.



# Program

## Sunday, 11 March 2018

| | | |
|---|---|---|
| 13:00 - 19:00 | | **Registration** |
| **13:30 - 16:30** | | **Workshops** |
| | 13:30 - 16:30 | **1. Computational and bioinformatics tools for proteomics (0.22)** – Martin Eisenacher, Dominik Kopczynski and David Bouyssié |
| | 13:30 - 16:30 | **2. Novel and established approaches to high-performance mass spectrometry imaging (0.23)** – Martin Eisenacher, Dominik Kopczynski and David Bouyssié |
| | 13:30 - 16:30 | **3. Cross-linking and HDX-MS: Towards the study of interacting domains in biological complexes (0.07)** – Noëlle Potier |
| | 13:30 - 16:30 | **4. Synthetic Polymers: 1) MS towards the megadalton range, 2) Ion mobility MSs (0.06)** – Laurence Charles |
| | 13:30 - 16:30 | **5. CE-MS coupling: new developments and applications (0.19)** – Yannis François |
| | 13:30 - 16:30 | **6. Data analysis in metabolomics (0.18)** – Ivan Protsyuk, Andrew Palmer, Theodore Alexandrov, Daniel Petras, Pieter Dorrestein |
| 17:00 - 17:30 | | **Opening session** (Audimax) - Andrea Sinz, Aura Tintaru, Dietrich Volmer, Frédéric Aubriet |
| 17:30 - 19:00 | | **Opening plenary session** (Audimax) – Andrea Sinz and Aura Tintaru |
| 17:30 - 18:15 | | What is the future of MS as a "Science" - DGMS Wolfgang Paul Lecture - Alain van Dorsselaer, France |
| 18:15 - 19:00 | | High resolution tailored metabolomics in health related and environmental research - Philippe Schmitt Kopplin, Germany |
| 19:00 - 21:00 | | **Welcome reception** |

## Monday, 12 March 2018

| | | | |
|---|---|---|---|
| 8:30 - 10:00 | **Plenary session** – Dietrich Volmer (Audimax) | | |
| 08:30 - 09:15 | Mass spectrometry and various shades of food fraud - Saskia Van Ruth, The Netherlands | | |
| 09:15 - 10:00 | Discovery of cancer neoantigens using a proteogenomics approach - Pierre Thibault, Canada | | |
| 10:00 - 10:30 | **Coffee break** | | |
| **10:30 - 12:00** | **Imaging MS (Audimax)** – Bernhard Spengler | | |
| | 10:30 - 11:00 | [Keynote] | Multimodal Imaging Workflows: How to integrate mass spectrometry imaging approaches and (vibrational) spectroscopy techniques - *Andreas Roempp, University of Bayreuth, Germany* |
| | 11:00 - 11:20 | [O1] | Implementation of a high-repetition-rate laser in an AP-SMALDI MSI system for enhanced measurement performance - *Max Müller, Justus-Liebig University Giessen, Germany* |
| | 11:20 - 11:40 | [O2] | On-tissue Paternò-Büchi reaction for localization of lipid carbon-carbon double bond positions in MALDI MS imaging - *Antonin Bednarik, University of Münster, Germany* |
| | 11:40 - 12:00 | [O3] | Error-free data visualisation and processing through mzML and imzML validation - *Alan Race, Universität Bayreuth, Germany* |
| **10:30 - 12:00** | **Targeted and non-targeted analysis – Structural identification and annotation (0.19)** - Jürgen Gross | | |
| | 10:30 - 11:00 | [Keynote] | Selective phosphatidylcholine double bond fragmentation and localization using Paternò-Büchi reactions and ultraviolet photodissociation - *Sven Heiles, Justus-Liebig University Giessen, Germany* |
| | 11:00 - 11:20 | [O1] | An Integrated Mass Spectrometry-Based Approach to Probe the Structure of the Full-Length Wild-Type Tetrameric p53 Tumor Suppressor - *Christian Arlt, Martin Luther University Halle-Wittenberg, Germany* |
| | 11:20 - 11:40 | [O2] | Novel aspects on chemical protein-RNA cross-linking coupled with mass spectrometry - *Alexander Wulf, Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany* |
| | 11:40 - 12:00 | [O3] | Investigation of the processes of oxidative modifications on the Reg3α liver protein and of some its shortened peptides by radiolysis and mass spectrometry - *Guillaume van der Rest, Laboratoire de Chimie Physique, France* |
| **10:30 - 12:00** | **Health - Clinical (0.18)** – Olivier Laprévote | | |
| | 10:30 - 11:00 | [Keynote] | Insights into the insertion mechanism of membrane proteins into nanodiscs using native mass spectrometry - *Oliver Peetz, Goethe-University Frankfurt am Main, Germany* |
| | 11:00 - 11:20 | [O1] | Microproteomics of FFPE tissues: from biomarker discovery to routine molecular diagnosis. - *Rémi Longuespée, Institute for Pathology Heidelberg, Germany* |
| | 11:20 - 11:40 | [O2] | Proteomic profiling of melanoma cell lines and tissue to predict treatment response and patient survival - *Christoph Krisp, University Medical Center Hamburg-Eppendorf, Germany* |
| | 11:40 - 12:00 | [O3] | Mass and Charge Distributions of Amyloid Fibers Involved in Neurodegenerative Diseases: Mapping Heterogeneity and Polymorphism - *Mohammad Abdul Halim, CNRS et Lyon 1, France* |
| 12:00 - 13:00 | **Bruker Workshop** (0.06) | | |
| 12:00 - 14:30 | **Lunch and Posters** | | |
| 13:30 - 14:30 | **Agilent Workshop** (0.23) | | |
| 14:30 - 15:15 | **Plenary session** – Emmanuelle Leize-Wagner (Audimax) | | |
| 14:30 - 15:15 | Challenges of protein PTM analysis by mass spectrometry - Joelle Vinh, ESPCI, France | | |
| 15:15 - 16:00 | **DGMS Mattauch Herzog Award session** – Andrea Sinz (Audimax) | | |
| 15:15 - 16:00 | New Methods and Instrumentation for Nanomaterials Characterization - Carsten Engelhardt, Universität Siegen, Germany | | |

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| 16:00 - 17:30 | **Coffee Break and Posters** |
| 17:30 - 19:30 | **DGMS Business Meeting** (0.19) |
| **17:30 - 20:30** | **Young mass spectrometrists meeting session (0.18)** – Laurent Laboureur |

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|---|---|---|
| 17:30 - 17:45 | **Introduction** | |
| 17:45 - 18:00 | [O1] | Protein Interaction of a of p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass-Spectrometry – *Friederike Leßmöllmann - Martin-Luther University Halle-Wittenberg, Germany* |
| 18:00 - 18:15 | [O2] | Proteomics applied to the study of archaeological ceramics (II$^{nd}$ century) preserved in submarine context – *Sergui Mansour – Université des Sciences et Technologies de Lille, France* |
| 18:15 - 18:30 | [O3] | Spectral library based approach to protein arginine phosphorylation in Staphylococcus aureus – *Sabryna Junker – University Greifswald, Germany* |
| 18:30 - 18:45 | [O4] | Characterization of Physical and Chemical Properties of Synthetic Polymers using Ion Mobility Mass Spectrometry – *Shinsuke Kokubo – University Göttingen, Germany* |
| 18:45 - 19:00 | [O5] | Probing peptide's conformational dynamics by a simple measure of its internal proton transfer time constant – *Mathilde Bouakil – Institut Lumière Matière de Lyon, France* |
| 19:00 - 19:15 | [O5] | Development of metabolite based MALDI MS assays – *Christoph Krisp, David Weigt – Mannheim University of Applied Sciences, Heidelberg University, Germany* |
| 19:15 - 19:30 | **Vote and results** | |
| 19:45 - 21:00 | **Young MS cocktail** | |

## Tuesday, 13 March 2018

| | | |
|---|---|---|
| 8:30 - 10:00 | **Plenary session** – Frédéric Aubriet (Audimax) | |
| 08:30 - 09:15 | Hyphenated MS in Pharmaceutical Applications: Data Independent Acquisition, Ion Mobility and Electron Based Dissociation - Gérard Hopfgartner, Switzerland | |
| 09:15 - 10:00 | Nanocraters, soft desorption and 3D-resolved organic mass spectrometry using cluster ion beams - Arnaud Delcorte, Belgium | |
| 10:00 - 10:30 | **Coffee break** | |
| **10:30 - 12:00** | **Omics - Proteomic, PTM and structural biology (Audimax)** – Noëlle Potier | |
| 10:30 - 11:00 | [Keynote] | Investigation of protein-DNA interactions in chromatin assemblies with chemical cross-linking mass spectrometry - *Fanni Bazsó, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany* |
| 11:00 - 11:20 | [O1] | The First "Zero-Length" Mass Spectrometry-Cleavable Cross-Linker for Protein Structure Analysis - *Christoph Hage, Martin Luther University Halle-Wittenberg, Germany* |
| 11:20 - 11:40 | [O2] | timsTOF Pro with PASEF for shotgun proteomics - *Pierre-Olivier Schmit, Bruker Daltonique, France* |
| 11:40 - 12:00 | [O3] | Absolute quantification of metabolic enzymes in C. elegans - *Bharath Kumar Raghuraman, Max Planck Institute of Molecular Cell Biology and Genetics, Germany* |
| **10:30 - 12:00** | **Health - Clinical toxicology and forensics (0.18)** – Markus Meyer | |
| 10:30 - 11:00 | [Keynote] | Development and validation of a multidimensional gas chromatography/ combustion/ isotope ratio mass spectrometry method for the investigation of carbon isotope ratios of urinary steroids in sports drug testing - *Marlen Putz, German Sports University Cologne, Germany* |
| 11:00 - 11:20 | [O1] | Detection and Identification of Performance Additives in Fuels for Forensic Applications - *Lisa Scharrenbroch, Bundeskriminalamt, Germany* |
| 11:20 - 11:40 | [O2] | Quantitation of Acetaminophen Covalent Protein Binding in Vivo by LC-MS/MS - *Timon Geib, Université du Québec à Montréal, Canada* |
| 11:40 - 12:00 | [O3] | Quantitative Lipidomic Fingerprinting for Antiepileptic Drug Monitoring - *Laura Bindila, Johannes Gutenberg-University Mainz, Germany* |
| **10:30 - 12:00** | **Targeted and non-targeted analysis: Petroleomics and synthetic polymers (0.19)** – Laurence Charles | |
| 10:30 - 11:00 | [Keynote] | How primary amine commonly used as dopant can be useful analytical tool for the semi-targeted analysis of complex matrixes by ESI FT-ICR MS - *Jasmine Hertzog, Helmholtz Zentrum München, Germany* |
| 11:00 - 11:20 | [O1] | Comprehensive mass spectrometric evolved gas analysis in the context of Petroleomics - *Christopher Rüger, University of Rostock, Germany* |
| 11:20 - 11:40 | [O2] | Graphical Decoding of Messages Stored in Sequence-Controlled Synthetic Polymers using Kendrick Mass Defect Analysis - *Salomé Poyer, Aix-Marseille Université, France* |
| 11:40 - 12:00 | [O3] | Characterization of a complex polymer mixture by Atmospheric Solid Analysis Probe (ASAP) MS combined with Kendrick mass defect analysis - *Gabriel Gaiffe, Institut Parisien de Chimie Moléculaire, France* |
| 12:00 - 13:00 | **Thermo Workshop** (0.06) | |
| 12:00 - 14:30 | **Lunch and Posters** | |
| 13:30 - 14:30 | **Shimadzu Workshop** (0.23) | |
| 14:30 - 15:15 | **Plenary session** – Gereon Niedner-Schatteburg (Audimax) | |
| 14:30 - 15:15 | Coupling mass spectrometry with ion mobility spectrometry, helium droplet isolation and infrared spectroscopy (Audimax) - Gert von Helden, Germany | |
| 15:15 - 16:00 | **SFSM PhD Award session – Young MS plenary session** – Aura Tintaru (Audimax) | |

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| 15:15 - 15:45 | | | Glycan Analysis by IR Spectroscopy Integrated into Mass Spectrometry - Baptiste Schindler, Université de Lyon, France |
| 15:45 - 16:00 | | | Best talk of the young mass spectrometrist session |
| 16:00 - 17:30 | | | **Coffee Break and Posters** |
| **17:30 - 19:00** | | | **Omics - Proteomic, PTM and structural biology (Audimax) –** Simone König |
| | 17:30 - 18:00 | [Keynote] | The forgotten proteome – proteomics approaches for the identification of short open reading frame encoded peptides - *Andreas Tholey, Christian-Albrechts-Universität zu Kiel, Germany* |
| | 18:00 - 18:20 | [O1] | Structural analysis of peroxisomal translocon complexes using cross-linking and native mass spectrometry - *Friedel Drepper, University of Freiburg, Germany* |
| | 18:20 - 18:40 | [O2] | In-depth glycoproteomics analysis of human C1-inhibitor using C18-PGC-LC-QTOF-MS/MS - *Kathrin Stavenhagen, VU University Amsterdam, The Netherlands* |
| | 18:40 - 19:00 | [O3] | Characterization of the virulome of Staphylococcus aureus by a highly multiplex approach with Scout-MRM - *Nicolas Mouton, Institut des Sciences Analytiques, France* |
| **17:30 - 19:00** | | | **Fundamental of MS - Instrumentation (0.18)** – Ralf Zimmermann |
| | 17:30 - 18:00 | [Keynote] | Chemical and topographical 3D-surface imaging of non-planar objects on the micrometer scale using AP-SMALDI MSI - *Mario Kompauer, Justus-Liebig University Giessen, Germany* |
| | 18:00 - 18:20 | [O1] | Hybrid SIMS: A new instrument for high resolution organic imaging with high mass-resolving power and MS/MS - *Matthias Kleine-Boymann, IONTOF GmbH, Germany* |
| | 18:20 - 18:40 | [O2] | Development and optimization of a membrane-inlet-photoionization mass spectrometer for real-time analysis of (poly)aromatic compounds in aquatic systems - *Christian Gehm, University of Rostock, Germany* |
| | 18:40 - 19:00 | [O3] | Ion mobility mass spectrometry of phosphoric acid cluster ions - *Hélène Lavanant, Normandie Univ, INSA Rouen, France* |
| **17:30 - 19:00** | | | **Omics - Lipidomics (0.19)** – Harald Koefeler |
| | 17:30 - 18:00 | [Keynote] | Study of acetogenins in a natural extract using SFC-HRMS/MS and post-column metal cationisation - *David Touboul, Institut de Chimie des Sunstances Naturelles, France* |
| | 18:00 - 18:20 | [O1] | In-depth Lipidomics using Laser Capture Microdissection - *Olga Vvedenskaya, Max Planck Institute of Molecular Cell Biology and Genetics, Germany* |
| | 18:20 - 18:40 | [O2] | An adaptive and dynamic approach to generate unique SMILES for lipidome homology - *Fadi Al Machot, Research Center Borstel, Germany* |
| | 18:40 - 19:00 | [O3] | The effect of double bond functionalization for their localization in lipids by means of MS/MS - *Viola Jeck, University of Münster, Germany* |
| 19:00 - 20:00 | | | **DGMS Award Session** (Wolfgang Paul and Life Sciences Awards) (Audimax) – Johanna Hofmann, Alessandro Vetere, Michal Sharon |

## Wednesday, 14 March 2018

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|---|---|---|
| 8:30 - 10:00 | **Plenary session** – Vincent Carré (Audimax) | |
| 08:30 - 09:15 | Advances in Ultrahigh Resolution Mass Spectrometry Enable Exploration of Complex Biological Systems - Ljiljana Pasa-Tolic, USA | |
| 09:15 - 10:00 | Localomics: Combining Mass Spectrometry Imaging & Microproteomics - Liam McDonnell, Italy | |
| 10:00 - 10:30 | **Coffee break** | |
| **10:30 - 12:00** | **Fundamental MS: Tandem mass spectrometry and data processing (0.19)** – Patrick Chaimbault | |
| 10:30 - 11:00 | [Keynote] | Data Independent Acquisition Coupled to Visible Laser-Induced Dissociation at 473 nm (DIA-LID) for Specific Targeting of Cysteine-Containing Peptide Subset - *Marion Girod, Institut des Sciences Analytiques, France* |
| 11:00 - 11:20 | [O1] | Programmed Inter-Byte Fragmentation to Facilitate MS/MS Reading of Digital Information Molecularly Encoded in Sequence-Controlled Synthetic Polymers - *Jean-Arthur Amalian, Aix Marseille Univ, France* |
| 11:20 - 11:40 | [O2] | A new R package for deconvolution of DIA data - *Michael Witting, Technical University München, Germany* |
| 11:40 - 12:00 | [O3] | Ion Mobility Spectrometry Enabled Fragmentation of Doubly Charged Peptide Ions in MALDI-TOF for Protein Identification - *Jens Sproß, Universität Bielefeld, Germany* |
| **10:30 - 12:00** | **Health - Environment (0.18)** – Cédric Guignard | |
| 10:30 - 11:00 | [Keynote] | A New Single Particle Aerosol Laser Mass Spectrometer for Dual TOFMS Analysis of the Individual Airborne Aerosol Particles: Detection of Polycyclic Aromatic Hydrocarbons and Inorganic Compounds - *Ralf Zimmermann, University of Rostock and Helmholtz Zentrum München, Germany* |
| 11:00 - 11:20 | [O1] | Polarity extended chromatography coupled with HRMS for non-target screening in the aqueous environment - *Stefan Bieber, Technische Universität München, Germany* |
| 11:20 - 11:40 | [O2] | Non-targeted characterization of PAXHs contaminated soil using API FT Orbitrap MS - *Ruoji Luo, Max-Planck-Institut für Kohlenforschung, Germany* |
| 11:40 - 12:00 | [O3] | Metal speciation and mobility in clay - From ICP-MS batch and CE-ICP-MS Speciation to miniaturised clay column experiments (MCCE) using LC-ICP-MS - *Ralf Kautenburger, Saarland University, Saarbrücken, Germany* |
| **10:30 - 12:00** | **Omics - Metabolomics and Fluxomics (Audimax)** – Nicolas Sommerer | |
| 10:30 - 11:00 | [Keynote] | Single-cell mass spectrometry of metabolites extracted from live cells by Fluidic Force Microscopy - *Patrick Kiefer, ETH Zurich, Switzerland* |
| 11:00 - 11:20 | [O1] | High-resolution mass spectrometry to decipher microbial origin of sulfonolipids inside mammalian gut- *Alesia Walker, Helmholtz Zentrum München, Germany* |
| 11:20 - 11:40 | [O2] | Determination of stable isotope labeled glucose tracers by high-resolution mass spectrometry in dual- and triple-tracer studies - *Harald Koefeler, Medical University of Graz, Austria* |
| 11:40 - 12:00 | [O3] | Planetary Scale Metabolomics - Molecular Imaging of a Phytoplankton Bloom in the California Current Ecosystem - *Daniel Petras, University of California San Diego, USA* |
| 12:00 - 13:00 | **Waters Workshop** (0.06) | |
| 12:00 - 14:00 | **Lunch and Poster** | |
| 13:00 - 14:00 | **Sciex Workshop** (0.23) | |
| **14:00 - 15:30** | **Health - Pharmaceutical applications and nutrition (Audimax)** – Carsten Hopf | |
| 14:00 - 14:30 | [Keynote] | Multiplexed proteome dynamics profiling reveals mechanisms controlling protein homeostasis - *Nico Zinn, Cellzome GmbH, Germany* |
| 14:30 - 14:50 | [O1] | Analysis of brain tumors after fluorescence-guided resection by the complementary use of HILIC-ESI-MS/MS and MALDI-MS/MS - *Sabrina Kröger, University of Münster, Germany* |

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| | 14:50 - 15:10 | [O2] | Volatile Compound Fingerprinting by Headspace Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS) for the Authenticity Assessment of Honey as Benchtop Alternative to 1H NMR Profiling - *Philipp Weller, Mannheim University of Applied Sciences, Germany* |
| | 15:10 - 15:30 | [O3] | Advanced glycation end-products in the bovine milk proteome - *Sanja Milkovska-Stamenova, Universität Leipzig, Germany* |
| **14:00 - 15:30** | **Fundamental of MS - Instrumentation (0.18)** – Heiko Hayen | | |
| | 14:00 - 14:30 | [Keynote] | Extending the gas phase structural biology toolbox: Circular Dichroism mass spectrometry of G-quadruplexes. - *Steven Daly, Institut Européen de Chimie et Biologie, France* |
| | 14:30 - 14:50 | [O1] | Comparison of TLC-LTP-MS and TLC-FAPA-MS: Fundamental Studies and Selected Applications - *Christopher Kuhlmann, University of Siegen, Germany* |
| | 14:50 - 15:10 | [O2] | Development of Micro-Time-Of-Flight mass spectrometer for Chemical Threat in situ detection - *Frédéric Progent, Commissariat à l'Énergie Atomique et aux Énergies Alternative, France* |
| | 15:10 - 15:30 | [O3] | Laser-spark ionization mass spectrometry - *Andreas Bierstedt, Federal Insitute for Materials Research and Testing, Germany* |
| **14:00 - 15:30** | **Fundamental of MS - Ion chemistry (0.19)** – Gereon Niedner-Schatteburg | | |
| | 14:00 - 14:30 | [Keynote] | Photo-induced linkage isomerization in the gas phase probed by tandem ion mobility and laser spectroscopy - *Fabien Chirot, Institut des Sciences Analytiques, France* |
| | 14:30 - 14:50 | [O1] | Mechanistic Insight in the Copper Catalyzed Oxidative Derivatization of Tetra-hydroisoquinolines - *Marianne Engeser, Universität Bonn, Germany* |
| | 14:50 - 15:10 | [O2] | Structure and Reactivity of Gas-Phase Phenylhydroxycarbenes: Hydrogen Tunneling at Room Temperature - *Mathias Schäfer, University Cologne, Germany* |
| | 15:10 - 15:30 | [O3] | Mass Spectrometry and Ion Mobility Spectrometry for Investigating the Interlocked Nature of Catenanes - *Anneli Kruve-Viil, Freie Universität Berlin, Germany* |
| 15:30 - 17:00 | **Coffee Break and Poster** | | |
| 17:30 - 23:00 | **Gala Dinner** | | |

## Thursday, 15 March 2018

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|---|---|---|---|
| 9:00 - 10:30 | **Plenary session** – Hans Maurer (Audimax) | | |
| 09:00 - 09:45 | Ambient Laser Desorption Ionization – instrumentation and applications - Zoltan Takats, United Kingdom | | |
| 09:45 - 10:30 | Mass Spectrometry in Forensics - Thomas Kraemer, Switzerland | | |
| 10:30 - 11:00 | **Coffee break** | | |
| **11:00 - 12:30** | **Omics - Proteomic, PTM and structural biology (Audimax)** – Andrea Sinz | | |
| | 11:00 - 11:30 | [Keynote] | Site-specific N-glycosylation analysis of the HIV-1 glycan shield - *Anna-Janina Behrens, University of Oxford, UK* |
| | 11:30 - 11:50 | [O1] | Displacement Chromatography Mode – High protein sequence coverages and identification rates for low µg-range proteomics using online 2D-LC-MS - *Marcel Kwiatkowski, University of Groningen, The Netherlands* |
| | 11:50 - 12:10 | [O2] | Protein and proteoform profiling of saw-scaled viper, Echis carinatus, venom using a top-down approach - *Parviz Ghezellou, Justus Liebig University Giessen, Germany* |
| | 12:10 - 12:30 | [O3] | Cross-Linking and Mass Spectrometry Give Insights into the bMunc13-2/Calmodulin Interaction - *Christine Piotrowski, Martin Luther University Halle-Wittenberg, Germany* |
| **11:00 - 12:30** | **Fundamental of MS - Ionization (0.18)** – Klaus Dreisenwerd | | |
| | 11:00 - 11:30 | [Keynote] | Mass spectrometric analysis of disease triggering amyloids: combining ESI-IMS and LILBID-MS- *Tobias Lieblein, Goethe-University Frankfurt/Main, Germany* |
| | 11:30 - 11:50 | [O1] | Silicon nanostructures for versatile SALDI-MS peptide analyses - *Christine Enjalbal, Université de Montpellier, France* |
| | 11:50 - 12:10 | [O2] | Combination of 2,5-Dihydroxybenzoic acid and 2,5-Dihydroxyacetophenone Matrices for unequivocal assignment of Phosphatidylethanolamine Species in complex Mixtures - *Jenny Schröter, Leipzig University, Germany* |
| | 12:10 - 12:30 | [O3] | Direct Ionization and Reaction Monitoring via Acoustic Levitation Ambient Mass Spectrometry - *Elizabeth Crawford, Saarland University, Saarbrücken, Germany* |
| **11:00 - 12:30** | **Imaging MS (0.19)** – David Touboul | | |
| | 11:00 - 11:30 | [Keynote] | Fourier Transform Infrared Microscopy guided Mass Spectrometry Imaging of Tissue Morphologies - *Jan-Hinrich Rabe, Center for Biomedical Mass Spectrometry and Optical Spectroscopy, Germany* |
| | 11:30 - 11:50 | [O1] | Towards Higher Sensitivity in MALDI-FTICR Imaging of CNS Drugs Using a Matrix Matching Unique Analyte Properties - *Ignacy Rzagalinski, Saarland University, Saarbrücken, Germany* |
| | 11:50 - 12:10 | [O2] | Evaluation of non-supervised MALDI MSI combined with microproteomics for determination of glioblastoma heterogeneity - *Lauranne Drelich, Univ. Lille 1, France* |
| | 12:10 - 12:30 | [O3] | A 3-D image of cereal cell walls provided by MALDI MS imaging - *Hélène Rogniaux, French National Institute for Agricultural Research, France* |
| 12:30 - 13:00 | **Poster Prizes/Closing Session (Audimax)** | | |

European Mass Spectrometry Conference

# EMSC 2018

Saarbrücken · Germany · 11-15 March

## Sunday, 11 March

# Workshops

# Workshop 1

## Computational and bioinformatics tools for proteomics

Proteomics investigates the identity, quantity, spatial and time dependent distribution of proteins in biological systems in a comparative manner. Therefore, and due to the massive amounts of measured MS data, it is inevitable that the developed methods and tools provide quick and reliable interpretations.

This short course gives insight into several aspects of proteomics data processing by going through the basic workflow; that is i) database acquisition, ii) peptide matching, iii) protein identification and iv) protein quantification. We will introduce the following tools: MS-Angel, SearchGUI, PeptideShaker, Knime and PIA.

The short course is for users who already have basic knowledge in analytical proteomics. Attendees should be familiar with topics such as target-decoy approach and false discovery rate. Please note that the complete workshop is organized as a hands-on session. please bring your own (modern) laptop (min. requirements: dual-core 1.8GHz, 4GB main memory / better 8GB, 40GB HDD).

After registration, we will inform you with further details. The space is limited to 25 participants.

This short course is supported by ProFI, de.NBI and ELIXIR.

Organizers: Martin Eisenacher (Ruhr-Universität Bochum), Dominik Kopczynski (ISAS Dortmund), David Bouyssié (University of Toulouse)

# Workshop 2

## Novel and established approaches to high-performance mass spectrometry imaging

The workshop will discuss the performance and relevance of alternative approaches in mass spectrometry imaging (MSI). Singular and hyphenated ion formation and detection principles will be discussed, in relation to the well-established approaches based on MALDI, DESI and SIMS. Besides informational richness and contents of the different MSI approaches, the practicability shall be a key parameter of a methodological overview and evaluation.

Organizers: Bernhard Spengler (Justus-Liebig-Universität Giessen), Andreas Römpp (Universität Bayreuth)

# Workshop 3

## Cross-linking and HDX-MS: Towards the study of interacting domains in biological complexes

This workshop presents two emerging methodologies based on mass dpectrometry in the context of structural biology research. It is now well establish that proteins carry out their function through interaction with other partners. Although important protein complex structures have been elucidated by traditional high resolution techniques such as X-crystallography or nuclear magnetic resonance, the requirement for large amounts of high purity homogeneous samples has limited their application and the emergence of recent mass dpectrometry-based technologies has gained strong interest in the community of structural biologists. In particular, strategies based on cross-linking and on H/D exchange combined with MS show a high potential for generating structural data on supramolecular topologies.

Basic information about cross-linking and HDX- MS strategies will be presented as well as technical obstacles related to interdependent choices of reagents, protocols and softwares. Cross-linker reactivities and their applications to study protein-protein or protein-nucleic acid interactions will be shown.

13.00 - 13.15 **Small introduction**
*Dr. Noelle POTIER (University of Strasbourg, France)*

13.15 - 14.05 **Introduction into Cross-linking/Mass Spectrometry for Protein Structure Analysis**
*Dr. Claudio IACOBUCCI and Christine PIOTROWSKI (Martin-Luther University Halle-Wittenberg, Deutschland)*

14.05 - 14.40 **Protein-protein and protein-nucleic acid cross-links analysed with MS - Principals, Applications, Pitfalls**
*Dr. Henning URLAUB (Max-Planck-Institute Göttingen, Deutschland)*

*14.40 - 15.15* **Data analysis for protein cross-linking/mass spectrometry**
*Pr. Juri RAPPSILBER and Dr. Francis O'REILLY (Technische Universität Berlin, Deutschland)*

15.15 - 15.45 **Break**

15.45 – 16.20 **Introduction to HDX/Mass Spectrometry for protein structure analysis**
*Dr. Julien MARCOUX (University of Toulouse, France)*

16.20 – 16.55 **Applications of HDX/Mass spectrometry and complementarity to native MS appraoches**
*Dr. Sarah CIANFERANI (University of Strasbourg, France)*

Organizer: Noelle Potier (Université de Strasbourg)

# Workshop 4

## Synthetic Polymers: 1) MS towards the megadalton range, 2) Ion mobility MS

The workshop will discuss different technical approaches to characterize synthetic polymers in the megaDalton range, as well as strategies to assess conformational features of synthetic polymers in the gas phase using ion mobility and appropriate models for collision cross section determination. The workshop is for users who already have basic knowledge in mass spectrometry of synthetic polymers.

Further details on the workshop and the lectures:

### Part I
### Mass Spectrometry of Synthetic Polymers: Towards the MegaDalton range

13.00 - 13.45 **MALDI-TOF of Mega Dalton Dendrimers - Obstacles and Benefits**
*Dr Hans Joachim RÄDER – Max Planck Institute for Polymer Research, Mainz, Germany*

13.45 - 14.30 **Hyphenated Laser-Charge Detection MS Approaches for Ultra-High-Molecular-Weight Polymers: Playing with Heavier Things**
*Rodolphe ANTOINE – Université Lyon I, Lyon, France*

14.30 - 15.00 **NEMS-MS Characterization of Megadalton Polymer Nanoparticles**
*Dr Mohammad Abdul HALIM & Dr Christophe MASSELON – CEA, Grenoble, France*

15.00 - 15.30 **Break**

### Part II
### Ion Mobility Spectrometry for Synthetic Polymers

15.30 - 16.30 **Calibration Strategies to Assess Conformation of Synthetic polymers**
*Pr Edwin DE PAUW – University of Liège, Belgium*

Organizer: Laurence Charles (Université de Aix-Marseille)

# Workshop 5

## CE-MS coupling: new developments and applications

The workshop will focus on the latest instrumental developments and cutting-edge CE-MS applications for small molecules, proteomics and biotherapeutics.

The provisional program is as follows:

**New instrumental developments of CE-MS coupling**
*Pr. Christian Neusüß from Aalen University*

**Insight from CE-MS approaches for pharmaceutical applications**
*Dr. Julie Schappler from Geneva University:*

**CE-MS-based proteomics in biomarker discovery and clinical applications**
*Dr. Rabah Gahoual from Paris Descartes University*

**Overview of CE-MS coupling for biotherapeutic applications**
*Dr. Yannis Francois from Strasbourg University:*

Organizer: Yannis François (Université de Strasbourg)

# Workshop 6

## Data analysis in metabolomics

This short course is aimed at explaining how to use the METASPACE and GNPS platforms for large scale data analysis in mass spectrometry (MS) based metabolomics experiments. METASPACE couples advanced bioinformatics with high performance cloud computing to provide MS based annotations of Imaging MS and LC-MS datasets. GNPS is an open-access knowledgebase for public sharing, processing, curation and annotation of tandem mass spectrometry datasets, combining molecular networking with a community driven spectral library.

This course aims to explain how to use the METASPACE and GNPS platforms for large scale data analysis in mass spectrometry (MS) based metabolomics experiments. METASPACE couples advanced bioinformatics with high performance cloud computing to provide MS based annotations of Imaging MS and LC-MS datasets [1]. GNPS is an open-access knowledgebase for public sharing, processing, curation and annotation of tandem mass spectrometry (MS/MS) datasets, combining molecular networking with a community driven spectral library [2,3]. GNPS enables large scale metabolite identification through spectrum library matching and propagation of these annotations in spectral networks. Both platforms leverage the community as a resource, making cross referencing of samples and studies possible to enable researchers to perform analyses that would be impossible in a single lab.

For each platform, we will present some principles of modern data science, explain the bioinformatics for metabolite annotation as well as give a hands-on step-by-step tutorial on retrieving and annotating data, browsing annotations, and exploring the wealth of public data submitted by others. By the end of the course you should expect to understand the principles of false-discovery-rate controlled molecular annotation [4,5], be able to submit your data to our services, as well as to browse and export the metabolite annotations.

For the hands-on tutorials you need to bring your own laptop.

The course received funding from the European Union's Horizon2020 programme under the grant agreement No. 634402 (METASPACE).

References

1. http://metaspace2020.eu/
2. https://gnps.ucsd.edu/
3. Wang, M., et al., Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nature biotechnology, 2016. 34(8): p. 828-37.
4. Palmer, A., et al., FDR-controlled metabolite annotation for high-resolution imaging mass spectrometry. Nature methods, 2017. 14(1): p. 57-60.
5. Scheubert, K., et al., Significance estimation for large scale metabolomics annotations by spectral matching. Nature Communications, 2017. 8(1): p. 1494.

Organizers: Ivan Protsyuk, Andrew Palmer, Theodore Alexandrov (EMBL Heidelberg), Daniel Petras, Pieter Dorrestein (UC San Diego)

# Opening Plenary Lectures

## What is the future of MS as a "Science"

Alain Van Dorsselaer*, Fabrice Bertile, Christine Schaeffer,
Laurence Sabatier, Christine Carapito, Sarah Cianferani[1]

[1]Laboratory of Bio-Organic Mass Spectrometry (LSMBO), Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France. E-mail: vandors@unistra.fr

Alain Van Dorsselaer is Emeritus CNRS Research Director at the University of Strasbourg. His group has constantly developed new analytical methods and strategies based on mass spectrometry, first for large synthetic molecules and supramolecules, and then for peptide and protein characterization, including posttranslational modifications. He has developed new methods for the study of noncovalent interactions in chemistry for supramolecules and then in biology for protein/protein and protein/ligand interactions. His group is now focusing on structural mass spectrometry and on the development and the application of proteomic methodologies in a large variety of domains, including the characterization of biopharmaceuticals in collaboration with pharmaceutical companies.

**Abstract:**
This presentation is dedicated to young scientists interested in mass spectrometry. Some of them could fear that mass spectrometry will not allow any more basic research programs and will become a "service science for other disciplines". Indeed, after almost 30 years of constant efforts and progresses of the mass spectrometry community, it seems that it is now possible to identify and quantify rather accurately thousands of proteins from very low sample amounts. This can be achieved with instrumentation and software tools that are getting commonly available in core facilities of biology institutes, such as for genomics, transcriptomics, metabolomics, ...
However, proteomic analysis is far from producing a full description of proteomes since a full characterization of most proteins present is not yet possible. All proteoforms from one gene are usually detected and quantified under the same name and post translational modifications are rarely systematically detected. Almost nothing is known on their combination when they are multiple. While MS/MS fragmentation of digestion peptides may enable determining their full sequence, this is still hardly achievable for, as an example, multiply phosphorylated proteoforms using top down approaches. There is clearly a long way to go before this will be possible and a large room for the development of innovative approaches in separation sciences and mass spectrometry is open.
Besides proteomics, there are many other fields in which major developments will offer opportunities to extend the use of mass spectrometry-based structure determination methods both in biology and chemistry.
The main research fields our laboratory has contributed to over the past 30 years will be presented to illustrate the attractiveness of mass spectrometry and the importance of technical developments. For example: - From Hopane triterpenes as biomarkers in sediments and oils, to protein biomarkers for diagnostic of lymphopathies. - How the characterization of molecular self-assembled supramolecules led to the characterization of large multi-protein complexes. - How the determination of the assembly order of supramolecules subunits led to the elucidation of multiprotein complexes. - How, thanks to bioinformatics developments, proteomics analysis of organisms with unsequenced genomes was made possible for the study of new metabolic pathways in exotic animal models. - Performing differential display of peptides induced during the immune response from a single Drosophila. - How the use of proteomic analysis-derived

methods and Ion Mobility were used to guide the production of biologics, and in particular monoclonal antibodies, with high purity and batch-to-batch consistency.
This series of examples will illustrate that mass spectrometry remains a fascinating and attractive science for young scientists that are willing to evolve in highly stimulating multidisciplinary contexts.

# High resolution tailored metabolomics in health related and environmental research

Philippe Schmitt-Kopplin

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2.Technische Universität München, Analytical Food Chemistry, Freising/Weihenstephan, Germany

Prof. Ph. Schmitt-Kopplin´s team performs tailored and comprehensive metabolomics in food, health and geosciences. He has a strong profile in analytical chemistry with integrated approaches combining (ultra)high resolution mass spectrometry, separation sciences, NMR-spectroscopy with (bio)informatics for the description of complex organic systems on a molecular level. A focus in the last decade was to implement magnetic resonance mass spectrometry into applications for a rapid and robust tool for deep metabotyping and small molecules profiling. One focus is on the chemical understanding of microbiomes in health and environments and integrating these information with existing biological, metaproteome or metagenome data. His interdisciplinary studies are related to relevant geochemical questions (C and S-cycling, halogens, origin of life) and important biology/health issues related to environmental factors (allergies, nutrition, metabolic diseases) at the interface of chemistry and biology. He is director of the research unit analytical BioGeoChemistry of the HelmholtzZentrum Muenchen, Germany and is member of the Technische University Munich and heads the Comprehensive Foodomics Platform of the Institute of analytical Food Chemistry.

**Abstract:**

From a traditional definition in the field of human health, metabolomics measures the concentrations of the large number of naturally occurring small molecules (called metabolites), that are produced as intermediates and end-products of all metabolic processes. They are analyzed from biological samples and body fluids such as urine, saliva, blood plasma, tissue sample; even the simple breath (exhaled breath condensates) can carry the information about the state of health. The total number of different metabolites is still unknown; some estimation ranges from few ten thousands to about one million, but even this latter estimate may be conservative; including plant and bacterial metabolites that are not necessary to keep the organism alive, also referred to as secondary metabolites, the number is enormously larger. The probable number of metabolites is also considerably larger than the number of corresponding genes, so it seems that the currently available databases cover at best 5% of the total number of existing metabolites. With our integrated analytical approaches(LC-MS, NMR and ICR-FT/MS) data we annotate from databases around 10% of the experimental signals.

Metabolomic, as the comprehensive study of metabolic reactions is growing very rapidly and integrates the knowledge of earlier developed Omics-branches. Especially ICR-FT/MS describes highly complex mixtures in complex systems on the level of the elementary composition distribution and is shown in this presentation as an dedicated and innovative mass spectrometry tool to understand the composition and processes on a molecular level in various study fields from food chemistry, biology, microbiomes towards the discovery of new bioactives.

European Mass Spectrometry Conference

# EMSC 2018

Saarbrücken · Germany · 11-15 March

# Monday, 12 March

# Monday Morning
# Plenary Lectures

# Mass spectrometry and various shades of food fraud

Saskia M. van Ruth[1], Valentina Acierno[1], Ningjing Liu[1], Isabelle Silvis[1], Jing Yan[1]

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Prof. Saskia van Ruth heads research on food authenticity at Wageningen University and Research in the Netherlands since 2005. She also teaches on the topic as professor in Wageningen and in University College Cork in Ireland. She received her PhD in Food Chemistry from the Wageningen University in 1995 and worked for industry and in the academic world since. Her present research deals with fraud risks, i.e. factors impacting on the risk of food fraud in supply chain networks. Furthermore, she is active in research on novel methodology for fraud detection, both in and beyond the laboratory.

**Abstract:**

Food fraud is a form of criminal behaviour, no matter the definition of crime. Its consequences are devastating. The interaction between motivated offenders, and the opportunities presented by victims and lack of control measures favour occurrence of food fraud. Control measures help to counteract fraud opportunities and motivations. Analytical testing is one of those control measures. Traditional measurements have focused on the analysis of one or a few product characteristics. However, nowadays analytical techniques generating detailed analytical fingerprints are used to determine the identity of foods, and we have many spectrometry techniques available. Sensitive targeted methods will be presented, for instance for authentication of olive oil grades, as well as various fingerprint approaches. They include for instance broad anomaly methodology for authentication of spices, as well as for the characterization of organic products. Furthermore, MS-based breath analysis will demonstrate the importance of the origin of cocoa beans for a distinct identity of chocolates.

# Discovery of cancer neoantigens using a proteogenomics approach

Pierre Thibault[1,2*], Diana Granados[2,3], Anja Rodenbrock[2], Joel Lanoix[2], Jean-Philippe Laverdure[2], Caroline Côté[2], Mathieu Courcelles[2], Hilary Pearson[2,3], Chantal Durette[2], Éric Bonneil[2], Denis-Claude Roy[4], Jean Sébastien Delisle[4], Sébastien Lemieux[2], Claude Perreault[2,3,4]

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Pierre Thibault is professor of chemistry and principal investigator at the Institute for Research in Immunology and Cancer (IRIC) at Université de Montréal where he leads the proteomics research unit. He holds a Canada Research Chair in proteomics and bioanalytical mass spectrometry at Université de Montréal. His group uses quantitative proteomics approaches to study the molecular mechanisms and the post-translational modifications regulating the functions of proteins involved in cell immunity and in the signaling of cancer cells.

**Abstract:**

The extent of genomic polymorphisms across human individuals can vary significantly even for monozygotic twins who can develop somatic mutations during development. However, the impact of these polymorphisms on the repertoire of peptides presented by major histocompatibility complex class I (MHC I) remains unknown. This presentation will highlight a novel proteogenomic approach that combines transcriptomic and MS-based proteomic data to profile MHC class I peptides and identify minor antigens (MiHAs) and other variants that harbour non-synonymous nucleotide polymorphisms. Using this approach we detected more than 45,000 MHC I peptides from B lymphocytes of 18 individuals and identified a subset of 39 MiHAs that share optimal features for immunotherapy of hematological cancers. These analyses also revealed that the immunopeptidome is represented by a limited number of source proteins with distinctive features, and that approximately 8% of MHC I peptides are derived from non-canonical reading frames. The notion the only a small fraction of the protein-coding genome is presented to our immune system has profound implications in autoimmunity and cancer immunology.

# Imaging mass spectrometry 1

# [Keynote] Multimodal Imaging Workflows: How to integrate mass spectrometry imaging approaches and (vibrational) spectroscopy techniques

Andreas Roempp *† [1], Rico Scheier [1], Heinar Schmidt [1], Axel Treu [1], Nicolas Desbenoit [1,2]

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[2] University of Bordeaux, Centre de Génomique Fonctionnelle de Bordeaux – CGFB – 146 rue Léo Saignat, 33000 Bordeaux, France

Multimodal imaging studies involving mass spectrometry typically face two major challenges: sample preparation and data analysis. Both will be discussed in detail in this presentation. Sample preparation is increasingly important since the spatial resolution of modern imaging systems can only be fully exploited if all data sets in a multimodal workflow are acquired from a single tissue section (as opposed to adjacent tissue sections). We give a brief example on the combination of SIMS and MALDI-MS imaging. The main part will focus on a workflow that combines atmospheric pressure MALDI-MS, Fourier-transform infrared (FT-IR) and Raman spectroscopy and histological staining for a single tissue section. All four techniques revealed a high spatial correlation of structural features. FT-IR provided a rapid overview about lipid and protein levels while Raman spectra revealed more specific information about lipids and heme species. MS provided the most detailed information about the spatial distribution of lipid species. The spectroscopic raw data was converted to the imzML format using a newly developed 'ASCII_to_imzML' converter. The converter will be made available on www.imzml.org and can be used for any ASCII data as demonstrated for statistical analysis results and mass deviation evaluation. Using our approach, spectroscopic and MS data can be directly combined "pixel-wise" on a spectral basis (as opposed to overlaying graphical images), enabling real multimodal data analysis. Our data processing workflow is flexible and can be easily adapted to multimodal imaging studies that include MS, vibrational spectroscopy and additional imaging techniques such as XRF or AFM.

*Speaker
†Corresponding author: Andreas.Roempp@uni-bayreuth.de

# [O1] Implementation of a high-repetition-rate laser in an AP-SMALDI MSI system for enhanced measurement performance

Max Müller *† [1], Mario Kompauer [1], Bernhard Spengler [1]

[1] Justus-Liebig University Giessen – Germany

Matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) at atmospheric pressure is a powerful technique to analyze biological samples and surfaces. Measuring large image sizes, however, is very time consuming, hindering high throughput applications.
A high-repetition-rate diode-pumped solid-state laser system, generating frequency-tripled 343 nm laser light with repetition rates of up to 2 kHz, was implemented in an AP-SMALDI10® MSI system in order to increase measurement speed and sensitivity. The setup was coupled to an orbital-trapping mass spectrometer with a mass resolution of 240,000 at $m/z200$ and a mass accuracy of
In order to increase sensitivity, a 'full pixel' scan mode was developed that allows ablation of rectangular spots of $\geq$25 $\mu$m size with a Gaussian 5 $\mu$m laser focus. Using 'full pixel' MSI, signal intensities increased up to 10-fold for lipids and peptides, allowing to analyze low-abundant species and to improve on-tissue MS2I measurements, while preserving scan speed.

*Speaker
†Corresponding author: max.a.mueller@anorg.chemie.uni-giessen.de

# [O2] On-tissue Paternò-B′uchi reaction for localization of lipid carbon-carbon double bond positions in MALDI MS imaging

Antonin Bednarik * [1], Stefan Bölsker [1], Jens Soltwisch [1,2], Klaus Dreisewerd† [1,2]

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[2] Interdisciplinary Center for Clinical Research (IZKF), University of Münster – Domagkstr. 3, 48149 Münster, Germany

Lipids have numerous essential functions in living organisms; for example, they act as structural components of cell membranes, source of energy, and as signaling molecules. Along with this variety of functions comes an immense structural complexity. Mass spectrometry (MS) is routinely used to unveil lipid class and overall composition and MALDI-MS imaging (MSI) helps us to understand how lipids are organized in biological tissues. However, resolving the physiologically important carbon-carbon double bond position in the alkyl chains of glycero- and glycerophospholipids remains challenging. One powerful approach for tackling this question is exploiting the Paternò-B′uchi (PB) reaction in combination with MS/MS of the products. In this work, we introduce a new "on-tissue" PB photo-derivatization protocol that enables subsequent MALDI-MSI and MS experiments. PB reaction is carried out in a reaction chamber filled with the vapor of a reactant. In order to precisely control vapor condensation, sample temperature is adjusted with a Peltier element. PB reaction is induced by irradiation with the 266 nm light of a Q-switched Nd:YAG laser. In our contribution we will discuss the selection of most suitable PB reagents and present data on the influence of the sample temperature, reaction time, and laser fluence. The capability of the combined on-tissue PB/MALDI-MSI technique to resolve double bond isomers of phosphatidylcholines (PC) will be exemplified with carboxymethyl cellulose (CMC)-based artificial tissues, spiked with known mixtures of egg yolk PCs, and with genuine animal tissues.

*Speaker
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# [O3] Error-free data visualisation and processing through mzML and imzML validation

Alan Race * [1], Andreas Römpp† [1]

[1] Universität Bayreuth – 95447 Bayreuth, Germany

Since their introduction, open vendor-neutral mass spectrometry (MS) and MS imaging (MSI) formats, mzML and imzML, have become increasingly prevalent, and are now the formats of choice for sharing and processing MS(I) data. This success has also facilitated the use of (i)mzML in data repositories and even storing multimodal data in multimodal studies.
Occasionally inconsistencies still occur which prevent successful visualisation of data, and it is not always obvious as a user what has caused the issue. One way to mitigate against this is to verify that every (i)mzML file adheres to the published standard.

Here we present an open source (i)mzML validation and editor tool. The primary purpose of which is to enable users to ensure that any given (i)mzML file is valid according to the specification and will therefore function correctly in any (i)mzML compatible software. The tool additionally provides an easy, user friendly, way to view and edit the imzML metadata, without requiring the user to understand XML.

We also present a means of performing conditional validation on imzML files, where validation rules are included based on the presence of other metadata. This enables thorough automated validation of (i)mzML data, necessary to validate against MS(I) data minimum reporting guidelines or perform validation during submission to data repositories.

The imzML format has also been updated to improve clarity and specificity. Full details can be found on the imzML website (https://imzml.org/).
Use of the validator results in a more reliable, error free, data processing workflow for the user.

*Speaker
†Corresponding author: andreas.roempp@uni-bayreuth.de

# Structural identification and annotation

# [Keynote] Selective phosphatidylcholine double bond fragmentation and localization using Paternò-B'uchi reactions and ultraviolet photodissociation

Sven Heiles * [1], Simon Becher [1], Mario Kompauer [1], Fabian Wäldchen [1], Patrick Esch [1]

[1] Justus-Liebig University Giessen – Germany

Double bond positions in phospholipids are believed to impact cell-membrane stability, protein-lipid interactions and lipid metabolism. Mass spectrometry based localization of double bonds in intact phospholipids, however, is challenging due to the inability to selectively cleave double bonds in fragmentation experiments. Therefore, the effect of double bond functionalization for selective double bond localization by ultraviolet photodissociation (UVPD) of phosphatidylcholines was investigated and results for complex phosphatidylcholine mixtures will be presented. Paternò–B'uchi reactions in nanoESI emitter tips enable attachment of acetophenone to double bonds of unsaturated phosphatidylcholines after 100 s of 254 nm light irradiation with about 50–80% reaction yield. Functionalized phosphatidylcholines dissociate upon 266 nm UVPD yielding double bond selective fragment ions in contrast to results for UVPD of unmodified lipids. UVPD of Paternò–B'uchi modified lipids results in a selectivity increase of up to 2.2 towards double bond localization compared to collision-induced dissociation (CID) experiments. Double bond localization is also possible with UVPD when alkali metal ion attachment to Paternò–B'uchi modified phosphatidylcholines occurs in contrast to CID experiments. The developed methodology is used to localize double bond and differentiate lipid double bond isomers in egg yolk allowing to identify 15 phosphatidylcholines. Results from this study demonstrate that locally depositing energy in close vicinity to cleavable bonds via UVPD can result in increased dissociation selectivity. This method can help to disentangle contributions from different structural elements in complex tandem mass spectra of lipids and aid to the structural characterization of phospholipids in a "top-down" approach.

*Speaker

# [O1] An Integrated Mass Spectrometry-Based Approach to Probe the Structure of the Full-Length Wild-Type Tetrameric p53 Tumor Suppressor

Christian Arlt * [1], Christian Ihling [1], Andrea Sinz† [1]

[1] Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Halle (Saale) – Germany

The tetrameric tumor suppressor protein p53 is a transcription factor that binds specific response element DNA sequences. P53 plays a crucial role in cancer prevention and is often referred to as "guardian of the genome". P53 is one of the most prominent representatives of intrinsically disordered proteins (IDPs) that are inherently difficult to be studied by conventional methods for protein 3D-structure analysis. Here, we present an integrated approach for deriving structural information of full-length wild-type p53 by cross-linking MS [1]. Cross-linking was performed using the MS-cleavable cross-linkers discuccinimidyl dibutyric urea (DSBU) and carbonyldiimidazole (CDI) [2]. The cross-links identified in the presence and absence of response element DNA were highly similar, indicating comparable conformations of free and DNA-bound p53 tetramer. To determine whether the DNA-binding capabilities of the p53 tetramer are retained after cross-linking; we combined cross-linking with native MS in one workflow. DNA binding of the p53 tetramer was retained when using DSBU [1]. In conclusion, integrating chemical cross-linking with native MS as complementary techniques is a powerful tool for protein structure elucidation. For the first time, the two techniques were combined in one workflow and allowed gaining structural insights into the p53 tetramer.

Arlt *et al.,* Angew. Chem. Int. Ed., 2017, 56, 275-279

Hage and Iacobucci *et al.,* Angew. Chem. Int. Ed., 2017, 56, 14551-14555

*Speaker

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# [O2] Novel aspects on chemical protein-RNA cross-linking coupled with mass spectrometry

Alexander Wulf * [1], Luisa Welp [1], Alexandra Stützer [1], Seychelle Vos [2], Timo Sachsenberg [3], Oliver Kohlbacher [3], Patrick Cramer [2], Henning Urlaub [1,4]

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[2] Molecular Biology Group, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany – Germany
[3] Quantitative biology center, University of Tübingen, Tübingen, Germany – Germany
[4] University Medical Center Göttingen Bioanalytics Department of Clinical Chemistry, Göttingen, Germany – Germany

Protein-RNA interactions play a pivotal role in copious cellular processes such as DNA transcription, mRNA translation, and RNA splicing. Understanding these biological processes on an interacting level requires the correct identification and exact location of RNA-protein interaction sites. Protein-nucleic acid cross-linking in combination with mass spectrometry (MS) has matured into a valuable tool to correctly identify these intricate sites by either two methods: Cross-linking can be performed using UV light, resulting in a "zero length" cross-linking event, where the interacting moieties form a chemical bond directly. Alternatively, the cross-link may be formed chemically by means of a cross-linking reagent.
Here, we present a method for cross-linking protein to RNA chemically using either 1,2,3,4-diepoxybutane (DEB), Traut's reagent or nitrogen mustard (NM). The yeast RNA-binding splicing protein Hsh49 and the negative elongation factor complex are assessed in a novel context of cross-linking coupled with mass spectrometry at amino acid resolution. Following enzymatic digests, chromatographic enrichments of cross-linked peptides, and subsequent mass spectrometric analyses, data analysis is performed in OpenMS using the RNP*xl* software tool. Our data demonstrate that chemical cross-linkers expand our knowledge on well-established identifications of cross-linked uracil adducts to all nucleotides found in RNA in a reliable, sensitive, and reproducible manner.
Identified chemical cross-linked amino acids are consistent with data from the literature and fit well into models of published crystal structures, as well as complement cross-links inferred from UV-cross-linking experiments. In this case, chemical cross-linking outperforms the UV standard method in terms of identified cross-linking sites.

*Speaker

# [O3] Investigation of the processes of oxidative modifications on the Reg3$\alpha$ liver protein and of some its shortened peptides by radiolysis and mass spectrometry

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[2] Physiopathologie et traitements des maladies du foie – Institut National de la Santé et de la Recherche Médicale - INSERM : UMR1193, Université Paris Sud - Paris XI – Centre Hépato-biliaire, Hôpital Paul Brousse, 94870 Villejuif Cedex, France

Reg3$\alpha$ is a C-type lectin that has been identified as a human pancreatitis-associated protein (PAP), and its cDNA was also found in hepatocellular carcinoma. It has been explored as a possible treatment for acute liver failure (ALF), and was shown to accumulate in the hepatic inflamed extracellular compartment where it limits the oxidative damages of surrounding proteins and lipids. The curative properties of Reg3$\alpha$ are therefore associated with its capacity to scavenge free radicals within the inflamed extracellular matrix (ECM). In this context, recombinant Reg3$\alpha$ protein was submitted to free radical generating conditions (generated by Fenton reactions and by 60Co irradiation radiolysis) to probe the molecular modifications induced by exposure of Reg3$\alpha$ to free radicals. An initial approach combining irradiation, protein separation and top-down mass spectrometry led to identify one systematically modified methionine residue and a 4kDa region in which further oxidation was located. This region could therefore be the active site of the antioxidant properties of the Reg3$\alpha$ protein and has been further studied. Under radiolysis conditions, this region was shown to possess a high number of oxidation sites, leading to a highly complex mixture of reaction products, that could not be resolved by high resolution MS alone. On smaller peptides, encompassing the regions of interest of the 4 kDa peptide, HPLC separation of the various oxidation products was developed and characterization by combined UV and MS approaches performed, in an attempt to tackle the complexity by progressing from a simplified system towards the full system.

*Speaker

# Clinical mass spectrometry

# [Keynote] Insights into the insertion mechanism of membrane proteins into nanodiscs using native mass spectrometry

Oliver Peetz *† [1], Erik Henrich [2], Aisha Laguerre [2], Jan Hoffmann [1], Volker Dötsch [2], Frank Bernhard [2], Nina Morgner‡ [1]

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[2] Goethe-University Frankfurt am Main - Department of Biophysical Chemistry – Max-von-Laue Str. 7 60438 Frankfurt am Main, Germany

Membrane protein (MP) complexes are particularly challenging to investigate with Mass Spectrometry (MS) as they require e.g. detergents for solubilization. However some complexes are not stable in detergents. For those, we focus on nanodiscs (ND) as a MP complex carrier. NDs consist of lipids which mimic the lipid bilayer of the membrane.
In LILBID (Laser-Induced-Liquid-Bead-Ion-Desorption), a piezo-driven droplet generator produces droplets of 30 $\mu$m diameter, which are transferred to high vacuum, irradiated by an IR laser operating at 2.94 $\mu$m, leading to an explosive expansion of the droplet which releases the solvated membrane proteins and protein complexes [1].

We developed a very successful method to investigate various numbers of membrane proteins in NDs using our homebuilt LILBID-MS. This method is suited to answer various questions on MPs, not just the more classical question on the oligomeric state. For instance, LILBID and activity assays revealed the activity of the translocase MraY is linked to the lipid induced oligomeric state [2].

Moreover, using LILBID-MS, we can reveal insights into the co-translational MP insertion process into NDs. Our method shows MPs enter the ND as monomers, subsequently forming complexes within the ND. During the MP insertion into NDs, lipids get kicked-out of the ND, confirmed by a combination of LILBID-MS and NMR [3].

Morgner et al., *Australian Journal of Chemistry*, 59:109–114, 2006.

Henrich & Peetz et al., *elife*, 2017;6:e20954, 2017.

Peetz & Henrich et al., *Analytical Chemistry*, 89 (22), 12314-12318, 2017.

*Speaker
†Corresponding author: peetz@chemie.uni-frankfurt.de
‡Corresponding author: morgner@chemie.uni-frankfurt.de

# [O1] Microproteomics of FFPE tissues: from biomarker discovery to routine molecular diagnosis.

Rémi Longuespée *† [1]

[1] Institute for Pathology Heidelberg (IPH) – Im Neuenheimer Feld 224 69120 Heidelberg, Germany

**Aim**

Shotgun proteomic approaches represent future directions for molecular pathology. We present a laser microdissection (LMD)-based method for microproteomics of FFPE tissues and applications for biomarker discovery.

**Methods**

The method was designed to avoid sample loss during processing of small FFPE tissue pieces for proteomics. It relies on heat-induced antigen retrieval and on-tissue digestion of proteins using highly concentrated trypsin. Proteolytic digests are analyzed by liquid-chromatography coupled to mass spectrometry. Data are processed for normalization and statistical analysis.

**Results**

The method was developed on a breast cancer tissue from which more than 1400 protein identifications from a single tissue piece containing less than 3000 cells were retrieved. The method was robust enough to distinguish very similar tissue types.

We applied the method to distinguish two types of anal cancer (squamous and transitional zones). We analyzed 5000 cells from 10 cases of each tissue type and retrieved 4500 protein identifications and 500 markers with at least 2-fold changes. The markers KRT7 and 19 were validated by immunohistochemistry.

We also analyzed a human liver infected by *Echinococcus granulosus (Eg).* The results suggested the presence of proteins related to Eg infection in the liver tissue itself, and specific immune mechanisms occurring in the cyst. Those markers will be of great interest for future diagnosis.

**Conclusions**

The method can be applied to a wide panel of biomarker discovery studies for pathologies that are difficult to diagnose. In the near future, microproteomics could represent one of the cornerstones for molecular pathology.

*Speaker

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# [O2] Proteomic profiling of melanoma cell lines and tissue to predict treatment response and patient survival

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[2] University Medical Center Hamburg-Eppendorf (UKE) – MARTINISTRASSE 52 20246 HAMBURG, Germany
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[4] Melanoma Institute Australia (MIA) – 40 Rocklands Rd, Sydney, NSW 2065, Australia

Melanoma is the most frequent cause of skin cancer-related deaths, and is the most common lethal malignancy in young people (< 40yrs). Inhibition of crucial survival pathways has become a routine in melanoma, however, response rates vary. We conducted mass spectrometric screening to detect cellular processes that might predict MEK inhibition (MEKi) response.
Ten subcutaneous melanoma derived cell lines (BRAFmut, NRASmut, MAPKwt,) and 32 fresh frozen NRASmut or BRAFmut regional lymph node metastatic melanoma specimens were obtained. Lysed cells and tissues were digested and analyzed by Data-Independent Acquisition (SWATH-MS) using a TripleTOF 5600/6600 mass spectrometer. Cell line viability over ten days in presence of a MEK inhibitor was determined using a PrestoBlue assay.

Across all cell lines, about 2500 proteins were quantifiable. Principal component analysis segregated melanomas based on in vitro MEKi sensitivity, whereas genotype alone did not. In total, 63 proteins were highly correlative with MEKi response. Survival analysis demonstrated significantly better survival (p=0.01) for patients with MEKi sensitive versus patients with MEKi resistant cell lines.

About 2000 proteins were quantifiable in the tissue cohort. The MEKi proteome phenotype was also a dominant signal in tissues, however, neither this phenotype nor genotype correlated directly with survival. Dividing patients into active and low or inactive melanogenic tumors and combining it with expression level of two plasma membrane proteins, allowed for accurate segregation between patients with good and poor post-biopsy survival.
SWATH-MS profiling demonstrated MEKi phenotypes in cell lines and fresh frozen metastatic melanoma and identified a marker panel predicting survival.

[*]Speaker
[†]Corresponding author: c.krisp@uke.de

# [O3] Mass and Charge Distributions of Amyloid Fibers Involved in Neurodegenerative Diseases: Mapping Heterogeneity and Polymorphism

Mohammad Abdul Halim * [1], Jonathan Pansieri [2], Rodolphe Antoine† [1], Vincent Forge‡ [3]

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[2] Univ. Grenoble Alpes, CNRS – Univ. Grenoble Alpes, CNRS, Grenoble INP, SIMaP, F-38000 Grenoble, France. – France
[3] BIG, CEA Grenoble – Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) - Grenoble – France

Authors: Jonathan Pansieri§a, Mohammad A. Halim§b, Charlotte Vendrelyc, Mireille Dumoulind, François Legrandd,e, Marcelle Moulin Sallanonf, Sabine Chiericig, Simona Dentig, Xavier Daganyb, Philippe Dugourdb, Christel Marquettea, Rodolphe Antoine*b, Vincent Forge*a
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c. ERRMECe, I-MAT FD4122, Université de Cergy-PontoiseF-95302 Cergy-Pontoise Cedex, France.

d. Enzymology and Protein Folding, Centre for Protein Engineering, InBIOS, University of Liège, 4000 Liège 1, Belgium

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f. Radiopharmaceutique biocliniques (INSERM U1039), Faculté de Médecine de Grenoble, F-38700 La Tronche, France

Heterogeneity and polymorphism are generic features of amyloid fibers with some important effects on the related disease development. We report here the first characterization, by charge detection mass spectrometry ,of amyloid fibers made of three polypeptides involved in neurodegenerative diseases: A$\beta$1-42 peptide, tau and $\alpha$-synuclein. Beside the mass of individual fibers, this technique enables to characterize the heterogeneity and the polymorphism of the population.

*Speaker
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In the case of A$\beta$1-42 peptide and tau protein, several coexisting species could be distinguished and characterized. In the case of $\alpha$-synuclein, we show how the polymorphism affects the mass and charge distributions. In the near future, such approach will be particularly powerful to characterize amyloid deposits extracted from patients.

# Bruker Workshop
# Monday 12:00 – 13:00
# Room 0.06

# Agilent Workshop
# Monday 13:00 – 14:00
# Room 0.23

# Monday Afternoon
# Plenary Lectures

# Challenges of protein PTM analysis by mass spectrometry

Giovanni Chiappetta, Massamba Ndiaye, Shakir Shakir, Joelle Vinh

Biological Mass Spectrometry and Proteomics SMBP CNRS USR3149 ESPCI Paris, PSL Research University. Email: joelle.vinh@espci.fr

Joëlle Vinh is specialized in analytical chemistry and biological mass spectrometry. She is presently the head of the Biological Mass Spectrometry and Proteomics laboratory at ESPCI Paris, PSL Research University. After a postdoctoral position in proteomics as an HFSP fellowship for 2 years at the Southern Denmark University in Protein Research group headed with Pr.Peter Roepstorff and Pr. Ole Jensen, she joined the Neurobiology and Cellular Diversity laboratory (ESPCI Paris) in 2000 as a CNRS research scientist. She set up the ESPCI biological mass spectrometry facility, with a first national recognition in 2001 from the National French Network (RIO, CNRS, Inserm, INRA and CEA). She worked as group leader of the biological mass spectrometry group since 2002, coordinating the RIO platform for proteomics at ESPCI. She co-authored more than 80 publications in international journals all involving biological mass spectrometry implementation. Working on the characterization of proteins (identification, post-translational modifications study, sequencing) using the MS proteomics toolbox, she focused more specifically on nano separations coupling with mass spectrometry for multidimensional analyses. Joelle Vinh coordinated the Network Analytics in Ile de France and is director of the CNRS Group of Scientific Interest for Analytical Sciences. She is member of the steering committee of Labex Institut Pierre Gilles de Gennes for microfluidics

**Abstract:**

Very rapid technological developments in separative sciences and mass spectrometry have made it possible to always better characterize proteins in biological models. The proteoforms are the final products of genes, and result from alternative RNA splicing (transcriptome) and from maturation of protein isoforms (proteome) associated to covalent post-translational modifications (PTMs). They interact with the molecular environment (proteins, ligands, oligonucleotides) and regulate many cellular processes. Their function, location, stability is often dynamically altered by PTMs. A protein can be declined into several proteoforms with multiple functions. PTM's characterization is therefore essential to understand and act on living organisms.

There are many PTMs (more than 1000 were listed in Uniprot in 2017). In order to finely characterize these changes, proteomic approaches in mass spectrometry are gradually developing with specific strategies to take into account their great molecular diversity. In order to better describe the underlying biological phenomena, it is necessary to identify and locate them and then quantify the proportion of proteoforms that are present. The control of many biological processes can result from the regulation of very low abundant and labile species. Diverse analytical strate-

gies for the characterization of these species were proposed, ranging from sample preparation to data processing, but still based on mass spectrometry analysis for the quantification and the structural characterization of multiple PTMs. Here we will present the evolution of some of these strategies, their similarities and differences, and the challenges and limitations associated with them, illustrating in particular this approach on the analysis of redox PTMs.

# New Methods and Instrumentation for Nanomaterials Characterization

Carsten Engelhard,[1] Bastian Franze,[2] Ingo Strenge,[1,3] Darya Mozhayeva[1]

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Professor for analytical chemistry at the University of Siegen.
Independent research at University of Münster (05/2010 – 03/2013).
Postdoctoral Research Associate (Prof. Gary M. Hieftje), Indiana University, Bloomington, USA, 01/2008-04/2010.
Adolf-Martens-Award, Adolf-Martens Fonds e.V. and Federal Institute for Materials Research and Testing (BAM), Award of the Analytical Chemistry Division of the German Chemical Society.
Editorial Board Member of the Journal of Analytical Atomic Spectrometry,

**Abstract:**

In this presentation, recent advances in inductively coupled plasma quadrupole mass spectrometry (ICP-Q-MS) instrumentation for nanomaterials analysis will be reviewed and an approach for direct, fast, and high-throughput nanomaterial characterization on a single particle level will be presented.

In the first part, the current state-of-the-art in single-particle (sp) ICP-MS instrumentation for the detection and characterization of single nanoparticles (NP) and our efforts to push the boundaries of MS instrumentation will be discussed. A custom-built high-speed data acquisition (DAQ) unit specifically tailored to the needs of spICP-MS will be presented. The DAQ was built in-house and enables continuous acquisition of signals from discrete ion clouds in the ICP with a time resolution of five microseconds and 100% duty cycle. With a time resolution much higher than the typical duration of a particle-related ion cloud, the probability of measurement artifacts due to particle coincidence could be significantly reduced and the occurrence of split-particle events was almost eliminated. Time-resolved ion cloud profiles of single 10-nm sized AuNPs were obtained for the first time using an ICP-Q-MS even in the presence of a significant background of ionic gold.

In the second part, it will be discussed how separation methods coupled to ICP-Q-MS can aid the detection of NPs in complex mixtures. Specifically, a method for nanoparticle fractionation, size characterization, and quantification of gold and silver nanoparticles using capillary electrophoresis (CE) and ICP-MS will be discussed and our recent efforts to expand this approach to CE-spICP-MS and different surface modifications of NP will be presented.

# Young MS Session

1st European Mass Spectrometry Conference
EMSC 2018
Saarbrücken • Germany • 11-15 March

DGMS
Deutsche Gesellschaft für Massenspektrometrie

SFSM
Société Française de Spectrométrie de Masse

# Youth Session of the EMSC

## Monday 12 March 2018

## 5:30 pm

## Room 0.18

## 6 oral presentations of young mass spectrometrists

Master and PhD students, young PhD graduates, will give six oral communications. You will have the opportunity to vote for the best presentation. The winner will have the opportunity to present his/her works during the EMSC conference.

**Friendship drink**

Club Jeune
Société Française de Spectrométrie de Masse

## Programme

- **5:30-5:45 pm - *Short introduction***

- **5:45-6:00 pm - *Talk #1***
  **Protein Interaction of a of p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass-Spectrometry**
  *Friederike Leßmöllmann - Martin-Luther University Halle-Wittenberg, Germany*

- **6:00-6:15 pm - *Talk #2***
  **Proteomics applied to the study of archaeological ceramics (II$^{nd}$ century) preserved in submarine context**
  *Sergui Mansour – Université des Sciences et Technologies de Lille, France*

- **6:15-6:30 pm - *Talk #3***
  **Spectral library based approach to protein arginine phosphorylation in *Staphylococcus aureus***
  *Sabryna Junker – University Greifswald, Germany*

- **6:30-6:45 pm - *Talk #4***
  **Characterization of Physical and Chemical Properties of Synthetic Polymers using Ion Mobility Mass Spectrometry**
  *Shinsuke Kokubo – University Göttingen, Germany*

- **6:45-7:00 pm - *Talk #5***
  **Probing peptide's conformational dynamics by a simple measure of its internal proton transfer time constant**
  *Mathilde Bouakil – Institut Lumière Matière de Lyon, France*

- **7:00-7:15 pm - *Talk #6***
  **Development of metabolite based MALDI MS assays**
  *David Weigt – Mannheim University of Applied Sciences, Heidelberg University, Germany*

- **7:15-7:45 pm - *Vote and results***

- **Drinks and food**

Youth Club of French Society of Mass Spectrometry
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# Application for a presentation

## Protein Interaction of a of p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass-Spectrometry

Leßmöllmann, Friederike[1]; Arlt, Christian[1]; Lewitzky, Mark[2]; Feller, Stephan M.[2]; Sinz, Andrea[1]

[1]Institute of Pharmaceutical Chemistry & Bioanalytics, Martin-Luther University Halle-Wittenberg
[2] Tumor Biology Section, Institute of Molecular Medicine, Martin-Luther-University Halle-Wittenberg

The tumor suppressor p53, also referred to as the “guardian of the genome”, is a transcription factor that plays a key role in preventing the development of cancer. It binds to specific response element DNA as a tetramer of initial dimers and enhances the rate of transcription of selected genes that are involved in cell cycle arrest, apoptosis or senescence. The structure of the p53 monomer comprises about 40 % of intrinsically disordered regions to allow the effective binding of a variety of different proteins [1]. Despite the broad spectrum of already known p53 interaction partners, the identification of p53 binding proteins has never been performed in the context of the full length p53 in a proteomic scale. We will perform p53 interaction partner analysis with lysate of LoVo colon adenocarcinoma cells [2] using a combined affinity enrichment cross-linking/MS approach utilizing the MS-cleavable cross-linker disuccinimidyl dibutyric urea (DSBU). The experiments will be performed by using the cytosolic fraction as well as the nuclear fraction to localize specific interaction networks depending on the compartment. Candidate p53 binding partners will be subjected to detailed structural analyses employing a multi-disciplinary approach combining chemical cross-linking/MS and native MS. These studies will offer further insights into the interaction network of p53 in colon carcinoma cell lines and the conformational versatility of the so far understudied IDRs in p53 upon binding to different protein interaction partners.

[1] Joerger and Fersht, Annu Rev Biochem, 2016, **85**, 375-404

[2] Drewinko *et al.*, J. Natl. Cancer Inst., 1978, **61**(1), 75-83

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**Proteomics applied to the study of archaeological ceramics (IInd century) preserved in submarine context**

Sergui Mansour*[1], Fabrice Bray[1] , Nicolas Garnier[2] , Franca Cibecchini[3], Christian Rolando[1] , and Caroline Tokarski[1]

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*2- SARL Laboratoire Nicolas Garnier – LNG – 63270 Vic le Comte, France, France*
*3- Département des Recherches Archéologiques Subaquatiques et Sous-Marines – DRASSM – 147 Plage de l'Estaque, 13016 Marseille, France, France*

Abstract Ceramic material represents essential witness of human activities through years. The objective of this study is to identify protein residues trapped into archaeological amphorae preserved in submarine context during several thousand years. The focus was given to the study of Dressel 14 amphorae from shipwrecks (IInd century). These amphorae were supposed to store and transport fish derivate products such as Garum and Liquamen. The proteomics workflow was firstly adapted to the study of Garum model fish sauces prepared from various fish species (e.g. sardines, mackerels). After the optimization of extraction, digestion and data-treatment procedures, both muscle proteins (e.g. tropomyosin, myosin) and blood proteins (e.g. hemoglobin) were successfully identified. Due to the lack of fish protein sequences in proteomic/genomic databases, most of the proteins were identified using sequence homologies to other fish species (e.g. Danio renio, etc.). De novo sequencing allowed the identification of new un-sequenced peptides from muscular and blood proteins and several peptides could be used to discriminate the different fish species. The developed protocol was applied on several hundred micrograms of archeological amphorae. For the first time, 157 proteins were successfully identified among which 19 muscular proteins (e.g. myosin light chain 1,2 and 3). Based on the current databases that include 21299 fish myosin sequences (19 for Thunnus genus, 21 for Scomber genus) and internal databases (fresh Thunnus and Scomber muscles), 4 myosin peptides could be attributed to Scombridae family. This result represents the first analytical confirmation of the use of Dressel 14 for Scombridae fishes transportation.

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# Application for a presentation

15 minutes with questions – **Slides and talk in English**
(Deadline for submission on February, 16th 2018 to clubjeunesm@gmail.com)

**Spectral library based approach to protein arginine phosphorylation in *Staphylococcus aureus***

Sabryna Junker, Sandra Maaß, Michael Hecker, and Dörte Becher

*Institute for Microbiology, University Greifswald, Greifswald, Germany*

*Staphylococcus aureus* emerged as an important human pathogen and is the causative agent of a high number of nosocomial infections. Therefore, investigation of the phosphoproteome will help to decipher molecular and cellular mechanisms that underlie pathogenesis and virulence. In addition to the well-known phosphorylations at ser/thr/tyr/cys/his/asp residues, the phosphorylation at arginine residues plays an essential, but mostly still unknown role in bacteria. Therefore, we decided to study arginine phosphorylations in greater detail.

*S. aureus* COL possesses the protein PtpB which is assumed to be an arginine phosphatase. Hence, we applied a gel-free method to analyze the changes in the phosphoproteome of the deletion mutant *ΔptpB* and the wild type, thereby focusing on arginine phosphorylations. This way, we could identify eight arginine phosphorylations in wild type samples (Bäsell *et al.* 2014) and 207 arginine phosphosites exclusively within the mutant (Junker *et al.* 2018). This identification of putative targets of PtpB allows further investigation of the physiological relevance of arginine phosphorylations.

In order to enhance the reliability of identified phosphorylation sites at arginine residues, a subset of arginine phosphorylated peptides was chemically synthesised. Combined spectral libraries based on phosphoenriched samples, synthetic arginine phosphorylated peptides and classical proteome samples contain 960 phosphopeptides (396 at arginine) and provide a sophisticated tool for the analysis of phosphorylations. Furthermore, the combination of proteome and phosphoproteome quantifications of wild type and mutant under control and stress conditions indicates drawbacks to amino acid metabolism and therefore reveals further insights into the physiological role of this important human pathogen.

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# Characterization of Physical and Chemical Properties of Synthetic Polymers using Ion Mobility Mass Spectrometry

Shinsuke Kokubo

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Only since very recently, ion-mobility mass spectrometry (IM-MS) starts to attract attention as a very powerful tool for obtaining microscopic physical and chemical properties of polymers. IM-MS simultaneously provides molecular weight and collision cross section (CCS), which corresponds to the spacious size of a polymer chain. The contribution of each individual monomer unit to the increment of CCS, which depends on the type of the monomer unit, can then be used to obtain information about the polymer coil. Following this line, I am thus enthusiastically working on the development of new IM-MS methods that are addressing various types of characters e.g. the dielectric property and the stiffness, i.e., the characteristic ratio ($C_n$) and of the polymer.

In this study, IM-MS of doubly-charged polyethylene glycol (PEG) and polypropylene glycol (PPG) were performed after electrospray-ionization mass spectrometry. The dependency of the effective collision cross section on the number of monomer units was evaluated with the help of MD simulations. Assuming a balance between elastic and coulomb forces inside short, asymmetric conformation of doubly-charged chains, a method was developed to evaluate the dielectric constant, which was found to be 7.87 for PEG and 6.18 for PPG. From the same experiment at higher chain lengths the characteristic ratio $C_n$ was evaluated with 4.30 for PEG and with 5.54 for PPG. Those values showed very good agreements with the ones were obtained by conventional methods [1].

The proposed methods are easy to perform and provide stunningly rapid access to various properties of synthetic polymers. Besides that, IM-MS provides the intrinsic properties of polymers, since the experiment is carried out under vacuum condition without the disturbances of solvent and neighbouring polymers. IM-MS has the potential for providing many other innovative strategies that may be important in polymer science and technology.

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Youth Club of French Society of Mass Spectrometry
EMSC 2018
Saarbrucken
11-15 March 2018
https://emsc2018.sciencesconf.org/

**Probing peptide's conformational dynamics by a simple measure of its internal proton transfer time constant.**

M.Bouakil, P.Dugourd, L.MacAleese
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Previous studies have shown that, in a certain family of peptides including a tryptophan and a histidine, it was possible to photo-induce an intramolecular proton transfer. It was also shown that, in the peptide sequence HGGGW, this proton transfer between tryptophan and histidine lasts over a few hundreds of microseconds[1]. Eventually, it was shown by comparison with molecular dynamics, that this internal proton transfer was kinetically limited by the peptides conformational dynamics. Building on those previous results, we want, in the study reported here, to use the proton transfer time constant as a signature of the peptide conformational dynamics. In particular, we propose to analyze the effects of different parameters – peptide size, amino-acid steric hindrance and backbone rigidity – on the conformational dynamics of those peptides.

A pump-probe setup was designed, coupling two lasers and an LTQ linear ion trap. The peptide of interest, which contains both a tryptophan and a histidine, is isolated and trapped in the gas phase. Then it is irradiated with a 266nm "pump" laser pulse that creates a radical cation (the radical and charge are situated on the tryptophan). The intramolecular proton transfer from the indole group of the tryptophan to the imidazole group of the histidine is probed by a second, delayed, laser pulse at 545 nm. This wavelength corresponds to the maximum absorption of tryptophan radical cation. Thus, the probe pulse induces the specific photo-fragmentation of peptides containing a tryptophan radical cation. As a consequence, the photo-fragmentation ratio decay at 545 nm reflects the progressive disappearance of this species as the proton transfer occurs. It can be fitted with a mono-exponential decay and the extracted time constant indicates the proton transfer time scale.

In order to assess effects of backbone rigidity, we substitute glycines with prolines in the original peptide sequence (HPPPW) and then we measure the photo-induced proton transfer time constant in the modified peptide. The effect of steric hindrance is probed similarly by substituting glycines with alanines and isoleucines (HGGGW, HGAGW, HGIGW,). Preliminary results seem to indicate that the steric hindrance of amino-acids in between the proton donor and acceptor has a limited effect on the proton transfer, and thus on the peptide conformational dynamics.

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# Application for a presentation

## Development of metabolite based MALDI MS assays

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Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has become a promising tool in drug discovery. Mass spectrometers of the latest generation enable high throughput, label free analysis of assays in 1536-well format [1]. Munteanu *et al.* demonstrated feasibility of whole cell MALDI MS-based assays, i.e. the delineation of concentration response curves for histone deacetylase inhibitors incl. calculation of $EC_{50}$ values from histone acetylation-associated mass shifts [2]. While whole cell assays are well established for abundant, pharmacodynamic protein markers, analogous assays for metabolites are lacking. A central reason is the higher complexity of spectra in the low molecular mass range [3]. In this study, we employed a score-based method development approach to establish a small molecule fingerprinting workflow applicable for whole cells. Our IT-supported data acquisition and processing pipeline enabled the identification of lipid marker molecules that show reproducible concentration responses to tyrosine kinase inhibitors. Re-measurements of spots that exposed highest feature intensity using ultra high-resolution FT-ICR MS enabled structural elucidation of the respective marker molecules.

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# Monday Posters

# [P001] Proteomic analysis of extracellular vesicles derived from mesenchymal stem cells in patients with myelodysplastic syndromes and acute myeloid leukemia.

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The hallmark of myeolodysplastic syndromes (MDS) is an insufficient haematopoiesis resulting in cytopenias and possible transformation into acute myeloid leukemia (AML) (Geyh, Oz *et al.,* 2013). As frequently shown, mesenchymal stem cells (MSC) serve a decisive role in hematopoietic insufficiency by generating signals for differentiation and proliferation of hematopoietic stem and progenitor cells (HSPC) (Saleh, Shamsasanjan *et al.,* 2015). MSC not only reveal impaired stromal support for HSPC in MDS and AML, recent results also indicate that soluble factors secreted by MSC essentially mediate as bioactive molecules in cell-cell-communication (Lavoie, Rosu-Myles, 2013).
In this work we are focussing on the proteomic characterisation of extracellular vesicles (EV) as an important component of the secretome to provide an insight into the mechanisms of intercellular communication between MSC and HSPC. Therefore, we precipitate EV of the secretome of patients with MDS and AML. Validation of exosomal isolation is implemented by detection of exosome-associated markers such as CD9 and CD63 using Western Blot. For mass spectrometric analysis isolated EV were processed by in-gel digestion with trypsin and analysed on a RP-HPLC system (RSLCnano, Thermo Fisher, Bremen, Germany) online coupled to an orbitrap mass spectrometer (Q Exactive Plus, Thermo Fisher, Bremen, Germany). Protein identification was performed using Proteome Discoverer 1.4 in combination with the MS Amanda algorithm to search the UniProt-SwissProt human database.
With this workflow we are aiming at determining the proteomic compounds of MSC-derived EV in healthy, MDS- and AML patients in order to facilitate the detection of possible HSPC-stimulating proteins.

*Speaker

# [P002] UVPD as a unique fragmentation tool for complete structure determination and substructure identification of small molecules

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Plant and fungus secondary metabolites are involved in many functions such as pigmentation, UV filtration, and defense mechanisms. These polyphenolic antioxidants have been incorporated for thousands of years in Eastern medicine but have yet to be utilized in Western therapeutics despite their phenomenal record in providing health benefits.

Here we investigate the molecular structures of several phenylpropanoids using various fragmentation techniques (CID, HCD and UVPD) available on an Orbitrap TM Fusion TM Lumos TM Tribrid TM mass spectrometer. We performed CID, HCD and UVPD fragmentation experiments on these molecules for structural investigation. The data acquired from the different fragmentation techniques were used to reveal the molecular structures of each flavonoid using mzCloud and Xcalibur Qual Browser.

The structural diversity of conjugated flavanoids is extremely large, with the total number of combinations of conjugate sugars on different cores and their linking positions being unknown. When subjected to UVPD, flavone and flavanone both produce unique fragment ions from radical fragmentation channels not observed in either CID or HCD. A detailed UVPD study of luteolin and several of its mono glycosides showed that fragmentation of the core structure could be obtained concurrently with the losses of the glycosides typically observed by other fragmentation techniques. UVPD also provided unique fragmentation channels in both low and high mass range, since UVPD does not suffer the same low mass cut-off observed with CID. These results reveal that UVPD could be a powerful technique for the structural elucidation of conjugated flavonoid structures.

*Speaker

# [P003] Photon and electron interactions with trapped lead clusters

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Charged lead clusters are trapped and photoexcited within a Penning Trap [1]. The charged dissociation products remain in the trap and are time-of-flight analyzed. Whereas larger clusters evaporate neutral atoms, for smaller precursors one of the main fragments observed are clusters which lost seven atoms. This is presumably due to the break off of a neutral heptamer. In a further measurement, the time between photoexcitation and time-of-flight analyzation is varied to disentangle the fragmentation pathways by comparing the growth and decay times of the fragments [2]. In addition, negatively charged lead clusters are trapped and exposed to an electron beam [3]. This leads to electron attachment [3,4] as well as to fragmentation processes [3]. The investigated fragment sizes reveal first hints of fission processes, where doubly negatively charged clusters break up into two charged fragments. This interpretation is confirmed by a measurement of the fragment cluster sizes of photoexcited doubly negatively charged lead clusters [5].

S. K´onig *et al.*, *J. Phys. Chem. C* 121 (2017) 10858.

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*Speaker

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# [P004] He-CTD: A new radical-initiated tandem MS Fragmentation to deciphering the structure of complex oligosaccharides

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Deciphering carbohydrates structures challenges the field of analytical chemistry. Classical tandem MS based on low-energy collision-activated dissociation (LE-CAD) fails in many cases to achieve definitive structural assignments. This is due to the lack of informative fragments produced, or even to the generation of ambiguous or internal fragments that can lead to misinterpretation.
The presented work explored a recently introduced method for ion activation, based on Charge Transfer Dissociation using kiloelectronvolt accelerated Helium cations (He-CTD). The electron affinity of Helium cations (24.6 eV) is the largest of any singly charged cation, promotes an electron detachment and the production of a radical species. This intermediate fragments readily and leads to a variety of diagnostic ions.

On carbohydrates, He-CTD provided rich and informative spectra allowing the complete sequencing as well as the characterization of the linkage pattern between subunits (including branched species) and the positioning of important chemical modifications.

In contrast to other methods such as ETD or ECD, He-CTD can be used on singly charged cations which are the mainly produced ions for oligosaccharides. This method can be implemented on a conventional benchtop ion trap and, recently, we have shown the first He-CTD experiments in negative ionization mode showing that He-CTD can be operated in both ionization modes. The efficiency of fragmentation is fully independent of the family of polysaccharide, the polymerization degree or the charge state of ions.
We thus believe that the advances brought by He-CTD in tandem MS will meet a widespread interest well beyond the community of glycoscientists.

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# [P005] Hydroxyethyl-group acting as a pseudo-label in Disperse-Red-1 push-pull azobenzene

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Isotope labeling is the standard procedure in mass spectrometry elucidating fragmentation mechanisms. However, this method usually is expensive and it needs ambitious laboratory work. To overcome these disadvantages, we use an additional hydroxyl-group labeling an alkyl chain using the example of disperse-red-1.
All mass spectra were recorded on an APEX-Qe FT-ICR-MS (Bruker Daltonik) equipped with an Apollo-III ESI-source. Fragmentation of the stored disperse-red-1 ions was achieved either by SORI-CID with argon acting as collision gas or by photodissociation with an argon-ion-laser. Additionally, quantum chemical and kinetic calculations were carried out.

Our interest focuses on the fragmentation behavior of the diethylamino-group combined with different aromatic systems. The hydroxy-label in disperse-red-1 provides us to distinguish between six instead of three proposed mechanisms regarding the diethylamino-group. Each of them depends on the protonation position resulting in 12 possible mechanism. The combination of mass spectral analysis together with theoretical methods reveal three mechanisms being the most likely ones. First, the loss of a hydroxymethyl-radical species is due to alpha-cleavage of the respective C-C-bond if protonation occurs at the azo-group. A following loss of an ethyl-radical group results in the overall elimination of C3H8O. Second, the loss of an ethyl-radical occurs in the case of protonating the aniline-nitrogen followed by losing the complementary hydroxymethyl-radical. In this case, there are no sufficient differences between the labeled or unlabeled sidechain (hydroxyethyl- or ethyl-radical). Concerted mechanisms do not show significant influence regarding the loss of C3H8O.
Pseudo-labeling is introduced to distinguish between 12 fragmentation mechanism.

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# [P006] Loss of C3H8 from the Methylene-iminium Cations of Diethyl-ethylanilines after Collisional Activation

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Molecules with diethylamino sidegroups show unusual fragmentation, for example the loss of C3H8 after chemical or electrospray ionization. Distinctions between consecutive or concerted mechanisms are not easily feasible. Isotopic labeling experiments can be a helpful tool to gain a deeper insight into the fragmentation reactions. In this work, the loss of C3H8 from the methylene-iminium cations of different diethyl-ethylanilines is investigated using a VG-Micromass ZAB-2F, a double-focusing sector field instrument. The samples including isotopically labelled molecules are ionized via electron ionization. Mass-analyzed-Ion-Kinetic-Energy-(MIKE)-spectrometry provides values for the kinetic energy release (KER). Collisional activation in the second field free region is performed using argon as collision gas. None of the investigated diethylaniline derivatives show the loss of C3H8 from the molecular radical cation. Instead, the sequential loss of a methyl radical and ethylene is observed leading to the formal loss of a C3H7 radical in the mass spectra. Upon collisional activation, the loss of 44 Da can be observed not in the MIKE spectra of the molecular radical cation but in the MIKE-spectra of the methylene-iminium cations of diethyl-ethylanilines ([M-15]+). The intensity of this fragment signal depends strongly on the position of the substituted ethyl group on the aromatic system. For ortho substitution, a significant increase in intensity is observed that is due to the availability of a concerted mechanism. It competes with the sequential loss of a methyl and ethyl radical via a radical mechanism. The proposed mechanisms can be supported with isotopic labeling experiments.

*Speaker

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# [P007] Femtosecond-laser-pulse induced photodissociation of Indocyanine Green in the gas phase

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The dye Indocyanine Green (ICG) is widely used for medical purposes and as a fluorescent dye[1]. Investigations on the fluorescence of ICG showed very short fluorescence lifetimes [2] which leads to the requirement of femtosecond laser pulses for photodissociation experiments. The mass spectra have been recorded on an Apex III FT-ICR mass spectrometer (7.05 T) (Bruker Daltonik, Germany) using different fragmentation techniques. The ionization was performed via Electrospray Ionization from different solvent mixtures. For the fragmentation reactions, collision gas (Argon) or a femtosecond laser system (NIR region) were used.

Depending on the solvent mixture, the sodiated or the protonated ions and their clusters were observed in the mass spectra. Collision-induced-dissociation (CID) and photodissociation (PD) were applied to the different ions. The photodissociation process at a wavelength of 790 nm resulted in several intense fragments for the sodium adduct and less intense fragments for the protonated molecule. The CID-spectra showed poor fragmentation for the protonated molecule and no fragmentation for the sodium adduct. Thus, we can assume that the collision energy does not exceed the required energy that is reached by multiphoton absorption of the femtosecond laser pulses.

The comparison of the fragments form the protonated and the sodiated molecules gives a hint on the influence of the sodium cation on the molecular stability. [3]

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E. Mitrofanov, T. Muskat, J. Grotemeyer, EJMS **2018**, in print.

*Speaker

# [P008] Collision induced dissociation (CID) and higher-energy collision dissociation (HCD) mass spectrometry for structural elucidation of saccharides and clarification of their dissolution mechanism in DMAc/LiCl

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The dissolution mechanism of oligosaccharides in N,N-dimethylacetamide/lithium chloride (DMAc/LiCl), a solvent used for cellulose dissolution, and the capabilities of Collision Induced Dissociation (CID) and Higher-energy Collision Dissociation (HCD) for structural analysis of these same oligosaccharides were investigated. Comparing CID and HCD spectra shows that, generally, HCD can provide more structurally informative fragment ions from lithiated sugars. This is because HCD is a non-resonant activation technique and it allows a higher amount of energy to be deposited in a short time, giving access to more endothermic decomposition pathways as well as consecutive fragmentations. When working with *mono-lithiated* oligosaccharides, glycosidic bond cleavage is the main pathway, whereas *di-lithiated* sugars undergo predominately cross-ring cleavages. This difference indicates that the presence of the second lithium strongly influences the relative rate constants for cross-ring cleavages (rearrangement) vs. simple glycosidic bond cleavages, and favors the former. Regarding the dissolution mechanism of sugars in DMAc/LiCl, CID and HCD experiments on di-lithiated and tri-lithiated sugars reveal that intensities of product ions containing two Li+ or three Li+, respectively, are higher than those bearing only one Li+. In addition, comparing the fragmentation spectra (both HCD and CID) of LiCl adducted lithiated sugar and NaCl adducted sodiated sugar shows that while, in the latter case, loss of NaCl is dominant, in the former case, loss of HCl occurs preferentially. The compiled evidence implies that there is a strong and direct interaction between lithium and the saccharide during the dissolution process in the DMAc/LiCl solvent system.

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# [P009] Fast Analysis of Liquid Crystal Displays by Plasma-based Ambient Desorption/Ionization Mass Spectrometry

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Liquid crystal displays (LCDs) are one of the most commonly used display technologies worldwide. Quality control, particularly for monitoring/understating display failure, as well as further product development necessitates powerful analytical methods to rapidly analyze these complex materials. Chromatographic techniques coupled to mass spectrometry and microscopy methods are typically used for liquid-crystal analysis. Because these methods, and the associated sample pretreatment steps (*e.g.*, manual extraction of liquid crystals from a large-format display), are very time consuming and require appreciable amount of solvent, there is a need for alternative methods to increase sample throughput. Ambient desorption/ionization mass spectrometry (ADI-MS) is well suited for such a task, because it can be used to directly desorb and ionize analytes from the sample surface with minimal sample preparation or consumption of solvent. In this study, selected plasma-based ADI sources, direct analysis in real time (DART), flowing atmospheric-pressure afterglow (FAPA), and low-temperature plasma (LTP), will be compared for the direct analysis of liquid-crystalline materials by mass spectrometry. The main aim of this work is to minimize sample preparation for quick and cheap LCD failure analysis by differentiating between liquid crystals and corresponding degradation products. Additionally, further improvements in mass-spectral imaging of LCDs will be presented. In the present case, imaging is performed with laser ablation coupled to FAPA-MS. This instrumental setup enables the analysis of very low sample volumes that are present inside the LCD. Ultimately, this method shall help to differentiate between different LCD components and identify defects within a LCD by lateral profiling.

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# [P010] The Usage of a Direct Inlet Probe Prototype, coupled to a Chemical Ionization Source of a High Resolution Mass Spectrometer

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The direct probe technique is a well established, fast and convenient method to analyze solid and liquid samples using mass spectrometry. This supplement, designed and manufactured by Scientific Instruments Manufacturer (SIM) GmbH, is now available for the LECO Pegasus GC-High Resolution TOFMS, equipped with a chemical ionization source (PCI). The CI source can be switched to EI operation from run to run by turning off the reagent gas and loading a different set of ion source parameters. Losses of signal intensity are negligible due to the high sample amount introduced into the source by the DIP device. Electron impact is an energetic ionization technique causing an extensive molecule fragmentation with low yields of the molecular ion. Standard 70-eV EI mass spectra are reproducible and allow comparisons with commercially available mass spectral libraries. In contrast, CI gives high proportions of quasi-molecular ions. The molecular mass of the analyte becomes clearly defined (CI) and the chemical structure can be inferred from the fragment ions (EI). The information content can be further enhanced by embedding the LECO GC-High Resolution TOFMS into the hardware configuration. The Pegasus® GC-HRT+ provides the ultimate high-performance MS capabilities, such as mass resolution of 50,000 FWHM and mass accuracies less than 1 ppm. For this revised instrument version, LECO has developed Encoded Frequent Pushing$^{TM}$, a method of pulsing an orthogonal accelerator multiple times per transient with unique time intervals between each push pulse. This method is particularly well suited for improving the duty cycle of an Multireflecting-TOFMS.

*Speaker

# [P011] Beispiele f́ur den Einfluss des Śaulentyps auf die Qualit́at der LC/MS-Analytik

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Das Poster zeigt, wie der Śaulentyp die Qualit́at einer LC/MS-Methode entscheidend beeinflussen kann:

**Mehr Retention f́ur polare Analyten bei Multimethoden im RP-Modus**

Restek‘s Ultra Aqueous C18, eine polar modifizierte C18-Phase, verh́alt sich wie eine hydrophobe C18-Śaule, bildet aber zuśatzlich Wasserstoffbŕuckenbindungen aus. So ist sie in der Lage in Multikomponentenmischungen auch die polaren Substanzen zu retardieren und mit guter Peakform zu eluieren, was eine reine C18-Phase meist nicht kann. Sie ist typischerweise im Einsatz in der Pestizidanalytik, bei wasserĺoslichen Vitaminen und ́ahnlichen Anwendungen.

**Bessere Robustheit bei gleichbleibender Trennleistung in der UHPLC/MS durch Ersatz von $<$ 2$\mu$m vollpoŕosen Partikeln durch 2.7$\mu$m Core-Shell Partikel**

Aufgrund ihrer besonderen Struktur erreicht man mit Core-Shell Śaulen deutlich schmalere Peaks als mit vollpoŕosen Śaulenmaterialien der gleichen Partikelgŕoße. Dies kann man in der UHPLC/MS bei Problemen mit schnell verstopfenden Śaulen und Druckanstieg ausnutzen. Mit Raptor Core-Shell Śaulen mit 2.7$\mu$m Partikelgŕoße erreicht man die gleiche Trennleistung wie mit einer vollpoŕosen $<$ 2$\mu$m Śaule, hat aber nur ca. den halben Druck und eine wesentlich geringere Neigung zum Verstopfen. Dies wird anhand eines realen Beispiels aus der Pestizidanalytik gezeigt, bei der die Robustheit der Methode entscheidend verbessert werden konnte.

**Schmale Peaks und schnelle Trennungen mit 5 $\mu$m Core-Shell Partikeln**

Dass man auch mit 5 $\mu$m Śaulen eine hervorragende LC/MS-Analytik machen kann, zeigt ein Beispiel von perfluorierten Śauren / PFTs. Raptor 5$\mu$m Core-Shell Śaulen erreichen bessere Peakbreiten als 3$\mu$m vollpoŕose Śaulen, haben dabei aber den niedrigen Ŕuckdruck und die gute Robustheit von klassischem 5$\mu$m Material.

*Speaker

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# [P012] New Methods and Instrumentation for Nanomaterials Characterization

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In this presentation, recent advances in inductively coupled plasma quadrupole mass spectrometry (ICP-Q-MS) instrumentation for nanomaterials analysis will be reviewed and an approach for direct, fast, and high-throughput nanomaterial characterization on a single particle level will be presented.
In the first part, the current state-of-the-art in single-particle (sp) ICP-MS instrumentation for the detection and characterization of single nanoparticles (NP) and our efforts to push the boundaries of MS instrumentation will be discussed. A custom-built high-speed data acquisition (DAQ) unit specifically tailored to the needs of spICP-MS will be presented. The DAQ was built in-house and enables continuous acquisition of signals from discrete ion clouds in the ICP with a time resolution of five microseconds and 100% duty cycle. With a time resolution much higher than the typical duration of a particle-related ion cloud, the probability of measurement artifacts due to particle coincidence could be significantly reduced and the occurrence of split-particle events was almost eliminated. Time-resolved ion cloud profiles of single 10-nm sized AuNPs were obtained for the first time using an ICP-Q-MS even in the presence of a significant background of ionic gold.
In the second part, it will be discussed how separation methods coupled to ICP-Q-MS can aid the detection of NPs in complex mixtures. Specifically, a method for nanoparticle fractionation, size characterization, and quantification of gold and silver nanoparticles using capillary electrophoresis (CE) and ICP-MS will be discussed and our recent efforts to expand this approach to CE-spICP-MS and different surface modifications of NP will be presented.

*Speaker

# [P013] Tandem Ionisation for GC(xGC)-MS: Complementary soft ionisation spectra for confident identification

Gerhard Horner [1], Laura Mcgregor* [2], Thomas Zanni †‡ [3], Joerg Koehler [3], Dedishov Ruslan [3], Bukowski Nick [4]

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Time-of-flight mass spectrometry (TOF MS) has made considerable advances in recent years, with the elimination of mass discrimination (crucial for GC couplings), increased sensitivity and compact instrument designs making the technique more amenable. We will explore a number of advances in TOF MS technology which are set to further increase its applicability.
The term 'multiplexing' is used to describe the combination of multiple data streams into a single, comprehensive stream. Multiplexing can be applied to mass spectrometry in a number of ways but, for the purpose of this presentation, we will focus on fast-switching of electron energies to collect both hard (70 eV) and soft (10-20 eV) electron ionisation spectra within a single run. Fast multiplexing during acquisition greatly improves the performance of a TOFMS, enabling two complementary MS datasets to be acquired simultaneously.

The novel ion source used in this study enables soft electron ionisation to be performed with no inherent loss in sensitivity. Multiplexing between ionisation energies allows conventional 70 eV spectra to be obtained - for routine identification against commercial libraries - as well as simplified soft EI spectra for enhanced confidence in identification of compounds which may exhibit weak molecular ions and/or similar spectra at 70 eV.

These technological advances will be demonstrated using a number of real-world samples.

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# [P014] A multi-reflection time-of-flight mass spectrometer for metal-cluster investigations

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Due to the possibility of attaining high mass resolving powers (R_FWHM > 10^5) in short measurement times, multi-reflection time-of-flight mass spectrometers (MR-ToF MS) are successfully employed for ion separation [1,2] and precision mass measurements [3].
An MR-ToF analyzer for molecular physics has been built at the University of Greifswald to investigate small metal clusters (compositions of identical atoms) produced by laser ablation. Cluster ions are captured between two electrostatic mirrors by switching the voltage applied to an in-trap lift electrode [4] located between them. The ions' kinetic energy is thus lowered, leading to them revolving between the ion mirrors. After storage periods of up to several hundred milliseconds (several thousand revolution periods), the ions are ejected by reapplying the in-trap lift voltage or lowering the potential of the exit mirror [5] and subsequently guided towards an ion detector.

Measurements have been performed using both single-path and multi-reflection ToF operation to characterize the cluster production as well as the MR-ToF analyzer. The system's mass resolving power is not only sufficient for the separation of individual cluster sizes but also of their isotopologues.

R.N. Wolf *et al.*, *Phys. Rev. Lett.* 110 (2013) 041101.

P. Fischer *et al.*, *Rev. Sci. Instrum.* accepted.

F. Wienholtz *et al.*, *Nature* 498 (2013) 346.

R.N. Wolf *et al.*, *IJMS* 313 (2012) 8.

S. Knauer *et al.*, *IJMS* 423 (2017) 46.

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# [P015] Production and decay-mechanisms of poly-anionic clusters investigated in ion traps

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Radio-frequency and ICR traps are versatile mass spectrometric tools. In particular, they are used in interaction-techniques to change the charge state of stored ions. In this contribution, the production of poly-anionic metal clusters in both trap types is discussed. While simultaneous trapping of heavy cluster-anions and light electrons is performed in a Penning trap, leading to electron attachment [1,2], in digitally-driven radio-frequency ion traps [3] field-free time slots [4] allow controlled application of an electron beam [5]. As a result, poly-anionic metal clusters with up to 10 surplus electrons have been produced. Besides, laser-excitation studies of the trapped (poly)-anions are presented, which reveal several different decay pathways depending on the cluster material, particle size and charge state [6,7].

A. Herlert *et al.*, *Phys. Scripta* T80 (1999) 200.

F. Martinez *et al.*, *J. Phys. Chem. C* 119 (2015) 10949.

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F. Martinez *et al.*, *Int. J. Mass Spectrom.* 365–366 (2014) 266.

M. Wolfram *et al.*, *J. of Phys. B,* in press.

S. K´onig *et al.*, submitted.

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# [P016] Design and performance of a second-generation cyclic ion mobility enabled Q-TOF

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Interest in ion mobility (IM) separation continues to grow and is now utilised over a broad range of application areas. Over the last ten years or so there has been a notable advance in ion mobility technology coupled with mass spectrometry with significant increases in mobility resolution and the prospect of additional instrument functionality. One such system is a travelling wave (T-Wave) driven multi-pass cyclic ion mobility (cIM) separator embedded in a Q-ToF instrument. Here we report on the preliminary design and performance of a second-generation research platform with enhancements to the ion optics, ToF mass analyser, detection system and instrument control.

*Speaker

# [P017] A new PTR-ToF reaction cell combining linear and oscillating fields

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Proton Transfer Reaction – Mass Spectrometry (PTR-MS) is a technique of increasing popularity for online monitoring of volatile organic compounds (VOCs). The working principle of PTR-MS is chemical ionization upon reactions between primary H3O+ ions and the analyte VOCs. Such reactions occur within a reaction cell having very stable and controlled electric fields, pressure, and temperature. The electric fields within the cell move the primary and product ions towards the detector. Conventional reaction cell designs are based on a linear, DC field along the drift axis. Ion losses occur at the reaction chamber cell walls because of diffusion and scattering. This problem affects the instrumental sensitivities and limits of detection (LOD). In order to overcome this issue a novel approach to the drift cell design is discussed. The solution involves adding oscillating RF fields on top of the linear field. The additional ion focusing which is reached in this way increases the transmission of ions towards the detector region. The net gain in sensitivity exceeds one order of magnitude and previous boundaries in LODs are overcome thus providing unprecedented performances for a PTR-MS. The new reaction chamber has been coupled to state-of-the-art mass analyzers such as time of flight mass spectrometers (ToF-MS) exeeding 10,000 (m/$\Delta$m FWHM) in mass resolution.

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# [P018] Sheathless CE-MS interface as an original and powerful infusion platform for nanoESI study: from intact proteins to high molecular mass noncovalent complexes

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M. Mann and M. Wilm caused a revolution with the development of the nanoelectrospray ion source. As compared to the conventional electrospray source, flow rate reduction allows significant enhancement of sensitivity. Nevertheless, aqueous medium and presence of salt may cause loss of sensitivity. Here, we have evaluated the suitability of a sheathless capillary electrophoresis-electrospray interface (CESI-MS) with a porous tip as the nanoelectrospray emitter to analyze with high sensitivity intact proteins and noncovalent complexes.
First, a systematic study of ionization profile of an intact model protein has been realized to evaluate the accuracy of delivered flow rate, the robustness and the enhancement of sensitivity provided by the use of CESI-MS as a nanoelectrospray emitter.

The DNA processivity factor of *pseudomonas* was studied. Results obtained showed the capacity of the system to maintain noncovalent interaction as the stoichiometry of the complex could be obtained using CESI-MS.
Finally, CESI interface was used to observe the quaternary structure of native hemocyanins from *Carcinus maenas* crabs by using an infusion flow rate of 10 nL/min; this complex resulting in masses ranging from 445 kDa to 1.34 MDa. Results obtained exhibit the suitability of the CESI-MS to be used as a nanoESI emitter and especially for the study of noncovalent complexes. The system allowed to finely tune the flow rate used for the injection and the intrinsic characteristics of the capillary used as well as the ionization source demonstrated their practical advantages in native MS which may lack sensitivity due to the employed conditions.

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# [P019] Extending the gas phase structural biology toolbox: Circular Dichroism mass spectrometry of G-quadruplexes.

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Native mass spectrometry is now widely accepted as a useful tool for structural biology studies, particular for the study of non-covalent oligomers of biomolecules. Despite this, there are currently many unknowns within native mass spectrometry which make it unclear to what extent it can be used as a structural tool. Hence, the development of probes of the gas phase structure of biomolecules is of high interest. Spectroscopy based techniques are particularly attractive as they can provide the possibility for comparison of structure in both solution and gas phase, therefore providing a bridge between the two media and allowing to study more deeply the link between solution and gas phase biomolecular structure. To this end, we report here the first results of gas phase circular dichroism on several G quadruplex structures measured by monitoring of electron photodetachment. G quadruplexes with parallel and antiparallel strand topologies are considered, and circular dichroism in gas phase and solution phase are compared. The results show for the first time that gas phase circular dichroism of large gaseous biomolecules is feasible, and can be an important and versatile new tool for native mass spectrometry studies.

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# [P020] A (Ultra-)High Resolving Time-of-Flight Mass Spectrometer with MS^N Capability

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A multiple-reflection time-of-flight mass spectrometer (MR-TOF-MS) has been developed that allows for sub-ppm accuracy with (ultra-)high mass resolving power[1,2,3].
The device offers multiple-stage tandem mass spectrometry with very high mass resolving power in every stage using the novel technique of mass-selective ion re-trapping[4]. Separation powers of more than 70000 after 3.6ms flight time and re-trapping efficiencies of up to 35% have been reached for precursor selection of caffeine molecules. Glutamine and Lysine were successfully isolated from their respective isobaric contaminants within 0.23 ms and their isolated dissociation spectra have been measured[3]. Tandem mass spectrometry with two stages (MS/MS) and three stages (MS^3) has been performed on the protonated ion of lysine[3].

An improved atmospheric pressure inlet (API) for the MR-TOF-MS was designed and commissioned. Four separately heated inlet capillaries and a movable jet disruptor allow for simultaneous use of different analytes at different temperatures. An RF carpet as an alternative to funnels enables high transmission rate.

*[1]* T.Dickel, et al, Nucl. Instrum. Methods B 317 (2013) 779
*[2]* J. Lang, *PhD thesis*, 2016, Justus Liebig University Gießen
*[3]* W. Lippert, *PhD thesis*, 2016, Justus Liebig University Gießen
*[4]* T. Dickel et al, J. Am. Soc. Mass Spectrom. 28(2017) 1079

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# [P021] Single-photon ionization of nebulized and laser desorbed molecules in a dual ion-funnel MALDI/ESI ion source

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Single-photon ionization (SPI) represents a versatile and comparatively 'soft' ionization method yielding molecular ions from a broad range of small molecules, including non-polar compounds. In the most common SPI variant, atmospheric pressure photoionization (APPI), Krypton discharge lamps (emission lines: 117/124 nm) are most typically used. Moreover, the ionization of nebulized compounds is often supported by use of reactive dopants.
Here we introduce a new approach in which RF-Kr lamps (PKR100, Heraeus) are integrated into a dual ion-funnel MALDI/ESI source (Spectroglyph) operated at 5-15 mbar of N2. The injector was mounted on a Q-Exactive Plus Orbitrap mass spectrometer. Plasma ignition/light emission was controlled by custom-made RF (13.56 MHz) electronics that enable externally triggered pulsed operation with adjustable pulse width. In this way, LDI laser and VUV light pulses can be synchronized to optimize the SPI efficiency for laser desorbed molecules. Furthermore, the ion source was modified with a capillary for optional introduction of volatile analytes and/or dopants (e.g., acetone, toluene).

Preliminary data highlight that the SPI set-up not only enables efficient post-ionization of several classes of introduced volatile organic compounds but that it can also be used to post-ionize laser desorbed molecules (e.g., PAHs, physiologically relevant lipids and lipophilic vitamins). The influence of relevant experimental parameters (e.g., gas pressure, type of dopant, SPI pulse energy, duration as also delay relative to the laser pulse) will be discussed.
The described SPI-module constitutes a low-cost, yet valuable complementary ionization scheme that could show great potential for general MS as well as MALDI-MSI applications.

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# [P022] Simulation of ions in a laminar flow through a heated capillary

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Transfer capillaries are often used in mass spectrometry to transport ions from the ambient conditions into low pressure.
This project aims to understand the behavior of charged particles in a gas flowing through a capillary, to get a better understanding of the losses occurring in this part of the machinery. The effects taken into account are the entrainment due the carrier gas, the space charge effects due to the electrical charge of the particles and the diffusion of the particles in the gas.

Previously reported simulations showed that the nature of the flow might be one of the main factor influencing the transmission rate of the particles. A turbulent flow might cause much higher losses than a fully laminar one.

The developed implementation intends therefore to be able to simulate the entire process of transport of the particles through the capillary as well as the underlying flow, in order to test this hypothesis and if conclusive to propose some improvements.

The laminar flow is in itself complex due to the sonic conditions and strong heating of the capillary.
Laminar flow simulations are performed and then used to reproduce the transport of the particles. This way, the assumption that a laminar flow enables high transmission rates can be verified and the influence of the other parameters, especially heating and involved species, analyzed.

*Speaker

# [P023] A transmission microscopy-based method for determination of focal laser beam profiles in MALDI mass spectrometry

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Accurate determination of the focal parameters of the MALDI laser beam (such as FWHM and s), is not only essential in MALDIimaging applications but also constitutes a key factor for fundamental studies of the MALDI mechanisms. Most typically focal beam widths is estimated by optical inspection of the ablation craters (i.e., area of fully removed matrix material). However, if typical MALDI lasers with near-Gaussian beam profiles are used, crater size varies strongly with laser power and absorptivity of the matrix.

Here we present a refined approach that is based on evaluating the irradiated MALDI samples by standard optical transmission microscopy. ImageJ and MATLAB software were used for data processing. Because the brightness in line scans is directly proportional to the thickness of the (residual) matrix layer, Gaussian functions can be used for fitting the data around the edges of the ablation craters; central areas with full matrix removal were ignored.

We performed tests with a set of different matrices (e.g., DHB and 2,5-DHAP) and for a range of spot sizes and also compared the results with a fluorescence-based method. In all cases consistent values for FWHM and s were obtained with most exact values being achieved for laser spot sizes below 150 $\mu$m. Importantly, results were independent of the used matrix and applied fluence.
As long as homogenous matrix layers and a suitable microscope are available, the method is readily usable in combination with virtually any MALDI(-MSI) set-up.

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# [P024] Identification study by using LC-ESI-HRAMS

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Different workflows are available combining full scan MS and MS/MS in the same run, that have to meet the identification criteria. SWATH® acquisition is a data independent non-target acquisition mode that divides the entire analysed mass range into smaller segments. It was evaluated for pesticide multiresidue analysis in fruits and vegetables by using LC-QTOF-MS. The compounds were quantified in full scan MS mode and the MS/MS mode was used for identification purposes. QTOF-MS allows working using high resolution accurate full scan mass spectrometry and to obtain better selectivity as well as permit the retrospective analysis of the data, feature that cannot be afforded by QqQ-MS. Identification capabilities were studied in 20 different matrices for 141 compounds, paying special attention to some issues that can difficult the identification of the studied analytes working combining MS and MS/MS mode.
LC-X500R-QTOF using SWATH® mode is a very robust and effective acquisition method for identification using ion ratio and mass error of precursor and fragment ions.

The number of compounds that arrives to the collision cell can interfere during the fragmentation process, that could alter the ion ratio abundance in different matrices.

Furthermore, LC-X500R-QTOF MS can be used as a quantitative technique, achieving low LOQs similar to those obtained by TQ.

*Speaker

# [P025] Laser induced acoustic desorption (LIAD) for gas phase spectroscopy.

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In order to study the spectroscopic properties of isolated molecules in the gas phase, small and medium size compounds are commonly placed into the gas phase using their vapour tension. In some cases, the molecules of interest may be heated under vacuum in ovens. However, this procedure cannot be used for thermally fragile compounds. Hence, the search for new vaporization methods is active.
Laser induced acoustic desorption (LIAD) is a relatively new desorption method. The sample solution is firstly deposited directly on a thin metal surface. The analytes are then volatilized by subjecting the opposite side of the foil to a pulsed laser in the vacuum. The resulting ablation of the metal by the laser pulse creates a large-amplitude acoustic wave (shockwave) that propagates through the metal foil, resulting in desorption of neutral molecules from the opposite side of the foil (Habicht et al. 2010).

We have developed a LIAD setup to perform gas phase spectroscopy of isolated molecules. The setup has been coupled to electron impact ionization to measure appearance energy for amino acids. Subsequently, the system has been used on a soft X-ray beamline at the SOLEIL synchrotron radiation facility, for near edge X-ray absorption fine structure (NEXAFS) spectroscopy.

*Speaker

# [P026] Continuous vacuum ultraviolet Tandem MS

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MSMS techniques are widely used for both, structure and sequence elucidation. Thereby, fragmentation activation is realized by various methods, for example with lasers or neutral gas collisions. In this study, we present a new Tandem MS system using a commercially available vacuum ultraviolet lamp. On the one hand, this approach provides efficient fragmentation in both ionization modes, positive as well as negative. On the other hand, it enables an additional previously not achieved post ionization of the nascent fragments. While the first results in atypical fragment patterns and, thus provides orthogonal information, the second is crucial especially to identify low abundant ions. A continuous vacuum UV lamp with transmission maxima at 160 and 125 nm was connected to an LTQ ion trap via the existing backdoor. Samples from different categories (e.g. oligonucleotides, peptides) were isolated and activated with the vacuum UV lamp in the milliseconds to seconds time range. To investigate many different samples oligonucleotides and ATP were measured in the negative mode. Here, typical fragments were observed. Additionally, untypical fragments were also observed. Moreover, for multiply charged ions, a reduction of charges and additional fragmentation could be observed. In positive ion mode, small molecules like caffeine and polymers like peptides and polyethylene glycol were assessed. As before, known typical as well as uncommon fragments were generated. Furthermore, an increase in intensity was observed in the isolation step upon radiation with VUV light. This enables new possibilities as a new and upgradable fragmentation method especially regarding low abundant samples.

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# [P027] Extreme ultraviolet radiation: a means of ion activation for tandem mass spectrometry

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Mass spectrometry, and in particular tandem mass spectrometry has developed into a corner stone method in structural and analytical chemistry. Fundamentally, tandem mass spectrometry relies on providing precursor ions with enough internal energy to produce structurally informative product ions spectra. Collision induced dissociation (CID), which involves multiple low energy inelastic collisions of ions with inert gas molecules, continues to be the most common way of ion activation. Despite its universality, CID suffers from limitations inherent to its nature, such as dissociation of the weakest bonds and loss of efficiency for large molecular ions. These limitations have motivated the search for alternative activation means. Recently, the use of ultraviolet (UV) photons activation has attracted considerable interest. Photodissociation in the UV is usually achieved using lasers, even down to the vacuum UV region. However, synchrotron radiation studies have revealed that the extreme UV (XUV) domain is potentially appealing for tandem mass spectrometry. Unfortunately, this spectral region was hitherto restricted to accelerator based facilities. We report here the coupling of a XUV laboratory light source with a G2-Si (Waters, Manchester, UK) mass spectrometer. The coupling has been tested on small singly charge molecules such as acarbose and erythromycin, for which XUV activation has been compared to CID. Moreover, the position of this new method with respect to other activation means is discussed. XUV photon activation appears to advantageously complement the arsenal of available dissociation methods.

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# [P028] Assessment of Collision Induced Unfolding experiments for protein characterization: the case of of mAbs

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A recent ion mobility-based approach called *CIU* (Collision Induced Unfolding) is more and more used in the ion mobility-mass spectrometry (IM-MS) toolbox for structural biology. CIU experiments allow monitoring unfolding transitions in the gas-phase, by applying a gradual collisional activation in the ion trap compartment located before the IM cell. Ion activation induces a variation of the arrival time distribution (ATD) which can be represented by a 3D fingerprint showing the variation of IM drift time, peak intensity as a function of the trap collision voltage (trap CE). This approach has been developed in order to circumvent the lack of IM resolution. and seem to be of potential interest to distinguish monoclonal antibody (mAb) isotypes1 or to monitor mAb stabilization upon drug conjugation2.
Here we aimed at precisely evaluating the influence of parameters on the quality of the CIU data using trastuzumab as reference therapeutic mAb. We thus focused on the effect of parent ion selection in the quadrupole prior to CIU experiment and charge state selection on the CIU pattern. Altogether our data highlight that parent ion isolation is not a prerequisite for CIU experiments and that lower charge states are less prone to unfolding than higher charge states.

Finally, CIU experiments performed on different therapeutic mAb isotypes and on trastuzumab conjugated maytensin (T-DM1) will be presented.

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2. Botzanowski, T. et al. *MAbs* **2017,** *9* (5), 801-811.

*Speaker

# [P029] Combination of native and middle-down mass spectrometry for in-depth characterization of a site-specific antibody-drug conjugate.

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Top- and middle-down mass spectrometry have emerged as promising alternative strategies to the classical bottom-up approach for protein characterization1. In particular, the analytical characterization of mAb-related compounds usually follows a multi-level approach ranging from intact to the peptide level with an intermediate state at the subunit level. At the subunit level, middle-down experiments can also be performed to provide in-depth sequence information.
Peptide mapping is routinely used for primary sequence and post-translational modification (PTM) assessments. However this strategy requires an extensive enzymatic digestion, which can affect the quality of the final product (increased deamidation, oxidation, etc.)2 and is also time consuming. Conversely, subunit and middle-down assays performed on mAb-based compounds require less time and sample handling and could also be used for protein characterization without loss of key features.

Here we report the combination of intact native mass spectrometry and middle-down analysis of a site-specific antibody performed on high resolution Orbitrap platforms (Orbitrap Exactive EMR and Orbitrap Fusion Lumos Tribrid, respectively). Drug load distribution and antibody-drug-ratio of the site-specific ADC were assessed through native MS experiments, pinpointing a highly homogeneous ADC.
The site-specific ADC was subsequently digested with IdeS protease and completely reduced. Middle-down analysis on LC, Fd, and Fc/2 subunits (25 kDa) were performed using different fragmentation techniques (ETD, HCD, and UVPD) in order to identify the sites of conjugation. These three activation techniques were evaluated either as individual fragmentation techniques or in combination to get optimal sequence coverages of the LC, Fd, and Fc subunits.

*Speaker

# [P030] Identification and characterization of hydroxylysines in an immunoglobulin

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During peptide map characterization of purified mAb samples a suspicious MASCOT hit appeared: a modification labeled as "Oxidation (Lys) [+15.99]" had been assigned to the tryptic peptide XXXXSSASTK. Oxidized Lys" seemed to be unlikely as it has not been observed before in this context, thus making a mutation Ala → Ser rather likely (same exact mass, same MASCOT score). Subsequent, thorough inspection of all available data, however, proved the correct assignment by MASCOT: for the peptide XXSSASTKGPS (from thermolysine digest) localization of the modification at the Lys residue could be unambiguously verified . Hydroxylation of these lysines occurs via the lysyl hydroxylase enzyme, which recognizes the consensus amino acid sequence Xaa-Lys-Gly and converts lysine to 5-hydroxylysine (Hyl). As this consensus sequence is present more than once within a typical antibody sequence, a closer look into the data of the analyzed samples showed that Hyl is present in low abundance also at several other consensus sites. Very recently Hyl has been observed for a peptide with sequence SSASTK (Qing Xie, Benjamin Moore & Richard L. Beardsley (2015): Discovery and characterization of hydroxylysine in recombinant monoclonal antibodies, mAbs, DOI: 10.1080/19420862.2015.1122148). The authors stated to have found Hyl at no other Xaa-Lys-Gly motif in their protein. In our study, however, we demonstrate, that this unusual modification can occur even at multiple sites in a recombinant mAb

*Speaker

# [P031] Combining Genome Mining and Comprehensive LC-MS Analysis to Investigate a Rare Myxobacterial Trans-AT PKS Pathway

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Myxobacteria are an abundant and important source for natural products and novel structures. Their genomes frequently contain several biosynthetic gene clusters of which apparently only a fraction is active under standard cultivation conditions. Recent advances in sequencing whole myxobacterial genomes have made it appealing to use *in silico* methods to search for these 'silent' biosynthetic gene clusters. We present here a comprehensive approach to select and activate rare or unique myxobacterial biosynthetic gene clusters in order to assign their small-molecule products. *Trans*-acyltransferase polyketide synthases (*trans*-AT PKS) represent a promising subject for this endeavor due to their unusual domain architecture and their propensity to generate diverse and unexpected molecules. In this project we focused on a particular *trans*-AT PKS revealed by *in silico* annotation, which gathered our interest due to its size and the presence of a putative membrane associated transporter operon encoded nearby. Following gene cluster activation by insertion of heterologous promoters (inducible and constitutive), principal component analysis was applied to high-resolution LC-MS data to highlight metabolomic differences between wildtype and mutants. This approach led to the discovery of several candidate masses, which were unexpected both in numbers and m/z range covered and seem to coincide with different stages in the growing chain of the PKS product.

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# [P032] Determination of apparent gas phase activation energies and dissociation constants of epitope peptide – antibody complexes by electrospray-mass spectrometry – ITEM TWO

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Most important characteristics of antibodies are that they typically strongly bind to specific epitopes, thereby expressing low dissociation constants (KDs) and high Gibbs free binding energies (ΔG0s). We present an electrospray-mass spectrometry method, termed ITEM-TWO, which enables one to simultaneously identify the epitope peptide(s) for an antibody of interest and to obtain quantitative information on the respective binding strength in a single mass spectrometry experiment[1-3].
Immune complexes are obtained in solution by mixing antibody- and peptide mixture-containing solutions. The so generated immune complex-containing mixture is then electrosprayed without any purification steps. With the aid of a quadrupole mass analyzer the immune complex ions are separated from unbound peptide ions. Increasing the energy in the subsequent collision cell in a stepwise manner results in collision induced dissociation (CID) of the immune complex, hence release of intact epitope peptide(s) whose mass(es) is(are) then measured in a ToF analyzer. From the ions' normalized intensity ratios are deduced apparent activation energies of the gas phase dissociation processes as well as the calculated apparent gas phase dissociation constants. The order of the apparent gas phase dissociation constants that we determined with four antibody – epitope peptide pairs matches very well with the order that was obtained from in-solution measurements. Thus, we anticipate that binding strengths determined by our ITEM-TWO method may become as useful as those currently determined in solution.

**References**

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# [P033] Mass precision and Mass accuracy of Intact Monoclonal Antibodies: The Differentiation of deamidation by CE-CE-ESI TOF MS

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In principle, mass spectrometry is a powerful tool for the characterization of intact mAb variants and enables the discrimination of analyte species even with minor mass differences. Nevertheless, the isotopic envelope of high mass molecules such as mAbs is broad (_˜25 Da at FWHM) limiting the provided information of variants with small mass shifts caused e.g. by deamidation (+1 Da). On the other hand, electrophoretic separation based on high amounts of $\epsilon$-aminocaproic acid enables the robust, precise and quantitative separation of such charge variants and, thus, is routinely used in biopharmaceutical applications. However, these electrolytes interfere with the electrospray ionization process.
Here, a heart-cut CZE-CZE-MS setup with an implemented mechanical 4-port valve interface [1] was developed using a generic $\epsilon$-aminocaproic acid based background electrolyte as 1st dimension and acetic acid as 2nd dimension [2]. Interference-free, highly precise mass data (deviation $<$ 1 Da) of charge variants of Trastuzumab, acting as model mAb system, were achieved enabling the determination of deamidation on the intact protein level. The obtained mass accuracy (lower ppm range) requires a careful look on the in the isotopic distribution of carbon of the sample in order to determine the mass accuracy: A correction from the usually applied 13C-content based on the "representative isotopic distributions" of IUPAC is required for correct calculation of the highly precise observed masses. This important finding will be discussed in detail.

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*Speaker

# [P034] Full Validation of Therapeutic Antibody Sequences by Middle-Up Mass Measurements

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The regulatory agencies require comprehensive primary sequence validation both for innovator and biosimilar monoclonal antibodies (mAbs). Full sequence coverage (SC) is typically obtained by the combination of multiple LC-MS/MS datasets from various protease digests. In this work, we report a new middle-up LC-ESI-MS subunit analysis and middle-down LC-MALDI in-source decay mass spectrometry method applied to cetuximab, panitumumab and natalizumab as representative FDA and EMA approved mAbs.
The antibody drugs in their formulation buffers were deglycosylated with IgGZERO (Genovis), and Fc/2, Fd and LC subunits were obtained by IdeS or PNGAse F digestion followed by DDT reduction in Gua-HCl. The subunits were separated by HPLC and analyzed with an ultrahigh resolution QTOF (Bruker maXis II) providing isotopic resolution of all mAb domains at _˜25 kDa. Monoisotopic molecular weights were automatically assigned after Maximum Entropy deconvolution using the SNAP algorithm. In addition each mAb was reduced to obtain accurate mass for the light and heavy chain. LC-MALDI-MS datasets were acquired after C4 cap-LC separation and fractionation. Relevant fractions were Middle-Down sequence analyzed by MALDI-ISD on the Bruker ultrafleXtreme MALDI-TOF/TOF using sDHB matrix.

The goal was to unambiguously confirm their reference sequences and show the general applicability of the method. All antibody sequences were fully validated in this work by this combination of middle-up molecular weight determination and middle-down protein sequencing and multiple errors in the natalizumab reference sequences were corrected. MALDI-ISD spectra were compared to a database of reference sequences. The best matches were reported for more detailed analysis.

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# [P035] An integrated subunit LC/MS analysis for fast core fucosylation assessment of mAb products.

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Critical quality attributes (CQA) are constantly monitored throughout the production process to maintain the quality of therapeutic monoclonal antibody (mAb) products. Core fucosylation of N-glycan structuresis a CQA that affects the potency of the mAbs and can be analyzed at subunit level. The following subunit level analysis holds several key advantages over conventional peptide mapping or released N-glycan analyses. It reduces LC-MS run time and provides a simplified workflow. The applications, however, are limited in late stage development and QC, due to the extent of user intervention required. Here, we introduce a fully integrated, workflow-driven, LC-HRMS platform solution with automated data acquisition, processing and reporting capability for core fucosylation assessment. Trastuzumab subunit samples were prepared by digesting with IdeS and Endopeptidase S (Genovis) enzymes. Samples were analyzed by reversed-phase LC-HRMS. Data acquisition, processing and reporting were controlled by workflow method generated in UNIFI Scientific Information System. The enzyme mixture trims the N-glycan structures down to one GlcNAc (with or without analpha-1,6 Fucose). Reversed-phase separation of the digest showed three ScFc fragments: aglycosylated (no glycans), afucosylated (one GlcNAc), and fucosylated (one fucose and one GlcNAc). Fucosylated ScFc was the dominant peak and all scFc peaks were detected with less than 5 ppm mass tolerance. Aglycosylated form was observed at less than 1% (relative abundance) with a 10% RSD in repeated measurements. This methodology can be easily introduced into late stage process development for routine fucosylation/afucosylation CQA monitoring by LC-MS.

*Speaker

# [P036] Monitoring Multiple Critical Quality Attributes of mAbs at Subunit and Peptide Level Using a Compliant Ready High Resolution LCMS Platform

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The extension of mass spectrometry to late development and QC environments has promised significant advantages for multiplex monitoring critical quality attributes (CQAs) at the molecular level, providing an in-depth understanding of the biotherapeutic production process and facilitating implementation of Quality by Design (QbD) initiatives for upstream and downstream processing, stability, and formulation activities. Here, we demonstrate the applicability of HRMS for multi-attribute monitoring (MAM) at both the peptide and subunit levels, using an HRMS platform capable of operating under the compliance requirements typical to regulated development and QC organizations. Subunit MAM exhibited minimimal sample preparation artifacts, and enabled higher assay throughput, both key advantages in for routine monitoring of domain-specific CQAs. Peptide-map based MAM enables site-specific information and the ability to monitor deamidation and isomerization attributes , but resulted in greater sample preparation complexity, greater data analysis complexity, and longer analysis run times. In this work, we have evaluated and compared these two analytical workflows (Subunit MAM and Peptide Map MAM) to determine the capacity of MS-based methods for monitoring several common classes of biotherapeutic attributes.

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# [P037] Insights from CE-ESI-MS approaches for detailed characterization of monoclonal antibody and related products

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Biotherapeutic proteins (biologics), although introduced only since two decades, represents the class of therapeutic agent having the fastest growth regarding market shares and are nowadays available in different format: fusion proteins, monoclonal antibodies, biosimilars or antibody-drug conjugates. The complexity of such molecules requires advanced and comprehensive characterization in order to guarantee their potency and safety, therefore urging the development of innovative analytical strategies involving mass spectrometry. Here we presented an overview of different methodologies involving innovative capillary electrophoresis - tandem mass spectrometry coupling for the characterization of biologics structure and posttranslational modifications (glycosylation, asparagine deamidation, aspartic acid racemization) at different level (Top-down, middle-up and bottom-up). These methods were applied to perform biosimilarity assessment between two mAbs and characterization of drug-loaded peptides of antibody-drug conjugates. Such level of characterization is permitted by cumulating the specificities of both CE and high resolution tandem MS therefore renewing the interest for this type of coupling.

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# [P038] Top-down protein characterization for vaccine antigens: a powerful method ?

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As novel vaccine antigens are being tested in humans, better characterization is essential for vaccine safety and eliciting optimal vaccine response. Among several critical quality attributes, identity of the vaccine antigens is a key parameter (Dey *et al*, 2014). Protein identification using mass spectrometry (MS) is now widely used and recommended by pharmacopeias. Bottom-up approach by MS is commonly performed and easily provides identification but could be time consuming and has several drawbacks. Top-down approach is based on the direct analysis of intact proteins using MS providing fast identification and complementary information to previous approach (Catherman *et al*, 2014). Development of this method in our platform is on-going to evaluate its benefit for vaccine characterization and as an alternative to the classical method of N-terminal protein sequencing. Top-Down approach was developed on an hybrid instrument combining a quadrupole and a time of flight analyser (Q-TOF) using collision induced dissociation (CID) and electron transfer dissociation (ETD) as fragmentation modes. Key parameters were optimized and data processing was evaluated using different softwares. Top-down approach was also investigated after chromatographic separation (Capriotti *et al*, 2011). So far, an optimised workflow for top-down protein identification based on our MS instrument has been proposed and used for several vaccines antigens. This study demonstrates top-down approach for identification allowed access to sequence confirmation of terminal regions of proteins as identification of major modifications. Currently, top-down is complementary to bottom-up methods but should be in the near future a powerful method for protein characterization.

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# [P039] Advances in glycosylation characterization of vaccine antigens

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As the glycosylation can influence the efficacy, pharmacokinetics and/or immunogenicity of therapeutic glycoproteins, its characterization and control are more and more required by health authorities (Aich *et al*, 2016). Like most therapeutic proteins, vaccine antigen could also be glycosylated. Despite the importance of glycosylation, little attention has been paid to the glycosylation characterization of vaccine antigens such as viral glycoprotein antigens. However the glycosylation of viral proteins could influence the infectivity of attenuated or chimeric vaccines *via* virus/receptors interaction (Alen *et al*, 2012) or modulate immunogenicity of vaccine antigens (Wolfer and Boons, 2013).
Typically, advanced methods of glycosylation characterization and control are well described for monoclonal antibodies, but very few methods were developed for vaccine antigens due to several technical challenges. Glycosylation of vaccine antigens could present a high degree of heterogeneity with specific structures, a low amount of glycoproteins could also be available, and the presence of different vaccine excipients could interfere with the methods.

Specific methods were developed in our platform since several years for a better characterization of the glycosylation of different vaccine antigens (Dubayle *et al*, 2015). Sample preparation, mass spectrometry and liquid chromatography were combined to characterize several vaccine antigens. Our data bring new insights to decipher the role of N-glycans in the virus-host cell interaction and could guide further vaccine development.

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# [P040] Alzheimer disease and phophorylation of tau protein: Polyphenols potential inhibitors?

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Alzheimer's disease (AD), as other chronic diseases, is a result of multiple factors. Two of the most prominent pathological events in the appearance and development of AD are the formation of insoluble amyloid plaques and neurofibrillary tangles (NFT). NFT are insoluble aggregates of the microtubule-associated Tau protein, which were also shown to promote neurodegeneration.The formation of NFT, is proposed to be caused by hyperphosphorylation of Tau by Protein kinases.
We would like to describe how polyphenols could inhibit hyperphophosylation and prevent Tau aggregation.

Previous studies by NMR have showed that polyphenols in particular procyanidins can interact with peptide Tau model issued from Proline Rich region (Tau P2:201G-T220).

Then,the effects of these molecules on the phosphorylation of Tau P2 were determined using the kinase GSK3$\beta$, ATP and with and without polyphenols.

A method was developed by **Maldi** to study the kinetic monitoring of the reaction. Phosphorylation of Tau P2 model achieved 40% after 7 days and leads to a precipitation of the peptide into filaments. But, in the presence of some polyphenols, decreases of the percentage of phosphorylation were observed blocking the precipitation of the peptide.
Thus, this study leads to interesting and promising results showing a way to inhibit phosphorylation of Tau by this kind of compounds potentially able to modulate pathological mechanism of aggregation, offering an alternative therapeutic strategy in the patient care.

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# [P041] Ion Mobility MS reveals ligand influences in the gas phase unfolding behavior of amyloid beta (1-42)

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Amyloid-beta (1-42) (A$\beta$42), the protein involved in Alzheimer's disease (AD) has been demonstrated to generate beta sheet forming oligomers leading to fibrils and plaques. Whereas mature fibrils have long been suspected to cause neurodegenerative disorders, recent studies suggest misfolded oligomer intermediates as the origin of neurotoxic species in Alzheimer's disease. Hence the early oligomerization stage stands in focus of inhibitor development to suppress the formation of toxic oligomers possibly resulting in off pathway aggregates.
Additionally to laser induced liquid bead ion desorption mass spectrometry (LILBID-MS), which gives an insight in the early aggregation kinetics of A$\beta$42 in presence of ligands, electrospray ionization mass spectrometry coupled with ion mobility (ESI-IM-MS) is used to reveal the ligand's structural influences on A$\beta$42. Unbound oligomers and ligand bound oligomer complexes were activated by collision induced unfolding (CIU) to evaluate their gas phase stabilities and collision cross sections ($\Omega$) of compact and extended species. Several distinctions in the unfolding behavior were observed, caused by conformational influences due to ligand interactions. Those indicate structural rearrangements affecting distinct aggregation pathways which differ in their kinetic behavior.
The combination of LILBID to reveal the oligomerization and its kinetics with ESI-IM-MS to detect changes in the peptide's structure, provides promising results in understanding the aggregation of self-assembling peptides and their prevention by inhibitors.

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# [P042] Influence of EGCG on the aggregation of -Synuclein wildtype and its mutant A53T – A LILBID-MS study

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There are many different proteins with various structures and functions that can spontaneously selfassemble into $\beta$-sheet–rich fibrillar aggregates, so called amyloids. A diversity of neurodegenerative diseases, for example Alzheimer's and Parkinson's disease (PD), are associated with amyloid formation. The common characteristic of those diseases are intrinsically toxic oligomers formed on the aggregation-pathway through to fibrils rather than the fibrils itself.
For investigating the aggregation of amyloidogenic polypeptides one can generally employ light scattering or a fluorescence assay which is based on the ThT dye to tag ß-sheet structures. While these methods enable analysis of formed fibril structures they don't allow a detailed look at the interesting early steps of the aggregation-process. With laser-induced-liquid-bead-ion-desorption mass spectrometry (LILBID-MS) we are able to observe the early steps of -Synuclein aggregation, the protein of interest regarding PD.
Using LILBID-MS it is possible to detect -Synuclein oligomers up to the octamer. This enables to study the kinetics of the oligomerization process by time-resolved measurements. Thereby it is possible to compare the oligomerization kinetic of -Synuclein wild-type and mutants. In addition, it is possible to study interactions with small molecules that may affect -Synuclein aggregation. We are able to detect ligand binding and its influence on the aggregation behavior. Therefore we can show, for example, the effect of epigallocatechin-3-gallate (EGCG) on -Synuclein wild-type and its mutant A53T and how it modulates/inhibits the amyloidogenic oligomerisation.

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# [P043] An Immunoaffinity-HRAMS Assay for the Pre-Clinical Quantification of Trastuzumab in Rat Plasma

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Immunoaffinity sample preparation techniques have seen an increase in applicability for bioanalysis of biotherapeutics such as monoclonal antibodies. The reason for this increase in utilization is the significant increase in selectivity and sensitivity that can be achieved when analysing human matrices.
The other benefit of immunoaffinity techniques is the ability to adapt the assay by simply changing the mode of capture. For the clinical analysis either a biotinylated target (Her-2) or anti-drug antibody can be used. This creates a selective assay for the biotherapeutic of choice.

An alternative approach is to use a capture probe that targets the Fc region of antibodies. For example, using anti-human IgG (Fc specific) antibodies allows for a simple and generic methodology for pre-clinical analysis. Multiple candidates can be rapidly screened using the same capture process. In this study, we will use this generic approach to capture Trastuzumab from rat plasma.

Routinely immunoaffinity prepared samples are analysed by signature peptide quantification on triple quadrupoles. In this study, we look at High Resolution Accurate Mass (HRAMS) as a complimentary analytical technique for signature peptide quantification.
We will present an MRMHR assay for the quantification of Trastuzumab in rat plasma, measuring the sensitivity, linear dynamic range, precision and accuracy.

*Speaker

# [P044] BioPharmaViewTM software as a robust tool for automated quantitation of oxidation sites in monoclonal antibody characterization.

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Oxidation of methionine is one of the common PTM known to occur in recombinant mAb during manufacturing, formulation and storage process. Monitoring oxidation is of major concern because it can limit the product's clinical efficancy or stability. Peptide mapping methods using MS detection are commonly used for the determination of oxidation levels, however availability of suitable software tools for the automated quantitation is of high importance. Here we demonstrated BioPharmaViewTM software as a robust tool for the detection of oxidation for the automated quantitation of oxidation sites.
Human IgG were incubated with different concentrations of hydrogen peroxide and analysed on intact, subunit and tryptic digest level. Samples were either separated on a C4 or C18 high flow HPLC setup, coupled to a X500B QTOF system using DDA acquisition. Subsequent data analysis was done with BioPharmaViewTM software.

The increase of oxidation levels with increasing concentration of oxidation levels on a peptide level was consistent with obtained data at the intact and subunit level. BioPharmaViewTM software was able to automatically assign these different levels of oxidation at the intact, subunit and peptide level. In addition the ratio of modification for each pair of peptides based on the extracted MS ion chromatogram was calculated automatically.

Because of the high relevance of lower levels of methionine oxidation, the automatic software detection was investigated.
In addition, good linearity of the LC-MS method was observed for modified and non-modified peptides. Furthermore, excellent accuracy and reproducibility of the data acquisition and analysis was demonstrated by replicate analysis.

*Speaker

# [P045] Characterization of age-related changes in the pea root nodule proteome

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Legumes are rich in bioavailable protein. Thus, their protein productivity depends from the capacity of rhizobial symbiosis for nitrogen fixation. Therefore, the nodule functional state is of the principle importance for sustaining high biomass yields over the span of plant life. For this, the interplay of age-related molecular mechanisms needs to be considered on the nodule and plant levels. Here we address the functionality of pea root nodules in a temporal dimension. Thereby, we focused on age-related formation of toxic advanced glycation end-products (AGEs), typically originating from reactive $\alpha$-dicarbonyls. Indeed, formation of these compounds during plant ageing was recently shown for Arabidopsis leaf tissues. Protein profiles and patterns of AGE-modified proteins of pea root nodules from 4-7-week old plants collected from two independent field experiments (each performed in triplicates) were studied by bottom-up proteomic approach relied on nanoLC-ESI-Q-Orbitrap-MS/MS experiments using data-dependent acquisition. The obtained MS data were quantified with the Progenesissoftware. MS/MS spectra were searched with SEQUEST against a non-redundant protein database of three plants. Thereby, protein annotation and quantification relied on at least two and three peptides, respectively. About 400 age-dependently regulated proteins were found at least in one experiment. Among them, 49 proteins (8 up-, 41 down-regulated) were confirmed in both experiments. Qualitative patterns of dicarbonyl-derived AGEs in nodules contained 23-39 proteins with 30-55 modified sites therein. In both experiments, the methylglyoxal-derived modifications were more abundant in comparison to the glyoxal-derived modifications. The research was supported by the Russian Science Foundation (project 17-16-01042).

*Speaker

# [P046] Use of MALDI-TOF MS and Statistical Learning for Classification of Olive Oil Adulterated with Vegetable Oils

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Olive oil (OL) is one of the most often adulterated food products: the expensive OL is often mixed with various amounts of much cheaper vegetable oils, such as soybean oils (SB). Various spectrometry tools coupled with statistical data evaluation like PCA have been employed to differentiate adulterated oils from genuine OL. However, the unsupervised PCA does not provide a reliable way to for this goal. Here a MALDI-TOF method was developed for rapid detection of adulterated OLs. Various supervised statistical learning techniques were tested for their usefulness to build a model that can determine the class of adulterated OL down to the level of 10% other oils.

5 OL and 8 SB from local supermarkets were provided by a local FDA laboratory. The 10% SB adulterated OL samples (OS) were prepared by mixing the two oils 9:1 (v/v). All samples were 200x diluted with 2:1 MeOH/CHCl3. Seven common MALDI matrices were initially evaluated for their performance on oil analysis. Matrix was dissolved in MeOH/CHCl3 (2:1 v/v) at a concentration of 10 mg/ml. Analysis was carried out on a benchtop MALDI-TOF (microflex LRF, Bruker Daltonics).

The combination of the MALDI-TOF and statistical learning provides a rapid and robust determination of olive oil adulteration without the need for characterization of individual components. CHCAE as matrix yields richer MALDI spectra features than others for such oil analysis. Supervised statistical learning with sPLS-DA was useful in identifying potential biomarkers from complex spectral features, as in non-targeted "omics" studies.

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# [P047] Profiling of Wine using ultra-high resolution Flow Injection Magnetic Resonance Mass Spectrometry (MRMS) and 1H-NMR Spectroscopy

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The complexity of organic compounds in food products such as wine can be analyzed by mass spectrometry. Beside GC/MS and LC/MS, wine can be analyzed on the molecular level by Flow Injection Analysis (FIA) after solid phase extraction (SPE) when combined with ultra-high resolution magnetic resonance mass spectrometry (MRMS). The mass spectra are a fingerprint of these complex mixtures of organic compounds. Multivariate statistical analysis of FIA-MRMS and 1H-NMR spectroscopy resulted in similar results.
MRMS Analysis: Wine samples were analyzed after solid phase extraction (SPE) using a Bruker solariX XR 7T mass spectrometer using ESI (-) with a resolving power of 300,000 at m/z 400. Raw spectra were subjected to deisotoping and adduct collation. Statistical analysis such as PCA as well as molecular formula calculation based on accurate mass, isotopic fine structure and filtering based on elemental composition were carried out automatically in MetaboScape 3.0. Annotated features were investigated using filters for mass defects and DBE.

NMR Analysis: SPE wine extracts were subjected to a Bruker FoodScreener (400 MHz Avance III NMR spectrometer). Automatic solvent suppression of the solvent resonances enabled the detection of organic constituents present in the extracts.

Molecular formulas were generated, assessed by mass accuracy, isotopic pattern fit and isotopic fine structure by ultra-high resolution MRMS. This could be used to distinguish different wines on the molecular level. Specific compounds could be identified responsible for separation of wines. Multivariate statistical analysis of MRMS and 1H-NMR were similar.

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# [P048] Overcoming the challenges of analysing ionic polar pesticides in food

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Glyphosate, a non-selective broad spectrum herbicide, accounts for more than half of the global herbicide sales. While discussions on the toxicological concerns of glyphosate and associated compounds continue, maximum residue limits (MRLs) are enforced globally, requiring continued analytical testing to ensure consumer safety. In previous work, various methodologies have been presented, looking at underivatised options for the direct analysis of polar pesticides in food. Here the novel application of the Waters' Torus DEA column is presented, showing the improved performance of a UPLC-MS/MS method for the underivatised analysis of glyphosate and a selection of other anionic pesticides. Separation is achieved under Hydrophilic Interaction Chromatography (HILIC), after a simple column conditioning to activate the mechanism. Preliminary method performance, in the absence of isotopically labelled internal standard is summarized in accordance with relevant SANTE 11945/2015 guidelines.

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# [P049] Fully automated derivatization and quantification of Glyphosate and AMPA in beer using a standard UHPLC-MS/MS system

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Glyphosate is currently one of the most common pesticides used worldwide. Therefore, the strict control of Glyphosate and its metabolite Aminomethylphosphonic acid (AMPA) in food and environment is mandatory. The chromatography of glyphosate is challenging due to its high polarity. In order to overcome this, there exists a well established method including a derivatization step with 9-Fluorenylmethyl chloroformate (FMOC) followed by LCMS analysis. Here we report a fully automated derivatization followed by LC-MS/MS analysis of beer samples. The instrumental set-up does not require any additional hardware for sample pretreatment but uses the built-in pretreatment function of the Shimadzu Autosampler SIL-30AC.
After precipitation with methanol (50:50) and centrifugation the beer samples were set into the autosampler. The addition of internal standards as well as the derivatization of Glyphosate and AMPA with FMOC was done fully automated by the autosampler within 15 minutes. After derivatization the sample was injected directly to the LC-MS/MS and analyzed accordingly.

Using internal standards quantitation ranges from 2.5 to 100 ng/ml for Glyphosate and 5 to 100 ng/ml for AMPA were reached in beer. Accuracies were calculated with beer control samples using internal calibration: 86.5 to 115.3 % (3 ng/ml), 99.3 to 109.5 % (15 ng/ml), 90.5 to 113.7 % (75 ng/ml) for Glyphosate and 90.3 to 158.5 % (3 ng/ml, extrapolated), 95.7 to 112.5 % (15 ng/ml), 96.1 to 114.2 % (75 ng/ml) for AMPA.
In addition automatic derivatization and LC-MS/MS-analysis were overlapped to nearly double the sample throughput.

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# [P050] Rapid Identification of pesticides and mycotoxins in beer samples with the new HR-LC/MS/MS technology X500R QToF System

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LC/MS/MS instruments operating in Multiple Reaction Monitoring (MRM) are widely used for targeted quantitation on triple quadrupole and hybrid triple quadrupole linear ion trap (QTRAP®) systems because of their well-known selectivity and sensitivity. However the new QtoF technology X500R allows unparalleled LC/MS/MS approaches in identification and quantitation. The X500R QTOF from SCIEX is the first high-resolution mass spec system designed exclusively for routine testing labs tasked with detection of low levels of compounds in complex samples or required to profile the composition of samples full of unknowns. The simple, balanced hardware design combined with the new SCIEX OS software user interface makes the system easy to learn, and the robust performance enables you to get the right results quickly, reliably, and with maximum uptime for all your routine testing applications.
We will present a robust and ease-of-use method to screen beer samples by using X500R QTOF system in combination with an ultra-fast HPLC System. No intense sample preparation is needed just filtering, centrifuging and direct sample injection. Information of exact mass, retention time and isotopic pattern of detected molecular ions was used for identification via new software SCIEX OS.

**Results**

Using this method it is possible to analyze all possible ionizable compounds (Vielleicht noch was ´uber SWATH erw´ahnen?). For the screening and identification, we have chosen the IDA and SWATH approach to gain TOF-MS and TOF-MS/MS data simultaneously. Detection limits of various contaminants were found to meet the required values by EU standards.

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# [P051] Combining Non-Targeted SWATH® Acquisition with Highly Selective MRMHR for the Analysis of Veterinary Drugs in Tissue Using the SCIEX X500R QTOF System

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Veterinary drugs are commonly used in livestock breeding to prevent or treat infections of the animals and to ensure their optimal growth. European guidelines require to sensitively control residues of veterinary drugs in animal products to prevent any potential risks for human health. We present a versatile and sensitive workflow on the SCIEX X500R QTOF system which combines a non-targeted screening workflow using SWATH® data acquisition looped with highly selective MRMHR data collection.
Liver tissue extract was prepared and spiked with 27 veterinary drugs to a final concentration of 0.2-50 ng/mL (corresponding to 0.08-20 $\mu$g/kg liver). Following chromatographic separation, samples were analyzed on the SCIEX X500R high resolution LC-MS/MS QTOF system using positive mode electrospray ionization. Data acquisition was performed using TOF-MS mode looped with eight SWATH® MS/MS experiments and scheduled MRMHR acquisition. SWATH® mode is a unique MS/MSALL technology in which MS/MS spectra of all precursors are acquired across the entire mass range of interest. The latter is divided into SWATH® Q1 windows, which are, one after the other, transported through the Q1 into the collision cell, where fragmentation takes place. Variable SWATH® Q1 windows were used, based on the average ion density of the matrix samples. Confident identification of the veterinary drug residues according European Commission detection requirements can be achieved by accurate precursor and fragment mass measurement and their compound specific ion ratios.

*Speaker

# [P052] Rapid LC-MS/MS Method for the Analysis of Insecticides and Associated Metabolites in Egg and Other Poultry Products in Response to a Contamination Crisis

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In 2017, fipronil was detected in eggs produced from farms in Belgium and The Netherlands. 15 other European countries were also affected prompting the recall of millions of eggs from human consumption. Fipronil, a broad spectrum insecticide which belongs to the phenylpyrazole chemical family, is used in the application of red mite, flea, cockroach and ant control. For eggs, European legislation sets the maximum residue limit (MRL) in Regulation (EC) No. 396/2005 at 0.005mg/kg. The European Food Safety Authority (EFSA) defines an Acute Reference Dose (AFrD) of 0.009mg/kg body weight. AFrD refers to the maximum amount of a substance that can be ingested with no health hazard. At 0.72mg/kg, the EU Commission proposes this level of contamination of fipronil could present an acute health risk.
We describe a fast and sensitive multi-component single method using LC-MS/MS for the detection and quantitation of fipronil and its associated metabolites, along with amitraz and its metabolites in eggs and poultry products. The assay uses a modified QuEChERS sample preparation method for the extraction of the egg and poultry matrices. Chromatography was performed using a reversed phase water/methanol gradient with a total runtime of 7mins using a Phenomenex Kinetex Polar C18 column. Mass Spectrometry was performed on a SCIEX 6500+ Triple Quadrupole LC-MS/MS instrument using electrospray ionization, scheduled MRM detection with simultaneous positive/negative ionization switching throughout the run. Results from the assay easily meet the EU regulation MRL in terms of LODs, LOQs, signal to noise, MRM ion ratio accuracy and CV.

*Speaker

# [P053] Sensitive and Specific Allergen Screening Analysis Using LC-MS/MS

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Allergens in food can result in severe or fatal reactions. There is no known cure for an allergenic reaction and the only thing a person can do is to avoid the potential cause eg nuts, milk etc. Food allergens are of increasing interest due to food allergy recalls that have doubled in recent years. To help safeguard consumers from food allergies, warning labels on packaging and in restaurants are a must these days to allow people to make a decision on what they eat. However, food testing is also important and as a result, it is vital to have a robust and specific analytical method to reliably identify and quantitate allergens that may be present. This work presents data from a method that has been highly characterized and verified to determine several different types of allergens using a tryptic digest and LC-MS/MS analysis to measure allergen peptides with a high degree of flexibility, specificity, high sensitivity
Samples were trypticly digested and the resulting extracts analyzed using LC-MS/MS. Peptides that were identified to be associated with a specific allergen were measured using electrospray ionization and scheduled MRM data acquisition. In this presentation a comparison between traditional methods and the LC-MS/MS will be described. Important considerations for method development will be discussed. The ability of the method to identify allergens and to quantify them in food samples will be presented and discussed. The method results from several different food matrices will be presented to demonstrate the potential of this method.

*Speaker

# [P054] Mass spectrometry profiling of polyphenols gives new insights into the chemistry that explains color diversity of 300 worldwide Rosé wines

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Rosé wines have a large diversity of colors worldwide, ranging from the light-grey pink, through yellowish-pink, to purple. Wine color greatly impacts consumer preference during purchase process and wine tasting and can be a signature of Rosé wines from a vineyard or a geographical origin. As in red wines, the Rosé wine colors are mainly due to anthocyanins, a subclass of polyphenols composed of red pigments extracted from the grape skin, but also to the numerous phenolic derived pigments formed during the Rosé specific wine-making process. We wanted to better understand the correlation between the different subclasses of phenolics and the color parameters of a broad Rosé wine collection.
150 polyphenols from a collection of 300 Rosé wines from a single millesime were analyzed by UPLC-MS-MRM. Without preconcentration, filtered wines were injected on a C18 UHPLC column for a 30 minutes gradient and targeted polyphenols were quantified in SRM/MRM mode. Wine color was measured with the L*a*b* colorimetric model. PCA and non-directed chemometric strategies were used to analyze the data.

Light, brownish or darker Rosé colors were explained with differential amounts of pyranoanthocyanin pigments, native anthocyanins, stilbenes and gallic acid.
Within a homogeneous wine-making process, we were able to predict the color characteristics based on a polyphenol composition model and discriminate wine origin and style.

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# [P055] Quantification of methylated amines in human body fluids: a versatile LC-MS method for nutrition studies

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Methylated amines in human body fluids (blood, urine) are of high interest in the context of nutrition, physiology and physical activity. Several compounds are discussed as potential biomarker of food consumption, including trigonelline for coffee, stachydrine for citrus fruits, TMAO (Trimethylamine-N-oxide) for fish, or methylhistidine isomers and dipeptides (anserine, carnosine) for meat. In addition, concentrations of certain amines are increased after heavy exercise, e.g. b-aminoisobutyric acid. For some compounds, for instance ADMA (asymmetric N,N-dimethylarginine) or TMAO, associations with the risk for CVD (cardiovascular diseases) have been shown. On basis of a previous LC-MS method for plasma TMAO and related compounds, we developed a new targeted UPLC-MS/MS method including 29 amines and amino acids in urine or plasma, mostly N-methylated compounds. After dilution and protein precipitation, analytes were separated by HILIC (hydrophilic interaction chromatography) and detected by positive ESI-MRM using time windows. The duty cycle is 9 minutes. Deuterated internal standards and matrix-matched standards were used for calibration. First validation results show good precision and recovery, and a broad linear working range for most compounds. Preliminary data from the KarMeN study (Karlsruhe Metabolomics and Nutrition) show weak associations of selected methylated amines with the consumption of certain food groups. The new method is efficient and allows quantification of a variety of amines in short times. It proved to be suited for application in nutrition-related research, e.g. for human cross-sectional or intervention studies.

*Speaker

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# [P056] Multiresidue Pesticide Analysis in Fruit and Vegetable Commodities Using Both UPLC and APGC on a Single Mass Spectrometer Platform

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Pesticide residue analysis is an essential element for ensuring food safety due to consumer concerns and federal regulations. Comprehensive pesticide screening is typically performed using both LC-MS and GC-MS techniques on dedicated MS platforms. For this analysis, both UPLC-MS/MS and APGC-MS/MS pesticide residue analysis was performed on one single mass spectrometer to demonstrate the flexibility of the analysis.
Plant commodities were subjected to Quechers extraction and dSPE cleanup. Analysis was performed using a Xevo TQ-S micro equipped with both UPLC and APGC as alternating inlets. 200 pesticides were monitored by MRM transitions in both the UPLC and APGC methods. The extracted commodities were screened for pesticide residues and the residues identified were then quantified against a calibration curve.

Pesticide analysis was successfully performed using both UPLC and APGC as alternating inlets on a single mass spectrometer. The fast scanning capability of the instrument allowed for the generation and implementation of methods containing around 200 pesticides for analysis on each chromatography system. The compounds included in the study were detectable at 10 ppb, a common Maximum Residue Limit (MRL) for most pesticides. The guidelines outlined in the SANTE Document (11945/2015) for pesticide residue analysis in food were followed to ensure acceptable results.
Incurred pesticide residues were identified in the samples in both the UPLC and APGC sample screening analysis. The residues identified in the screening analysis were subjected to a confirmatory analysis to accurately quantify the amount of each pesticide identified in each sample.

*Speaker

# [P057] Impact of bifidobacteria-supplemented formula and breastfeeding on fecal metabolite profiles in infants

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The early-life metabolome of the intestinal tract is dynamically influenced by colonization of gut microbiota and diet, i.e. breast milk or formula. The aim of this study is to investigate the effect of bifidobacteria-supplemented formula on the infant fecal metabolome within the first year of life. Therefore, a trial with 106 healthy neonates receiving infant formula with or without probiotics or breast milk was designed and fecal samples were collected over a period of two years at seven time points (Bazanella *et al.*, Am J Clin Nutr 2017). Non-targeted metabolomics analysis was done using hydrophilic interaction chromatography (HILIC) and reversed-phase (RP) ultra-high performance liquid chromatography–time-of-flight mass spectrometry complementarily to cover polar and semi-polar metabolites. The data was noise subtracted, mass signal and retention time aligned. Peak annotation with the Human Metabolome Database (HMDB) was done after isotope filtering. Additionally, acquired data dependent MS/MS spectra were compared with MassBank of North America (MoNA), Global Natural Product Social molecular networking (GNPS) and an in-house database for metabolite identification. Finally, features were batch corrected applying a total abundance regression model based on quality control measurements. Metabolite profiles were clearly distinct between formula- and breast-fed infants were found. Discriminative features among the different feeding types and months were extracted by Partial Least Squares Discriminant Analysis (PLS-DA). We found several metabolites that were altered through the addition of bifidobacteria to infant formula, however, the differences between breast- and formula-fed infants converged over time.

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# [P058] Implementation of a novel scanning quadrupole DIA acquisition method for DESI imaging

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Mass spectrometry imaging (MSI) allows for the correlation of spatial localization and chemical information directly from biological surfaces. Typically untargeted MSI experiments are carried out in full scan MS. After mining of the MSI data, the next step is the identification of potential biomarkers which is usually a limited number of manual MS/MS experiments.
Recently a new Data Independent Acquisition (DIA) method called SONAR utilising a scanning quadrupole mass filter has been introduced with multiple functions: one with no collision energy (CE) and one with elevated CE to get precursor and fragment information within the same experiment.

We have assessed the applicability of this new method and optimisation of settings for a DESI imaging analysis.

The Sonar method for DESI imaging consisted of two alternating functions. In both cases the quadrupole was scanned multiple times across the mass range with a pre set quadrupole window. In the first function (precursor function) the collision energy was fixed at 6ev, in the second function collision energy was applied to fragment the ions (MS/MS function). The functions alternated between pixels to generate images of precursors and of fragments in a single experiment. The precursor and MS/MS functions were subsequently time aligned to relate the fragments to precursors for identification of multiple species from a single imaging run.

Proof of concept experiments have been performed analysing a mouse brain tissue section.

Reviewing the data with Driftscope and HDImaging a number of time aligned precursors / fragments could be identified

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# [P059] Multimodal Imaging Workflow: Combining Vibrational Spectroscopy and Mass Spectrometry

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Mass spectrometry provides a detailed insight into the chemical composition of biological tissue while vibrational spectroscopy yields quantifiable information about protein and lipid content. A combination of both techniques provides complementary information to gain deeper insight into the anatomical structure of biological tissue and to verify findings. Therefore, a workflow is presented combining atmospheric pressure matrix assisted laser desorption/ionization mass spectrometry (AP-MALDI-MS), Fourier-transform infrared (FT-IR) and Raman spectroscopy. Using mouse brain as specimen, FT-IR, Raman and MS images were acquired in this sequence on the same section. In a final step, the section was histologically stained. The spectroscopic raw data was converted to the imzML file format. As Raman and FT-IR data were saved in ASCII format, an 'ASCII to imzML' converter was developed and is presented herein. This converter, which will be made available for download on www.imzml.org, can be used for any ASCII data. We demonstrated this for statistical analysis results and mass deviation evaluation of a MS image. Spectroscopic and stained images revealed a high spatial correlation of structural features. FT-IR provided a rapid overview about lipid and protein composition while Raman spectra revealed more specific information about lipids and heme species. MS provided the most detailed information about the spatial distribution of lipid families. Using our approach spectroscopic and mass spectrometric data can be combined "pixel-wise" on a spectral basis (as opposed to overlaying graphical images). In the future, this approach can be generally applied for multimodal imaging combining spectrometry techniques and different vibrational spectroscopy techniques.

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# [P060] High resolution mass spectrometry imaging of food samples

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Mass spectrometry imaging (MSI) is a powerful tool to study the lateral distribution of a large variety of analytes without peak labelling. One of the most common MSI techniques is matrix-assisted laser desorption/ionization (MALDI) MSI. The laser beam is used to scan the sample to desorb and ionize the surface material [1]. This soft ionization process enables the detection of intact biomolecules in different application areas such as food chemistry. The spatial distribution of compounds in food can reveal additional information about its properties and functions.
In this study, MSI measurements were performed using a Q-ExactiveTM HF hybrid-quadrupole-orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen), on which the AP-SMALDI10 ion source (TransMIT GmbH, Giessen) is installed. Images can be created from every single $m/z$ in the mass spectra. For data analysis, we used the open format imzML which enables visualization of the ion images with different software tools (www.imzml.org).

In first experiments we could optimize the MSI workflow for selected foods in order to retain the spatial distribution of different analytes. Carrots and (sprouted) apple seeds are two applications, where it was possible to acquire data with high mass resolution (240,000 @ $m/z$ 200) and with pixel sizes between 10 and 50 $\mu$m. With the optimized workflow for food analysis, we are now able to investigate processes where lateral distributions of compounds are required, e.g. for food additives.

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A. Ŕompp and B. Spengler, Mass spectrometry imaging with high resolution in mass and space, *Histochem Cell Biol,* **2013** 139:759-783**.**

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# [P061] Insect neuropeptide MALDI Imaging: Simultaneous analysis and localization with spatial resolution at the micrometer scale

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There is increasing pressure to develop new insecticides that affect pests without harming beneficial species. Targeting neuropeptides is one strategy for novel insecticide development as they control most aspects of insect physiology, such as feeding, water homeostasis and reproduction. Current limitations to study complex neuropeptide interactions in insects include small tissue size, low abundance, and expression of many neuropeptides in a few cells. The spatial localization of neuropeptides has traditionally been studied with immunocytochemistry, but this cannot simultaneously discriminate between different neuropeptide precursors. In contrast, MALDI Imaging is an untargeted technique which allows visualization of many molecules in tissue sections.
A customized MALDI-MSI workflow was developed to examine the distribution of neuropeptides in the brain and retrocerebral complex (RCC) of the American cockroach, *Periplaneta americana*. After sacrificing the animals, brains and retrocerebral complexes were extracted and embedded in 120mg/ml gelatin/deionized water. Tissue samples were frozen at -50∘C, sectioned with at 14 $\mu$m on a cryo-microtome, and transferred to ITO slides. Tissue sections were washed, dried under vacuum, and coated with HCCA matrix by a spraying device.

MS data was acquired on a rapifleX MALDI-TOF mass spectrometer (Bruker, Bremen, Germany) over a mass range of m/z 600-3200. Pixel size was set to 15 $\mu$m at a laser shot rate of 5 kHz.
MALDI-MSI can be used for detailed analysis of insect neuropeptide distribution. The described protocol can be applied to different neuroendocrine tissues. This approach covers the majority of expressed neuropeptides in the nervous system.

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# [P062] Distribution of lipids and antibiotics in mouse lung tissue

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The rise of multi drug-resistant *Mycobacterium tuberculosis* strains creates an ever increasing need for novel anti TB drugs. The development of TB drugs however is impeded by the shortcomings of current preclinical animal models. In preparation for future studies on novel TB drugs, AP-MALDI imaging was used in this study to map the distribution of phospholipids and multiple TB drugs in mouse lung tissue.
MS imaging experiments were carried out on a Q-Exactive$^{TM}$ HF Hybrid-Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen), coupled to the AP-SMALDI10 ion source (TransMIT GmbH, Gießen). With this instrumental set up, mass spectra with high mass accuracy (better than 2 ppm) and high mass resolution (R=240,000 @m/z 200) can be acquired. Measurements were performed on lung tissue of BALB/c mice treated with anti TB drugs. Different matrices were applied using a pneumatic sprayer system. Mass images were generated using Mirion and MSiReader.

Sample preparation including matrix application and cryosectioning was optimized for several TB drugs. MS imaging measurements of mice lung sections were performed with pixel sizes between 10 and 40 $\mu$m. These experiments showed high spatial details in phospholipid images, which corresponded well with histological features. Phospholipid patterns differed substantially between different tissue regions. In addition, the distribution of several TB drugs could be shown in mouse lung tissue.

In this study, we adopted a high resolution MALDI MSI method to map the distribution of phospholipids and TB drugs in mouse lung tissue with a spatial resolution close to the cellular level.

*Speaker

# [P063] Matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) facilitates mapping of the peptide FR900359 in Ardisia crenata leafs

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We used matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) on a Thermo LTQ Orbitrap XL to detect the pharmacologically relevant nonribosomal peptide FR900359 (FR) in leafs of the plant *Ardisia crenata*. The peptide FR is produced by a yet uncultured bacterial endosymbiont of the genus *Burkholderia*. The detection of FR by MALDI-IMS corroborates previous results from scanning electron microscope (SEM) experiments and shows indirectly the spatially limited distribution of these bacteria in the leaf.

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# [P064] Combination of an AP-MALDI with a Hybrid Linear Ion Trap-Orbitrap Mass Spectrometer for accurate separation and location of isobaric lipids in mouse brain tissue sections

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Mass Spectrometry Imaging allows the combination of molecular analysis and spatial information. This technique is a robust tool for the location and identification of molecules of interest such as lipids on biological tissue sections. Most investigations have been performed using TOF-SIMS and MALDI-TOF instruments. TOF-MS offers the advantage of fast analysis but it remains limited in terms of mass resolving power and correct molecular identification. Indeed, it does not allow to perform structural identification such as MS/MS which constitutes an issue for formal molecular identification. Coupling an Atmospheric Pressure-Matrix Assisted Laser Desorption Ionization (AP-MALDI) source with a Hybrid Linear Ion Trap-Orbitrap Mass Spectrometer permits to combine the advantage of high mass accuracy for untargeted molecules screening with a sensitive structural identification by MS(n) for targeted analysis which is critical for reliable identification and location of compounds of interest desorbed directly on biological tissues. Several classes of lipids such as galactosylceramides, phosphatidylethanolamines or phosphatidylcholines were identified and located in the tissue with accurate mass (error $<$ 3 mmu). Isobaric lipids coming from different lipids classes were clearly separated and located in different parts of the mouse brain. The MS/MS allows to confirm the identification of these molecules with high confidence. Two types of AP-MALDI sources were used for lipids identification and location on mouse brain sections covering a lateral resolution range from 10 to 150 $\mu$m.

*Speaker

# [P065] Improvements in Imaging Mass Spectrometry for the Analysis of FFPE Tissue Sections

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Mass spectrometry imaging (MSI) is a label-free technique allowing direct measurements of molecular distributions in tissues in their native histological context. The acquired data can also be used for spatial segmentation or classification, offering the possibility of assessing phenotypic heterogeneity. Although formalin-fixed paraffin-embedded (FFPE) tissues are the principal samples generated in clinical institutions, MALDI MSI of FFPE tissue with high spatial resolution and reproducibility has been limited. We have developed sample preparation improvements for MALDI MSI leading to highly increased data quality.
FFPE mouse brain and human lung tumor tissues were cut at 5 $\mu$m, mounted onto conductive ITO slides (Bruker Daltonik GmbH, Bremen, Germany), and subjected to deparaffinization, rehydration and heat-induced antigen retrieval. Proteins were digested by spraying samples with trypsin solution in TM Sprayer (HTX Technologies, Chapel Hill, USA), and staying under humid conditions for 2h. ACCA matrix was deposited using the same sprayer. MS analysis was done using a rapifleX MALDI-TOF (Bruker Daltonik) at 50 $\mu$m spatial resolution.

Changes in MALDI MSI sample preparation has produced improved resolution of histological structures. MALDI imaging allows the analysis of molecular phenotypes which are characteristic for different tissue types, here used for the clear discrimination of organized structures within mouse brain by individual m/z species. These molecule species were found to be highly correlated to distinct histopathological entities in a cancer sample. Statistical grouping of similar spectra via hierarchical clustering and pLSA is able to separate these organized structures in mouse brain from disorganized structures in lung adenocarcinoma.

*Speaker

# [P066] Imaging analysis of phenethylamine derivatives 25C- and 25I-NBOMe on blotter papers

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The rising popularity of phenethylamine derivatives as illicit drugs since 2010 can be traced back to a shortage of lysergic acid diethylamide (LSD). Therefore, phenethylamine derivatives are often sold instead, sometimes even deceptively as LSD itself. These potent and toxic substances can lead to severe intoxication. Thus, a more profound understanding of the toxicological properties of the phenethylamines is required. The manufacturing process of spraying these compounds onto the common blotter paper increases the risk of overdose due to heterogeneous distribution. Besides rapid detection of the analytes, the manufacturing process was elucidated via three different imaging techniques and liquid chromatography-mass spectrometry (LC-MS). A blotter paper sample, containing two phenethylamine derivatives (iodophenethylamine; 25I-NBOMe and chlorophenethylamine; 25C-NBOMe) was analyzed via imaging techniques, namely, micro x-ray fluorescence ($\mu$XRF), laser ablation (LA)-inductively coupled plasma-optical emission spectroscopy (ICP-OES) and matrix assisted laser desorption ionization (MALDI)-MS and was validated with HPLC-MS/MS after extraction. Furthermore, LA-ICP-OES depth profiling was used to obtain three-dimensional information of the compounds in the blotter paper. These results helped to confirm the assumption that manufacturers spray the compounds onto the paper. Whereas $\mu$XRF and LA-ICP-OES detected signals for chlorine and iodine, MALDI-imaging showed the molecular distribution of both analytes. Finally, complementary HPLC-MS/MS measurements validated the results obtained by $\mu$XRF, LA-ICP-OES and MALDI-MS.

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# [P067] A new toolkit for rapid batch processing, analysis and visualisation of imaging data

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Most tools, which are used for imaging analysis, target a specific imaging technique and handle data in limited formats. The presented toolkit enables batch processing, analysis and visualisation of both simple text-based and complex spectral data (e.g. in the mzML or imzML formats) from multi modal imaging techniques with the same algorithms. A major goal is to generate high-quality charts and analysis results in a modern intuitive framework. For spectral preprocessing, raw data is imported to MZmine 2, which was originally designed for chromatography-based HRMS studies. MZmine 2 was modified to incorporate its various algorithms into imaging workflows, which include image generation, manipulation, visualisation and statistics. For region-of-interest (ROI) statistics and quantification, different ROI shapes (rectangle, ellipse, polygon and "freehand") and types (sample, quantifier and blank) are available. Additional exclusion ROIs cut out single pixels or areas from statistics. Further implemented functions include algorithms for a bicubic interpolation and a fast Gaussian blur approximation, overlaying of multiple images and a microscopic image, quantification and internal standard normalisation. Selected images of multiple data sets are stored in a project structure, thus allowing to apply parameters to other images, perform batch processing and to export results and thousands of print-quality images in a few clicks. For graphics export, options are available for vector-based (pdf, eps and emf) and pixel-based graphics (jpg and png). In order to save and share results, an open readable format comprises a whole project with its raw data and settings.

*Speaker

# [P068] Atmospheric-pressure MALDI mass spectrometry imaging for lipidomic analysis of late fetal mouse lungs

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Pulmonary surfactant contains ¸˜90% phospholipids and ¸˜10% proteins. The main function of the pulmonary lipidome is to reduce the alveolar surface tension at air-liquid interface, thereby preventing end-expiratory collapse of the alveoli. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) has widely been used, since 1) labeling is not necessary, 2) hundreds of molecules can be detect in a single run, and 3) spatial distribution of biomolecules in biological tissue is provided. So far, characterization of the lipidome of the late fetal stage 19-days (E19) mouse lungs has not been performed. In this study we therefore employed high-resolution atmospheric pressure scanning MALDI MSI (AP-SMALDI MSI) for the lipidomic analysis of E19 mouse lungs. Molecular species of different lipid classes were imaged in E19 lung sections at high spatial (10 $\mu$m per pixel) and mass resolution (140,000 @ $m/z$ 200) in positive- and negative-ion mode. The lipid species were characterized based on accurate mass ($\leq$ 3 ppm) and on-tissue tandem mass spectrometry. In addition, a dedicated tissue-sectioning method, homogenous deposition of different matrices on tissue surfaces and detailed data processing parameters were optimized for the comparison of signal intensities of lipids between different tissue sections from MSI data using PEX11$\beta$ knockout (peroxisome biogenesis factor 11$\beta$) E19 mouse lungs. Our study provides lipid information of the E19 mouse lungs shorty before birth, optimized experimental, instrumental and data processing strategies for the direct comparison of signal intensities of lipids and other cellular metabolites among the tissue sections from MSI experiments.

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# [P069] Elucidation of the development of defensive metabolites over time and their spatial distribution around damaged plant tissue

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Higher plants respond to wounding, caused by mechanical injury or herbivore feeding with a complex defense mechanism. This involves production of a multitude of secondary metabolites in and around the damaged tissue. These metabolites include healing accelerants, defense metabolites like toxins and repellents as well as antimicrobials to ward off pathogens. While their chemical nature, as well as the involved biosynthetic formation is well understood, this study aims to examine distribution of wound metabolites. Leaves of *A. thaliana* were mechanically wounded by a metal pin and after certain time covered with basic MALDI matrices using MALDI-Sprayer (SunCollect). AP-SMALDI-Orbitrap imaging experiments on wounded *A. thaliana* leaves show presence of precursors and intermediates of jasmonic acid (JA) signaling pathway as well as defensive phytohormones. Using super basic MALDI matrices e.g. 1,8-di(piperidinyl)naphthalene (DPN) we were able to visualize a distribution of these compounds in and around sites of injury. For instance we show a distribution of major precursors of jasmonic acid - linoleic or linolenic acid. JA biosynthesis starts via peroxidation of linoleic acid by lipoxygenases (LOX) and we could visualize a progress of this process over time. Apart from that we investigated a lateral distributions of glucosinolates (e.g. 4MSOB, 4MTB, I3M, 8MSOO) and amino acids on intact/wounded leaves.

*Speaker

# [P070] Mass spectrometry imaging of cotton leaves by the means of laser ablation electrospray ionization

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*Gossypium hirsutum* is a widely cultivated cotton species. In most of its tissues, pigment glands with detrimental effects on insect growth can be found (Kong et al., 2010; Stipanovic et al., 1977, 1978). The insecticidal effect is attributed to gossypol and related sesquiterpenoid aldehyde quinones (Stipanovic et al., 2008), isolatable from *Gossypium* species and other plants of the Malvaceae family (Fryxell, 1992). Here, we would like to present evidence for the localization of different gossypol-related compounds in cotton pigment glands by means of laser ablation electrospray ionization (LAESI) in a mass spectrometry imaging (MSI) setup. Parts of cotton leaves were subjected to profilometric measurements and subsequent LAESI-experiments with topographic correction, according to a method published earlier (Bartels et al., 2017). All mass spectra were acquired on a Synapt HDMS instrument in negative ion mode. Spatial context was provided by external trigger data from the infrared laser used for ablation and evaluated with a R script written in-house. Target compounds, namely gossypol, hemigossypolone and heliocide H1-4, were identified by *m/z*-value, based on previously conducted LC-MS/MS experiments (H'olscher et al., submitted) and correlation of *m/z* occurrence and laser activation times. The reconstructed ion maps for all target compounds were compared to optical images of the ROI to investigate the occurrence of black pigment glands with the distribution of all target compounds.

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# [P071] A novel membrane-based protocol for MALDI- and MALDI-2-MS imaging of inactivated bacterial colonies

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Bacterial growth is mediated by numerous classes of metabolites, lipids and, in some cases, peptides. MALDI-MS imaging is increasingly used to monitor this metabolic exchange. However, obtaining artefact-free snap-shots of the actual metabolic situation comes with several challenges. Pathogenic samples must be safely inactivated before further handling, whereas this step should not change morphology or chemical profile. Here we present a novel membrane-based workflow for cultivation, safe inactivation and subsequent highly-resolved MALDI-MS imaging of bacterial colonies.

*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* strains were investigated as representative, medically relevant Gram-negative and Gram-positive samples. Bacteria were grown in liquid LB broth and 1 $\mu$L-aliquots were transferred onto 150 $\mu$m-thick mixed cellulose ester membrane filters (Merck Millipore) with pore sizes of 0.45 or 0.22 $\mu$m, respectively, placed on LB Agar. Rapid inactivation was obtained by placing the removed membrane in saturated H2O atmosphere (_˜100 ∘C) for about 5 s. Application of MALDI matrix (e. g. 2,5-Dihydroxybenzoeic acid) was achieved with an airbrush sprayer. A modified Synapt G2-S mass spectrometer with MALDI-2 postionization option was used for data acquisition with a lateral resolution of _˜50 $\mu$m.

Smear tests proofed that safe inactivation was achieved for all three strains without notable difference in the morphology between inactivated and life colonies. MALDI-2-MSI provided a wealth of chemical information. For example, based on tentative data evaluation about several dozen different metabolites were recorded from *P. aeruginosa* in positive ion mode alone.

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# [P072] AP-SMALDI MSI of lipids in Schistosoma mansoni parasites

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According to the World Health Organization, neglected tropical diseases are an emerging problem, affecting about 1 billion people worldwide. Often, the metabolome of parasitic vectors is neither described nor understood. We have used mass spectrometry imaging to analyze lipids and metabolites in *Schistosoma mansoni*, a parasite causing bilharzia. It is crucial to characterize the interaction between female and male schistosomula to identify possible novel anti-schistosomal drug targets.
Longitudinal cryo-sections of adult *S. mansoni* worms, 1-5 mm long and up to 500 $\mu$m thick, were prepared prior to matrix application by pneumatic spraying. The worm sections were measured using an autofocusing AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen, Germany) coupled to a Q Exactive HF mass analyzer (Thermo-Fisher Scientific, Bremen, Germany), allowing to record high-resolution mass spectra at high spatial resolution including complex sample topographies. Data evaluation was performed using software packages MIRION and MSI-Reader and by database assistance derived from complementary LC-MS experiments.
Due to the structure of adult schistosomata, it is crucial to obtain longitudinal sections of the organs such as testes/testes-lobes, esophagus, oral sucker, ventral sucker, gynaecophoric canal where the paring contact between female and male is established, and tegument, the outer body cover of flatworms, in order to detect differentially expressed lipids in such organs. Combining the microscopic image with the ion images, organ-specific up-/downregulation of various lipids was observed. The lipid profiles were linked to gender as well as pairing status, enabling novel insights into male-female interaction in schistosomata.

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# [P073] Optimization of nano desorption electrospray ionization for the examination of biomolecules

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Desorption Electrospray Ionization Mass Spectrometry (DESI MS) is a common technique for ambient analysis, allowing measurements after minimal sample preparation. Nano-DESI MS is a development towards smaller sampling areas and better sensitivity, based on a liquid contact to the sample surface. The method dissolves small amounts of analyte from the sample surface and directs it into a second capillary via continuous solvent infusion. High voltage applied to the second capillary causes an ion spray at the tip of the secondary capillary. Liquid extraction from individual sample spots provides laterally resolved (imaging) analysis.
The ion source setup was optimized for ion signal intensities by variation of the capillary angles, relative to each other and to the sample surface, the distance between capillary and sample and the distance between the secondary capillary and the MS inlet. Experiments were performed to understand the transportation process and the delay between liquid extraction on the sample surface and signal detection. All experiments were performed on an Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany).
Imaging of rhodamine 6G was used for optimization of parameters. The analysis of porcine liver tissue demonstrates the high potential of nano-DESI MS for MSI. Lipid profiles of different tissue types were determined, and small metabolites were detected and identified. The achieved lateral resolution was in the range of 40-50 $\mu$m. It was found that the low liquid flow rate of the method results in less spreading of tissue components on the surface compared to conventional DESI MS.

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# [P074] Controlling matrix deposition: A sublimation chamber with adjustable reservoir and sample temperatures, background gas pressure and matrix deposition rates

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The success of a MALDI MS imaging experiment (e.g., in terms of lateral resolution and analytical sensitivity) strongly depends on an optimal sample preparation. Uniform microcrystalline matrix coating of tissue sections is typically achieved either via repeated pneumatically spraying of matrix solution or by vapor deposition of sublimated matrix molecules in vacuum. To improve analyte/matrix co-crystallization, the latter approach is often followed by recrystallization.
Here we describe a custom-made sublimation chamber that enables precise control of relevant physical parameters. The temperatures of the heated matrix reservoir (control range: 20 ∘C to 140 ∘C) and that of the sample (-25 ∘C to 25 ∘C) are adjusted using an electric heating element and a thermoelectric cooler respectively. The buffer gas pressure (10e-5 to 10e-3 mbar) is adjusted via a needle valve. Custom-made electronic feedback loops allow for precise control and read-out of all parameters. Deposition rates can, furthermore, be adjusted by use of different apertures in the opening of the heated matrix reservoir and by varying the reservoir-sample distance.

To monitor the matrix deposition rate, we utilized a quartz microbalance, mounted in close vicinity to the sample plate. 2,5-dihydroxybenzoic acid (DHB) was selected as the primary, well studied and established MALDI matrix.

The combined results highlight the relevance of systematic studies on the fundamental parameters effecting MALDI-MSI analysis.

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# [P075] Phospholipid-mediated ion suppression effects in MALDI mass spectrometry imaging investigated by use of spiked micro-arrays of artificial tissue

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MALDI-MS imaging (MALDI-MSI) is increasingly used to visualize the molecular distribution of lipids in biological tissue sections. However, a major hindrance for quantitative (Q)MALDI-MSI applications results from notorious ion suppression effects. To obtain a deeper insight into these phenomena for phospholipids (PL) under MSI conditions we developed "artificial tissue" (AT) substrates that mimic essential characteristics of soft tissue and that can be spiked with controlled concentrations of selected PLs.
Best results so far were obtained with a mixture of carboxymethyl cellulose and gelatin as the AT material. A micro-array mold with 24 vials of 20 $\mu$L volume was constructed and filled with lipid-spiked AT. For MALDI-MSI, cryosections of the material were coated with matrix using a sublimation/recrystallization protocol. To study the PL-derived ion suppression effects in a systematic manner, we used two-component systems. Molecular signal intensities of phosphatidylcholine (PC) were compared to those of a second PL (e.g., phosphatidylethanolamine (PE), and phosphatidylglycerols (PG)) in dependence of the respective lipid concentrations. Results show an intricate interplay between PL classes impeding the quantitative analysis of complex biological samples especially in MALDI-MSI applications.
Further experimental parameters (choice of MALDI matrix, influence of the MALDI laser wavelength, fluence and focal spot size) were investigated to some effect with respect to their influence on ion suppression. Finally, we present first data on ion suppression in laser-induced postionization (MALDI-2), investigated using an exemplary a PC/ PG mixture.

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# [P076] Application of MALDI Imaging in ovarian cancer for tracking biomarkers for therapy response

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High-grade serous ovarian cancer (HGSOC) is worldwide the predominant histological subtype of ovarian cancer. The majority of patients have initially a complete response to debulking surgery and platinum-based chemotherapy, but more than 70% will relapse, subsequently develop chemotherapy resistance and die of the disease. Due to lack of specific symptoms, HGSOC often presents at a late, advanced stage and have a significantly worse outcome than those with early stage disease. However, some patients even with early stage HGSOC will relapse and prediction of tumor recurrence is crucial for clinical management and patient survival. Hence, the aim of this study is to determine potential biomarker of recurrence using proteomics signatures via MALDI Imaging tissue assessment of early stage HGSOC. 15 FFPE specimens from early stage HGSOC tissue in 6$\mu$m thickness were transferred onto Indium-Tin-Oxide slides. After deparaffinization and rehydration the heat-induced antigen retrieval was performed on sections. Using MALDI-TOF-MS, peptide spectra were acquired at a mass range of m/z 600-3200 Da and lateral resolution of 50$\mu$m. The full dataset was evaluated with Receiver-Operating-Characteristic analysis (ROC) and Probabilistic-Latent-Semantic-Analysis (pLSA) with the software SCiLS Lab.The evaluation of proteomics signatures results in a clear discrimination of recurrence and no recurrence groups in compound 1 of pLSA. Moreover, single peptide values could determine significant difference for recurrence group. To summaries, MALDI Imaging is a valuable tool to discriminate recurrence and non-recurrence HGSC tumor. Limit of the low amount of patient samples the distinguished peptide markers need further validation through a larger sample number.

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# [P077] Identification of lipid markers of coccidian infections in bovine umbilical vein endothelial host cells

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Coccidian parasites are a subclass of obligate intracellular apicomplexan parasites which induce a variety of human and veterinary diseases worldwide. Given that their metabolism is barely investigated on the molecular level,the aim of this study was to analyze the lipidome of *Besnoitia besnoiti*-, *Eimeria bovis*- and *Toxoplasma gondii*-infected host cells by MALDI mass spectrometry imaging (MSI). We developed a method for identifying lipid markers of infection in cell pellets of bovine umbilical vein endothelial cells (BUVEC) and established a protocol for accelerated and partially automated data analysis.
Cell cultivation and infection was carried out at the Institute of Parasitology (Justus Liebig University). Atmospheric pressure MALDI MSI measurements were performed by ablating a predefined area for all samples. A Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to an AP-SMALDI10 ion source (TransMIT GmbH, Giessen, Germany) was used for MSI experiments.
In positive ion mode, 25 lipid biomarkers were determined for the parasite *B. besnoiti*. The markers comprised mainly the lipid classes of diglycerides (DGs), phosphatidylcholines (PCs),phosphatidic acids (PAs), phosphatidylglycerols (PGs) and phosphatidylserines (PSs). Individual lipid species were assigned by database search. In negative ion mode, 20 lipid biomarkers were determined and were mainly assigned to phosphatidylinositols (PIs), PGs and PSs. Similar molecule numbers were obtained for *E. bovis* and *T. gondii* host cell infections. Furthermore, we were able to transfer the cell pellet-derived results to the analysis of cell monolayers. As such, previously found lipid markers were also detected in monolayers and indicated parasite-infected cells.

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# [P078] Lipid Profiles as Marker for Antimycobacterial Treatment

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It is estimated that approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* (*Mtb*). An active progressive tuberculosis (TB) infection is developed in a proportion of chronically infected patients. Because of the increased portion of multidrug resistant TB worldwide, treatment regimens become more complicated and last prolonged time periods, up to 24 months.
There is urgent need to establish analytical approaches that help to monitor drug concentrations and find a biochemical correlate for therapy success. Therefore, we studied in a murine TB infection model how pathogen burden and antibiotics treatment influence the lipidome of the lung. For this purpose lipids from TB infected mice were extracted by the methyl *tert*-butyl ether (MTBE) protocol at different time points during antibiotics treatment. Shotgun lipid*omics* was performed with nanoESI-MS/MS using a Q Excative Plus coupled with Triversa Nanomate. Lipid identification and quantitation was implemented using LipidXplorer.
We found that mycobacterial glycerophospholipids, specifically tuberculostearic acid containing phosphatidylinositol PI (16:0/19:0) provides enough specificity and sensitivity to determine the *Mtb* burden. Additionally we searched for specific *Mtb* related imprints in the lipidome of the host. We show first results for integrating data from drug monitoring and shotgun lipid*omics* in a mouse model.

*Speaker

# [P079] LC-MS/MS method for the simultaneous quantification of endocannabinoids

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Endocannabinoids (EC) represent a group of diverse substances acting at cannabinoid receptors. EC are involved in multiple physiological and pathophysiological processes including analgesia, appetite regulation and motoric functions. Moreover, preclinical research points toward a profound anti-cancer activity of these compounds. To study biological functions of EC in a more detailed manner, appropriate analytical methods for simultaneous quantification of a panel of EC such as *N*-arachidonoylethanolamine (anandamide, AEA), 2-arachidonoylglycerol (2-AG), *N*-arachidonoylethanoldopamine (NADA), 2-arachidonoylglycerolether (noladin ether, 2-AGE) and *O*-arachidonoylethanolamine (virodhamine, VA) are needed. We herein present a high-sensitive LC-ESI-MS/MS method based on a triple quadrupole mass spectrometer to measure the aforementioned EC. A preceding liquid-liquid extraction with ethyl acetate was used. The method was validated for all analytes in accordance with the FDA guidelines. The following parameters were determined (results in brackets): lower limit of quantification (0.03 to 2.00 ng/mL), intra- and inter-day precision and accuracy (statistic and systematic error: $<$ 10 %), matrix effects ($<$ 25 %), extraction efficiency ($>$ 87 %), recovery ($>$ 58 %), stability over 2 months (30 to 95 %). In ongoing research the method is routinely applied to study the role of EC in tumorigenesis both in vitro and in vivo.

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# [P080] Set Up of a RPLC-MS method to the analysis of complex mixtures of Lipids

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The analysis of lipids by mass spectrometry presents some difficulties. First of all lipidomes are extremely complex; the LipidMAPS structure database, updated 30/01/2017, reports more than forty thousand of unique lipid structures. Furthermore, between them there is high incidence of isomeric (same elemental composition and therefore same molecular weight, but different structure) and isobaric (different elemental composition, but very similar molecular weight) compounds.
We developed and set-up a RPLC-MS/MS workflow to analyze complex mixtures of lipids.

Plasma from Human, extracted using the protocol reported by Matyashetal et al1, was chosen as it has a complex lipidome with large variance of classes and species. Lipids were separated using RPLC using an Ultimate 3000 HPLC system and 5 different columns. High mass accuracy MS analysis and data-dependent MS2 analysis were applied using a Q Exactive HF mass spectrometer to identify individual lipids molecular species. LipidSearch software version 4.1 SP1 was used for lipid identification. This workflow was applied to evaluate the separation capability of each column and to achieve a lipidomics profile of highly complex biological samples. The workflow used has successfully identified a large range of lipid molecules.
1. Matyash V, Liebisch G, Kurzchalia T V, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *J Lipid Res.* 2008;49(5):1137-1146. doi:10.1194/jlr.D700041-JLR200.

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# [P081] Lipidomic profiling with high spatial resolution in hippocampus of acute epileptic seizure model compared to controls

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***Introduction***

Lipids emerge as important candidates for biomarkers, drug targets, but also as therapeutic agents in many pathological processes, including neurodegenerative diseases such as epilepsy. Interrogations in neurological diseases often require qualitative and quantitative molecular profiling at higher spatial resolution in the brain, to localize the functional subareas of the brain involved in the disease.

***Method***

We applied quantitative lipidomic profiling based on LC/MRM at different spatial resolution in hippocampi and comparative lipidomic profiling via MSI across hippocampus obtained 1 h after kainic acid-induced epileptic seizures in mice vs. control. Three different protocols were applied: i) quantitative profiling of selected PLs and eCBs by LC/MRM in whole hippocampus homogenates ii) simultaneous quantitative profiling of PLs and eCBs by LC/MRM as well as mRNA by qPCR in brain punches from dorsal (dHC) vs. ventral (vHC) hippocampus and iii) MS imaging of PLs across cell layers/populations of the hippocampus.

***Result/Discussion***

We discovered that vHC and dHC exhibit distinct lipid and mRNA dynamics with epilepsy, which were masked when an entire hippocampus was investigated. Higher spatial resolution profiling by MSI revealed alternating lipid changes between control and epileptic mice across the dHC, supporting a fluctuating lipid metabolism at acute epilepsy.

These findings strongly underline the demand for quantification at higher spatial resolution to elucidate (sub)localized mechanisms and specific lipid mediators and transcript plasticity under pathological conditions.

Quantitative neurolipidomics at high spatial resolution is here highlighted as a valuable, necessary tool to expedite elucidation of neurobiological mechanisms, hence drug target and candi-

*Speaker

dates discovery.
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# [P082] Differentiation of constitutional isomeric phospholipids by hydrophilic interaction liquid chromatography coupled to ESI-MS/MS

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Phospholipids (PL) are important lipids which inherit multiple functions in biological systems. These lipids are divided into classes regarding their polar head group which is linked to the glycerol backbone or to the glycerophosphate backbone. Interesting representatives of phospholipids are phosphatidylglycerols (PG) bis(monoacylglycero)phosphates (BMP).
Especially BMP is an interesting PL. BMP is a constitutional isomer of PG, so thus it has been erroneously determined as PG for a long time. BMP was found in the most mammalian tissues with a share of 1 - 18% of the total phospholipids. Several reasons render BMP to an interesting lipid: Its biochemical properties (unusual selectivity and binding of fatty acids), its relevance for multiple biological activities (including cholesterol homeostasis), its specific cellular localization, and its important role in various diseases (including lysosomal disorders or breast cancer).

Important structural information can be obtained by MS/MS. However, the constitutional isomers PG and BMP show similar MS/MS spectra. Therefore, differentiation of these lipids solely based on MS is not possible and hence chromatographic separation is mandatory. Consequently, we developed an HPLC method based on hydrophilic interaction liquid chromatography (HILIC), which ensured baseline separation of PG and BMP.
The applicability of the developed phospholipid-profiling method based on HILIC-ESI-MS/MS is demonstrated by analysis of phospholipids from MCF7 breast cancer cells in oncogene-induced senescence cell culture model. Healthy cells and breast cancer cells could be differentiated by specific changes in BMP species distribution.

*Speaker

# [P083] LC-MS3 method for the analysis of Lipid Peroxidation Products (LPPs) positional isomers

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Lipid peroxidation is an enzymatic or non-enzymatic transformation of polyunsaturated fatty acids (PUFAs) leading to the formation of various lipid peroxidation products (LPPs) including low molecular weight aldehydes (i.e. 4-hydroxy-trans-2- nonenal, acrolein), truncated lipids (i.e. alkanals, alkenals), and hydroxy-alka(e)nals, isoprostanes, hydroperoxy-, hydroxy-, keto- and epoxy-derivatives. Numerous studies shown the involvement of lipid peroxidation in the onset and progression of inflammatory based diseases such as diabetes, Alzheimer and cardiovascular diseases. LPPs are known to modulate different cellular signaling pathways by inducing changes in the biological membranes, protein lipoxidation, and interaction with cell surface or intracellular receptors.

Various biological activities of LPPs are mainly determined by their chemical diversity. In order to understand LPPs structure-functional relationships, specific and sensitive analytical tools allowing separation and identification of structural isomers are required. Liquid chromatography coupled on-line to mass spectrometry (LC-MS) allows high-throughput characterization of LPPs. However, majority of the methods are not capable to distinguish LPP structural isomers.

Here a novel LC-MS method for analysis of isomeric LPPs was developed. MS and tandem MS analysis was performed in negative ion mode. Anions of oxidized PUFAs, produced by collision induced dissociation of LPP precursors, were further used for data-driven MS3 analysis yielding structure specific fragment ions necessary to assign type and position of oxidation within PUFA alkyl chain. Method was validated using in vitro oxidized standard PLs and further translated for analysis of complex biological samples. Thus, using novel LC-MS3 based approach it was possible to reveal structural diversity of LPPs in a high-throughput manner.

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# [P084] Separation of lipid oxidation products by thin-layer chromatography – the advantages of reversed-phase TLC

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Oxidized lipids are of paramount interest because they play important roles in human health and disease. Their impact on cells is often studied using *in vitro* systems. Even though the demand on oxidized lipids is high, their commercial availability is limited. Therefore, they need to be synthesized in the lab by suitable approaches. These artificial oxidations result in mixtures of different lipid oxidation products that can be divided into primary (long chain) oxidation products, such as peroxy, epoxy and hydroxy compounds, and secondary (short chain) oxidation products, such as lyso lipids, aldehydes and carboxylic acids. Identification and separation of these products are challenging even if defined lipids are oxidized and require a suitable chromatographic approach coupled to mass spectrometry (MS). There are many reports on the coupling of liquid chromatography (LC) to electrospray ionization (ESI). Nevertheless, thin-layer chromatography (TLC) coupled to MS is also a promising alternative method that possesses several advantages compared to LC - such as the simple equipment. Furthermore, the reversed-phase approach of TLC (RP-TLC), which has only been sparsely used so far, seems to be superior to normal phase TLC regarding the separation of lipid oxidation products according to their functional groups and an increased number of detected oxidation products (particularly aldehydes). Lipid oxidation products of single unsaturated phospholipids but also of phospholipid mixtures can be separated in a single step.

*Speaker

# [P085] CsCl addition enhances the detection of phospholipids in crude adipose tissues extracts

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MS analysis of mixtures is normally hampered by the ion suppression effect. Regarding lipid analysis, phosphatidylcholines (PC) and sphingomyelins are more sensitively detected than other lipids. In the case of adipose tissues, however, peaks of PCs are suppressed by an excess of TAG species.
MALDI MS has a high tolerance towards salts and this enables the application of selected salts as "auxiliary agents". Selected lipid mixtures of known compositions and organic adipose tissue extracts were investigated by positive ion MALDI-TOF MS. 2,5-dihydroxybenzoic acid (DHB) in methanol, in selected cases saturated by addition of NaCl, KCl, RbCl and CsCl, was used as matrix.

Saturation of the DHB matrix with solid CsCl leads to tremendous changes: a significant reduction of the TAG signal intensities and, concomitantly, a considerable increase of the intensity of weak phospholipid peaks. The decrease of the TAG intensity is particularly caused by (a) the increased fragmentation of the corresponding alkali metal adducts and (b) the considerable size of the Cs+ ion which prevents successful analyte ionization. The effect of CsCl addition on the TAG peak intensities was observed in artificial lipid mixtures of known compositions as well as in biologically relevant lipid mixtures. Using an excessive salt concentration is important to minimize the influence of the matrix. Nearly identical data can be obtained with equimolar ratios between salt and matrix.
It was found that the addition of Cs+ represents a rapid and simple approach to overcome ion suppression effects of triacylglycerols in selected biological samples.

*Speaker

# [P086] High-throughput analysis of complex lipid extracts using supercritical fluid chromatography coupled to high-resolution mass spectrometry

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Lipids are essential cellular constituents which play a crucial role in living organisms for membrane structuration, energy storage and cellular signaling. Lipids can also be considered as disease biomarkers in particular cases. Therefore, the access to lipid profiles is mandatory. For their high-throughput analysis, the main challenge to overcome is related to their large structural diversity, leading to an important disparity in terms of physicochemical properties, which complicate their simultaneous analyses in complex extracts. Liquid Chromatography (LC) is to date the most widely used technique for lipidomics analyses, but Supercritical Fluid Chromatography (SFC) is a very promising tool to performed sensitive, rapid, low cost and environment-friendly analyses of heterogeneous samples, especially with the use of sub-2$\mu$m columns.

In this context, we have developed a robust method, allowing the separation and identification of numerous classes of lipids present in complex biological samples using SFC coupled to high-resolution mass spectrometry (SFC-ESI-Q-TOF). In order to optimize the separation and detection, different stationary phases, modifiers, make-up solvents and ionization parameters have been investigated. Importantly, the use of a particular SFC-MS splitter has allowed a significant peak sharpening increasing the overall resolution and sensitivity.

Using this optimized method, 9 classes of lipid have been detected and separated in a human plasma extract and 11 classes in a brown algae extract, belonging to glycerolipids (TG, DG, MG, DGTS, MGDG, SQDG and DGDG), glycerophospholipids (PC, LPC, PG, PI), sphingolipids (Cer, SM), sterol (CE) and fatty acyls (FA) within a short acquisition time (less than 9 min).

*Speaker

# [P087] Absolute quantification of neuronal lipids using deuterated standards and short-acyl chain analogues

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Transfer of information through membranes can be accomplished by membrane fusion, e.g. in neuronal synapses during neurotransmitter release. Membrane proteins and their interplay with specific lipids trigger this process. However, the role of these lipids is poorly understood. Therefore, we studied neuronal lipids by mass spectrometry to establish a reliable quantification workflow in neuronal membranes. Standard lipids representing various neuronal lipid classes were subject to high-energy collisional dissociation (HCD). The performance of deuterated lipids for quantification purposes was examined by titration and isotopic cluster simulation for overlapping peak envelopes of unsaturated lipid isomers. The performance of short-acyl chain phospholipids for quantification was also tested .

We found that relative quantification by isotopic cluster simulation proofed reliable and was integrated in our quantification approach allowing quantification of overlapping unsaturated lipid isomers. Absolute quantification by spiking natural phospholipid mixtures with varying amounts of deuterated standard lipids was linear over a broad concentration range. For this, we first employed Phosphatidylglycerols (PG) and Phosphatidylserine (PS) with different fatty acid chain length and number of double bonds to show that chain length and saturation has no effect on the linearity of the titration curve. Finally, we showed that the application of short-chain lipid isomers, e.g. PG(8:0/8:0) can be used to absolutely quantify the composition of complex lipid mixtures.

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# [P088] Quantitation of Lipid Mediators and Lipidome Profiling within one LC-MS/MS Approach

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Recently, we developed a SPE-free approach to quantify lipid mediators (LM) utilizing Parallel Reaction Monitoring on a Q Exactive Plus instrument. The acquired full MS2 spectra were used for both, a quality control applying the dedicated spectral comparison score (SCS) and quantitation utilizing specific fragment ions. Experimental data indicate that a specific wash step is required to elute remaining membrane lipids and neutral lipids to maintain column performance. Therefore, a direct combination of LM quantitation and lipid profiling on basis of LC retention and accurate mass determination is possible. Generally, first experiments show that all major abundant glycerophospholipid classes like phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol and phosphatidylethanolamine (PE) are accessible as well as their lysoforms. Moreover, after a switch to positive ion mode neutral lipids like DAGs, TAGs, cholesterol and cholesterolesters can be detected. Applying specific retention time windows, LipidXplorer analysis were automatized to obtain lipidome snapshots. Following this strategy, we could decrease sample amount requirements and increase overall throughput. We applied this strategy to monitor the LM responses during *M. tuberculosis* infection and determine lipid metabolic perturbations in the mouse lung tissue.

*Speaker

# [P089] Wolfgang Paul Study Award for best Master's thesis: Characterization of the cuticular lipid profiles of parasitoid jewel wasps (Nasonia) by means of laser desorption/ionisation mass spectrometry

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The cuticules of insects are covered with a blend of cuticular hydrocarbons (CHC). In addition to serving as desiccation and infection barriers, individual compounds or blends act as chemical communication signals (e.g. sex pheromones). The standard method to analyze the CHC composition is GC/MS. In particular, EI-GC/MS offers the ability to structurally characterize CHCs (e.g. branching positions). However, CHCs with chain lengths > C40 and polar compounds are difficult to detect using GC/MS.
Here we applied two recently developed UV-laser mass spectrometry based techniques to characterize the CHC-profiles of three species of parasitoid *Nasonia* jewel wasps (*N. vitripennis, N. giraulti, N. longicornus).* Direct UV-laser desorption/ionization (LDI) [1] enabled the analysis of CHC profiles directly from the surface of individual animals. This method revealed several previously uncharacterized alkenes in addition to previously described ones [3] and a new series of very long chain alkanes (C41-C52).

To evaluate species, sex, age, and mating status-dependent differences, we applied our recently introduced Ag-LDI-MS-based method, where small amounts of heptane extracts from cuticle are spotted onto etched silver substrates. PCA of the generated [M+Ag]+ signals enabled differentiation between the three species, which were congruent with the known phylogenetic relationship. In particular, sex-specific differences in the production of the newly discovered CHCs were evident.

In conclusion, use of direct UV-LDI and Ag-LDI-MS in combination with PCA provides a powerful complementary tool for CHC analysis.
Yew *et al.*, *Curr. Biol.* (2009); [2] Schnapp *et al., Methods* (2016); [3] Buellesbach *et al., J. Evol. Biol.* (2013)

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# [P090] Steps toward QMALDI-MSI: Use of HILIC-based nano-HPLC-ESI mass spectrometry for phospholipid quantification in (micro-dissected) tissue sections

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Phospholipids (PL) exhibit numerous essential physiological functions. For example, they are the main structural elements of cell membranes and certain PLs serve as signaling molecules. This multitude of functions comes with a high structural diversity and sizable differences in expression levels. Exact knowledge about the PL composition in tissues provides information about health and disease states. This is one reason, why determination of the "lipidome" by dedicated LC-MS approaches finds a tremendous interest in the biomedical community. However, these methods are only established for standard LC-MS set-ups requiring larger quantities of material. MALDI-MSI imaging can provide qualitative information about the distribution of PLs in thin tissue sections, but then quantitation is severely hampered by ion suppression effects. Here we report about on-going work aiming at improving quantitative (Q-)MALDI-MSI by combining it with nano-HPLC-ESI-MS of extracts, obtained from small amounts of raw material (e.g., micro-dissected pieces of tissue sections). An Ultimate 3000 (Dionex) was used as HPLC system and coupled to a MALDI/ESI injector (Spectroglyph) mounted on a QExactive Plus Orbitrap. Different nano-LC columns and liquid phases were tested. So far, best results were obtained with DIOL-HILIC column as stationary phase. In this combination, all major PLs classes are efficiently fractionated, while the high mass resolving power and MS/MS capability of the orbitrap ensures differentiation of PLs according to their overall alkyl chain composition. Because, ion suppression is mainly mediated by the PL headgroups, only few exogenous PL standards per PL class are needed for quantification.

*Speaker

# [P091] 3D-Kendrick mass plots as a graphical analysis tool for lipid identification in complex matrices, utilizing liquid chromatography-high resolution mass spectrometry

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Recent technical advances regarding liquid chromatography and high resolution mass spectrometry enable the mapping of the lipidome of an organism with short data acquisition times and without resource and time-consuming derivatization steps. However, the interpretation and evaluation of the resulting multidimensional datasets are challenging and still the bottleneck regarding overall analysis times. Therefore, a novel tool for computational analysis of HRMS/MS has been developed and implemented in MZmine 2, using the Kendrick mass defect in combination with chromatographic characteristics for graphical analysis and visualization.
To demonstrate the developed tools, occurring phospho- and glycolipid classes of the green alga *Chlamydomonas reinhardtii* have been separated using HILIC. Phospho- and glycolipid species have been identified by exact mass database matching and graphical 3D-Kendrick mass plot analysis. LC-HRMS/MS datasets have been processed utilizing an optimized MZmine 2 workflow for feature list generation. Database matching was achieved by extending the already existing MZmine 2 module 'glycerophospholipid search' by adding phospho- and glycolipid classes (glycerophosphoglycerol, monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol). Furthermore, graphical analysis has been carried out by implementing a new visualization module in MZmine 2 named 'Kendrick mass plots', which adds chromatographic characteristics, such as retention time, to a classic Kendrick mass plot in form of a heatmap. The developed visualization module will be featured in a future update of MZmine 2.
Using the implemented analysis tools in combination with an optimized MZmine 2 workflow has accelerated the identification of various phospho- and glycolipid species in *Chlamydomonas reinhardtii.*

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# [P092] In-vivo study of GW501516 metabolism enables detection of its prohibited use in horse for doping control

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According to the International Federation of Horseracing Authorities (IFHA), the use of peroxisome proliferator activated receptor (PPAR)-$\delta$ agonists is forbidden at any time in a horse career. Indeed, PPAR-$\delta$ agonists improve athletic performances by modulating gene expression involved in oxidative metabolism and lipid consumption.
GW501516 is a PPAR-$\delta$ agonist developed in the 1990s to treat metabolic and cardiovascular diseases. Its clinical developments have been stopped because of tumor developments in rodents. However, GW501516, also named "Endurobol" or "Cardarine", is easily available on internet or the black market and is considered as the "future" of anabolic steroids.

To detect the administration of GW501516, we studied its metabolism in horse to identify and characterize the most relevant metabolites to target in urine and plasma. *In-vivo* study was performed using an oral administration (single dose 0.15 mg/kg to one horse). Urine and plasma samples were analyzed by LC-HRMS2 and LC-SRM after SPE-C18 sample prep.

This *in-vivo* experimentation in horse has shown the presence of the native molecule and two metabolites (sulfoxide/sulfone metabolites) in both urine and plasma. Our results reveal that the analysis of GW501516 metabolites in urine provides a larger detection time and consequently, should be the preferred strategy for doping control.
In accordance with this result, an analytical method was validated to detect the use of GW501516 based on its metabolites in urine. This enabled the Laboratoire des Courses Hippiques to report a case of a fraudulent administration of this forbidden molecule in a post-race control.

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# [P093] Separation of Isomeric Steroids by Trapping Ion Mobility Spectrometry (TIMS) - ToF

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A long-standing challenge for anti-doping laboratories is the ability to distinguish between structurally-similar isomeric dopants. Trapping ion mobility spectrometry (TIMS) is a recently introduced technology that is allowing ion mobility separations at substantially higher resolving powers than were previously accessible. Despite difficulties encountered using established tri-wave or drift tube ion mobility approaches, the advent of TIMS technology brings new hope to the possibility of achieving ion mobility separations of small molecule isomers, including sports dopants. We have found advantages to the use of anion attachment to form negative ion adducts of steroid isomers (identical m/z values) that may be separated by TIMS. In certain cases, separations of isomeric anionic adducts are observed at substantially higher resolving powers compared to the analogous MH+ pairs. We report here the attainment of peak resolving powers as high as 139 in ion mobilograms for chloride adducts of steroid pairs. Baseline separation of [M+ Cl]- adducts was obtained for the isomeric steroids prednisolone and cortisone. Interestingly, separation of the MH+ forms of these same two steroids was not achieved, even after extensive tuning and optimization. However, ion mobility separation of the [2M+H]+ species was obtained, a finding that complements recent results published by the Yost group wherein steroid epimer separation was achieved for sodiated dimer species. Computational energy-minimization (M06-2X/6-31G(d,p)) of [M + Cl]- adducts showed that [prednisolone + Cl]- can exist in a more compact form than [cortisone + Cl]- which rationalizes the former's higher mobility, and allows for baseline separation of these isomers.

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# [P094] Identification of Commercial and Military Explosives by Means of HPLC-HRMS

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Forensic scientists constantly develop improved methods for the detection and identification of hazardous materials employed by criminals and terrorists. In this field, explosive compounds are of particular interest, due to their destructive potential. The identification of confiscated explosives that do not appear to be homemade is an important step in uncovering distribution pathways of these materials. Commercial explosives formulations pose highly diverse matrices, which do not allow for a straightforward sample preparation and analysis. Earlier approaches therefore mainly focused on a few selected compounds, in order to identify these complex mixtures. More recently powerful statistical methods have enabled scientists to identify differences between a large number of samples, while taking into account more data. In this work high performance liquid chromatography (HPLC) in combination with high resolution mass spectrometry (HR-MS) was employed to analyse commercially available explosives. A relatively simple and effective dissolution and extraction strategy was developed to minimise possible sources of error. Several commercial explosives were analysed regarding their composition with a focus on explosive compounds as well as by-products and additives. Molecular formulae for various additives and by-products could be derived from exact masses, taken from HR-MS spectra. Pronounced differences in the amount and character of components between a set of samples could be shown, and thus a differentiation was possible.

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# [P095] Steroid Analysis in Sports Drug Testing - Friend or Foe

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Sports drug testing predominantly relies on chromatographic-mass spectrometric approaches, amongst which steroid analysis has been critical on numerous counts. Anabolic agents have been the most frequently detected class of prohibited substances for decades in routine doping controls, which highlights the importance of sensitive and comprehensive analytical methods covering a broad range of synthetic anabolic steroids. In addition, quantifying natural "endogenous" steroids and generating so-called steroid profiles for athletes (forming the steroidal module of the Athlete Biological Passport) allows detecting the misuse of substances such as testosterone. Moreover, steroid profiling has demonstrated great utility as analytical tool in cases of sample manipulation and, more recently, it's value as diagnostic instrument concerning pituitary adenoma was identified. In this presentation, three rather diverse examples of steroid analysis in sports drug testing are presented including the identification of sample manipulation, the indication of pituitary irregularities in an athlete, and sophisticated approaches towards the determination of long-term metabolites of new steroidal anabolic agents by means of chromatographic-mass spectrometric means. GC-MS/MS, LC-MS(/HRMS), GC-HRMS, and GC/C/IRMS are employed to provide the dataset providing sufficient information for specific drug metabolite identification, quantification, and characterization.

*Speaker

# [P096] Quantitative clinical toxicological screening comparing library ID from product ion scan MS/MS to MRM Spectrum mode ID

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Forensic toxicological sample measurement is commonly performed in a targeted analysis on selected panels of compounds. When using triple quadrupole platforms for analysis, typically two MRMs are used for compound measurement with a quantifier ion transition and reference ion transition. To help reduce false positive and false negative reporting two alternative approaches have been considered: MRM triggered product ion spectrum and MRM Spectrum mode. MRM Spectrum mode acquires a high number of fragment ion transitions for each target compound generating a fragmentation spectra that could be used in routine library searching and compound verification using reference library match scores. A generic method was developed for clinical toxicology and forensic analysis using a QuEChERS sample preparation method, a single LC analysis and methods for product ion spectrum identification. By combining MRM quantifier ions with either MRM or scanning product ion scan data both MS/MS methods result in higher confidence in compound identification as a result of library searching with robust quantitative data. Library identification added increased confidence to compound identification in situations where reference ion ratios were outside method tolerances or if concentrations measured were below or above LLOQ or ULOQ. Both MRM triggered product ion spectrum mode and MRM Spectrum mode generate quantitative data in agreement to a validated conventional MRM method.

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# [P097] AdipoR agonists and their implementation into sports drug testing

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Adiponectin receptor (AdipoR) agonists are substances with similar physiological effects as adiponectin, an adipokine with antidiabetic and antiatherogenic effects. Adiponectin also decreases insulin resistance caused by obesity, supports fatty acid utilization, reduces triglyceride content in cell and has anti-inflammatory effects. Additionally, adiponectin has proven to increase the mitochondrial DNA content and oxidative metabolism. Substances exhibiting the latter effects are also referred to as exercise mimetics.
AdipoRon and 112254 are two known adiponectin receptor agonists. These substances can potentially be abused by athletes for performance enhancing purposes, since they prove to have similar effects to adiponectin. As part of preventive doping research efforts, it is indispensable to be able to detect AdipoRon and 112254 in routine doping control with regard to the implied so called pseudo-exercise effects.

The adipoR agonists were synthesised and characterised by means of LC-MS/MS and $MS^3$-methods. Additionally, both *in vitro* and *in vivo* studies were conducted for the prediction of potential metabolites in human urine. Two monohydroxylated compounds found both *in vitro* and *in vivo* were synthesised and characterised using tandem-MS methods.
Matrices used for the detection of these substances were human blood plasma, urine and whole-blood by means of dried blood spots. While human plasma and urine are well-established matrices in doping control analysis, DBS analysis is a relatively new, easy und promising way of sampling and sample analysis. All methods were validated according to the guidelines of the World Anti-Doping Agency and have shown to be fit-for-purpose for use in routine doping control analysis.

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# [P098] Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System

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Quadrupole Time-of-flight mass spectrometry (QTOF-MS) provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. This is an ideal approach for forensic toxicology screening where unknown compounds in complex samples must be identified from information-rich data sets. The SCIEX X500R Q-TOF system provides the capability of switching between MS and MS/MS scans instantly, enabling the fast acquisition of detailed structural information for easier compound identification. Designed for routine, use, the benchtop SCIEX X500R QTOF system also provides the capacity for high-specificity, targeted quantitation as well as for non-targeted screening from single sample sets in a daily laboratory environment. Because of its straightforward design and intuitive software workflows, non-targeted data obtained on the X500R can be retrospectively mined for additional analytes missed in initial screens, which is important with the constant emergence of new synthetic drugs. Also, the ability of retrospective analysis on X500R has become increasingly popular in forensic work.

Herein, we present a single-injection method for screening 664 most up-to-date forensic compounds using the SCIEX X500R QTOF system and the SCIEX OS 2.0 software. The obtained data provides both structural information and retention times for enhanced identification accuracy, especially for structurally similar isomers. Sample preparation procedures for urine and whole blood samples, as well as library-search settings recommended here can help automate and confidently establish the identification of unknowns in an efficient, all-in-one workflow.

*Speaker

# [P099] Probing for mitochondrion derived peptide MOTS-c in human blood employing HPLC-US-MS/MS for doping control purposes

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In the field of preventive anti-doping research the development and improvement of detection methods regarding new substances with potential performance enhancing properties is of utmost importance. As peptidic hormones are an emerging class of illegally misused drugs, existing testing procedures were consecutively adapted as by the present study. Herein, the qualitative identification of the mitochondrion derived peptide named MOTS-c (Mitochondrial Open Reading Frame of the 12S rRNA type-c) by means of high performance liquid chromatography coupled via UniSpray$^{TM}$ Ionization to triple quadrupole tandem mass spectrometry (HPLC-US-QqQ-MS/MS) is demonstrated. Since MOTSc was discovered in 2015, it is known to regulate insulin sensitivity and metabolic homeostasis on a cellular level. As the skeletal muscle was observed to be the preferred target organ, MOTS-c is leading to AICAR accumulation and consequently AMPK activation. Levels of endogenous MOTS-c in plasma are described at about 200 pg/mL in the literature so that concentrations after administration are expected to be significantly higher. Within this assay, commercially available human MOTS-c and further animal analogues were extracted and purified from human plasma by protein precipitation and detected as multiply charged intact peptides after reversed phase chromatographic separation. Furthermore, *in vitro* generated metabolites were investigated and characterized by high-resolution MS/MS dissociation in consideration of degradation products due to metabolic processes.

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# [P100] Determination of Sotatercept in dried blood spots for doping control analysis by means of LC-HRMS

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Sotatercept (ACE-011) is a therapeutic fusion protein originally developed for osteoporosis therapy, which was also found to have an erythropoiesis stimulating effect. An elevated aerobic capacity due to increased red blood cell mass may lead to performance enhancement in sports. The protein dimer is composed of the extracellular domain of the human activin receptor type IIA (ActRIIA) and the Fc domain of human IgG1. Soluble Sotatercept competes with the membrane-bound ActRIIA for binding activin a and other members of the transforming growth factor beta superfamily, thus inhibiting downstream signaling cascades. Dried blood spot (DBS) sampling is considered a promising alternative to normal blood sampling for doping control purposes due to minimal-invasiveness, reduced costs, robustness and improved storage and shipment conditions. Herein, a sensitive detection method for Sotatercept in DBS was developed. The procedure was adapted from a recently published detection method for Sotatercept in conventional serum samples. It comprises a protein extraction from the DBS card with a minimal volume of 20 $\mu$L of dried blood, protein precipitation, affinity purification and enzymatic digestion. The assay was optimized in all parameters and comprehensively characterized. Proteolytic peptides from the ActRIIA and Fc domain unambiguously prove the presence of Sotatercept by liquid chromatography-high resolution mass spectrometry. For doping control analysis, the determination of other therapeutic proteins such as Fc fusion proteins or therapeutic antibodies in DBS can be realized adopting this approach.

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# [P101] Analysis of volatiles in fire debris by combination of activated charcoal strips (ACS) and automated thermal desorption – gas chromatography-mass spectrometry (ATD/GC-MS)

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**Aims:** The chemical analysis of fire debris for traces of accelerants can help investigators determine whether the fire was the result of an arsonist. We have developed a screening method combining automated thermo-desorption (ATD) of a small piece of activated charcoal strip (ACS) and GC-MS analysis. This technique was compared with the generally used desorption of ACS with pentane.
Method: Evidence samples collected by the police and different fire accelerants were analyzed. Samples were heated in glass jars with ACS at 60∘C for 16 hours. A fraction (ca. 4 mm2) of the ACS was then placed in a glass tube for ATD. Compounds were concentrated on a cold trap, desorbed and carried to GC-MS. Another fraction of the same ACS was extracted with 200 $\mu$L pentane, 0.5 $\mu$L were injected into the GC-MS system.

**Results:** Both methods allow the detection of medium sized n-alkanes (C6 – C16) and aromatic hydrocarbons. Heavy compounds (n-alkanes above C22, such as paraffin) could only be detected after solvent desorption. However, ATD of the ACS allows the detection of very small sized compounds such as ethanol, while this is not possible by solvent desorption due to the solvent delay for analysis by GC-MS. The detection of terpenes, which is important for the identification of wood residues, was only possible by ATD.
**Conclusion:** ACS ATD-GC-MS has proven to be a sensitive and rapid procedure for the detection of ignitable liquids in fire debris samples and an attractive alternative to the widely used solvent desorption of ACS.

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European Mass Spectrometry Conference

# EMSC 2018

Saarbrücken · Germany · 11-15 March

# Tuesday, 13 March

# Tuesday Morning
# Plenary Lectures

# Hyphenated MS in Pharmaceutical Applications: Data Independent Acquisition, Ion Mobility and Electron Based Dissociation

Anita Hidasi, Laura Akbal, Sophie Bravo-Veyrat, Michel Raetz, Thomas, Stricker and Gérard Hopfgartner

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Gérard Hopfgartner is full Professor for Analytical Sciences and Mass Spectrometry in the Department of Analytical and Inorganic Chemistry of the University of Geneva. His research interests focus around the development and application of novel mass spectrometry approaches with and without chromatography in the field of life sciences. His scientific interests include: hyphenation of separation sciences with MS, automation, bioanalysis, metabolism, metabolomics, analytical proteomics, ionization techniques, MS/MS fragmentation, ion mobility mass spectrometry and mass spectrometry imaging. He acts as an editor for Analytical Bioanalytical Chemistry and he is member of the scientific committee of the HPLC Conference Series.

**Abstract:**

The accurate and precise identification and quantification of pharmaceuticals and metabolites in biological samples relies mostly on the combination of separation sciences and mass spectrometric detection. One of the major challenges in is the chemical space and the dynamic range of the analytes of interest. Supercritical fluid chromatography (SFC) has experienced a renaissance in the last decade offering an additional separation dimension. In LC-MS the ionization conditions are predominantly controlled by the mobile phase composition, whereas in SFC-ESI/MS, the ionization can be tuned using the addition of a liquid make-up, which is independent of the chromatographic conditions. Significant improvement in MS response can be achieved when carefully selecting the make-up flow. Recent instrumental improvements in high resolution mass spectrometry (HRMS) have enabled data independent information acquisition (DIA) schemes, such as MSEverything or SWATH. With SWATH a collision induced MS/MS spectra can be generated for every precursor ion enabling simultaneous quantitative and qualitative analysis (QUAL/QUANT). Comparison with MS/MS database or the use of in-silico fragmentation tools can further improve compound and enables multianalytes quantification. However, improved selectivity, higher sample throughput or MS/MS structural information is needed. The implementing of mobility spectrometry (IMS) into the workflow as the use of electron based dissociation referred as ExD would significantly improve the performance of LC-MS analyses. In the present talk the benefits to apply multiple separation techniques (dual LC, SFC) with multiple MS and MS/MS techniques (DIA, IMS, ExD) will be presented for the analysis of pharmaceuticals and metabolites in biological samples.

# Nanocraters, soft desorption and 3D-resolved organic mass spectrometry using cluster ion beams

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**Arnaud Delcorte** is a Research Director of the Belgian National Science Foundation (FNRS) and a Professor at the University of Louvain (UCL) and University Saint-Louis (Brussels). He heads the management board of the surface characterization facility of UCL. He has been active in Secondary Ion Mass Spectrometry for about 20 years. His current research activities encompass the theoretical and experimental study of energetic cluster ion-surface interactions, with an emphasis on soft molecular emission, and of plasma-surface interactions, as well as the 2D/3D molecular characterization of surfaces. Overall, he has published 5 book chapters, 150 articles in peer-reviewed journals and has received 50 personal conference invitations. He is the secretary of the International Committee of the biennial SIMS conference, a member of the SIMS Europe Workshop Advisory Board and a member of the International Scientific Committees of the ECASIA and ISI conferences. He co-chaired the SIMS XXI international conference in Cracow (Summer 2017).

**Abstract:**

Over the past decade, secondary ion mass spectrometry (SIMS) has moved from probing and imaging surfaces with keV atomic ions to small clusters such as $SF_5^+$, $C_{60}^+$ or $Bi_n^+$ and, most recently, to nanoparticles like $Ar_n^+$ or $(H_2O)_n^+$ containing thousands of atoms. This shift in projectile size involved a shift of underlying physical processes, from linear collision cascades to collective atomic motions and macroscopic-like impact phenomena, and a step change in performance for the chemical and molecular analysis of solid samples at the nanoscale.

In this contribution, I first use our results of molecular dynamics (MD) simulations and experiments [1] to review the changes associated to this increase in projectile size, which translates in ever smaller projectile atom energies. The analytical consequences are illustrated, e.g. the possibility of soft desorption-ionization, “damageless” depth-profiling (Fig.1a,b) and 3D molecular imaging, giving unprecedented information on the chemistry of the top nanometers of organic films [2]. In addition, the MD models quantitatively describe the variations of sputter yield as a function of cluster size and energy, and serve as a guide for future developments [3]. The emission yield dependence on the molecular size and substrate nature are also observed in the experiments and explained by the models. While the issue of damage in sensitive molecular and organic materials (fragmentation and cross-linking, Fig.1a,c [4]) could be essentially

alleviated by using large $Ar_n^+$ clusters, two other important challenges are now being tackled. First, the very large sputter yield difference between inorganic and organic materials hampers 3D analysis of hybrid organic-inorganic samples. Second, though subcellular molecular imaging with excellent mass resolution and accuracy was demonstrated [5], the molecular ion yields often remain low for high lateral resolution mapping using $Bi_n^+$ and $Ar_n^+$ projectiles. New routes for improvement are envisioned.

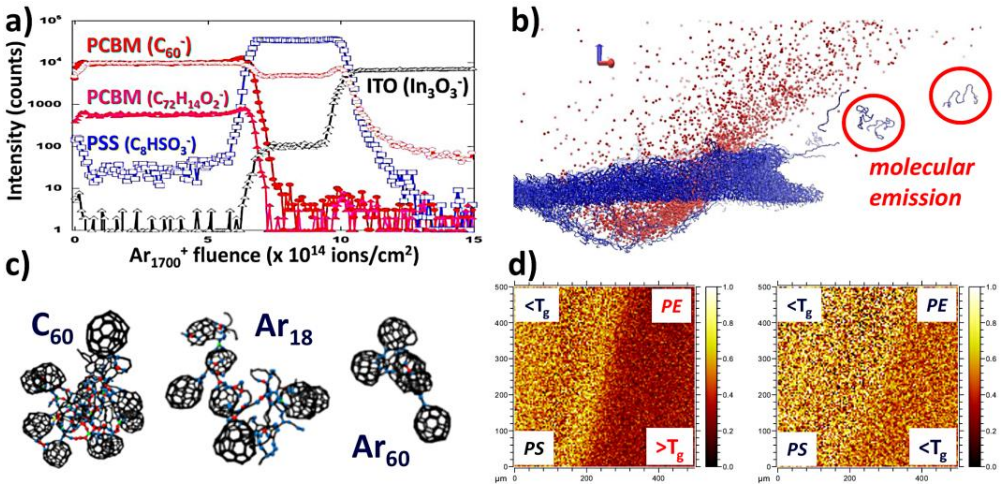


***Fig. 1. (a)*** *Molecular depth profiling of an organic photovoltaic multilayer device using $Ar_{1700}^+$ cluster sputtering.* ***(b)*** *Snapshot of the MD showing the cratering and molecular emission induced by a 5 keV $Ar_{5000}^+$ cluster in an organic sample (45° incidence).* ***(c)*** *MD Simulation of the crosslinking induced in fullerite by 2.5 keV clusters.* ***(d)*** *Images of the backscattered Ar cluster intensity ratio $Ar_2^+/(Ar_2^+ + Ar_3^+)$ for a mixed polystyrene/polyethylene surface at room temperature and at -150°C.*

Finally, I demonstrate that the backscattering of gas cluster ion fragments also provides information on the local physical properties of the bombarded organic surfaces, as illustrated with the glass transition of polymeric layers (Fig.1d) [6]. This potentially adds a new dimension to SIMS imaging with large clusters.


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# Proteomic, PTM and structural biology 1

# [Keynote] Investigation of protein-DNA interactions in chromatin assemblies with chemical cross-linking mass spectrometry

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Protein-DNA interactions mediate various cellular functions such as transcription, replication or DNA damage repair that are essential in all eukaryotic cells. A better understanding of these processes can be accomplished by studying protein-DNA complexes. Cross-linking mass spectrometry is one of the suitable methods for mapping of these interaction sites. During cross-linking, a chemical bond is formed between spatially close protein and DNA moieties, for example by UV irradiation or by using chemical cross-linkers. After enzymatic digestion of the protein and DNA moieties, the peptide-oligonucleotide cross-links have to be enriched. LC-MSMS and data analysis enable the identification of the cross-linked peptides. With this approach, we can assign DNA-binding protein domains, the cross-linked amino acids as well as the cross-linked nucleotides. UV cross-linking has a relatively low yield (around 1%), therefore, we aimed to develop a chemical cross-linking method in combination with mass spectrometrical methods for the comprehensive investigation of chromatin assemblies. During this study, we used *in vitro* reconstituted oligonucleosomal arrays as chromatin model and single linker histones with double-stranded DNA as cross-linking substrates. We identified cross-linked peptide-oligonucleotides heteroconjugates of all four core histones from the nucleosomal arrays as well as peptide-oligonucleotides of single linker histones. We further show that structural models of nucleosomes support the identified sites of interaction. Overall, our data demonstrates that chemical cross-linking mass spectrometry is a very efficient tool for characterization of protein-DNA complexes.

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# [O1] The First "Zero-Length" Mass Spectrometry-Cleavable Cross-Linker for Protein Structure Analysis

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Combining the properties of a "zero-length" cross-linker with cleavability by tandem mass spectrometry (MS/MS) poses great advantages for protein structure analysis using the cross-linking/MS approach. These include a reliable, automated data analysis and the possibility to obtain short-distance information of protein 3D-structures.
We introduce *1,1'*-carbonyldiimidazole (CDI) as an easy-to-use and commercially available, low-cost reagent that ideally fulfils these features. CDI bridges primary amines and hydroxyl groups in proteins with the lowest possible spacer length of one carbonyl unit (_˜2.6 Å). The cross-linking reaction can be conducted at physiological conditions in the pH range between 7.2 and 8. Urea and carbamate cross-linked products are cleaved upon collisional activation during MS/MS experiments generating characteristic product ions, greatly improving the unambiguous identification of cross-links. Our innovative analytical concept is exemplified and applied for bovine serum albumin (BSA), wild-type tumor suppressor p53, an intrinsically disordered protein, and retinal guanylyl cyclase activating protein-2 (GCAP-2).
Keywords: CDI, peptide-cross-linking, zero-length, CID- cleavable, p53, GCAP-2

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# [O2] timsTOF Pro with PASEF for shotgun proteomics

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**Background:** The "Parallel Accumulation - Serial Fragmentation" method (PASEF, Meier et al., JPR 2015, PMID: 26538118) for trapped ion mobility spectrometry (TIMS) coupled to a quadrupole time of flight (QTOF) instrument, has been described with the promise of achieving five to ten times faster data dependent acquisition of fragment ion spectra with improvements in sensitivity. Here we present results showing that the promise of PASEF has been realized.
**Material & Methods:** The performance of a timsTOF instrument with PASEF for shotgun proteomics has been evaluated by using tryptic digests of human cancer cell lysates (HeLa) spiked or not with an UPS peptide mixture, and separated by 90 min nanoLC gradients. Data were analyzed using DataAnalysis (Bruker), Mascot (www.matrixscience.com), and MaxQuant (Cox group, MPI of Biochemistry) or PEAKS (Bioinformatics Solutions).

**Results:** A standard 1.1 second PASEF acquisition cycle, which performs over 120 MS/MS at high sensitivity, identifies over 2900 protein groups from a 12 ng injection of a Hela cell digest separated with a 60 min gradient, and over 4800 protein groups from a 100 ng injection with the same gradient. In parallel, ion mobility separation allows to separate isobaric co-eluted peptides prior to fragmentation, further increasing the ID rate.

**Conclusion:** By enabling data dependent acquisition at very high speeds with improved sensitivity, the timsTOF Pro with PASEF enables researchers to dig deeper into the proteome, using less sample, yet finding more proteins of biological relevance.

*Speaker

# [O3] Absolute quantification of metabolic enzymes in C. elegans

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Studying metabolic pathways is key to understand bioenergetics of a cell. They are controlled and coordinated by various group of enzymes to regulate flow of energy. Substantial progress were made in identification of pathways and deducing their regulatory patterns but very less is known about the quantitative aspects of their components. Previously, pathways were quantitatively studied by correlating gene expression profiles (microarray or mRNAseq) to protein abundances. Owing to poor congruence between them, knowing absolute amount of proteins can contribute to better elucidation of pathways. To derive absolute molar amounts we used a novel MS Western method developed by Kumar *et al,* 2017 and understand how metabolic shift happens in *C. elegans.*
*C. elegans* are free living nematodes which can adapt to harsh conditions by tweaking its metabolism. They go through various larval developmental stages having distinct metabolic signature for each. Out of all stages metabolic rewiring is important when they enter a dormant stage called dauer. They rely on stored energy reserves to survive stress conditions and revert back to reproductive development when conditions gets suitable. Dauer worms switch to gluconeogenic mode by increasing gluconeogenic specific enzymes.

We quantified 53 metabolic enzymes spanning glycolysis and TCA cycle with a CV$<$ 10. With absolute molar amounts we were able to derive subunit stoichiometry of protein complex and ratio of different enzymes within a pathway. We also observed isoform dependency in rewiring metabolism.

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# Clinical toxicology and forensics

# [Keynote] Development and validation of a multidimensional gas chromatography/ combustion/ isotope ratio mass spectrometry method for the investigation of carbon isotope ratios of urinary steroids in sports drug testing

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The steroid hormone testosterone is misused for performance enhancement in sports and its administration is prohibited by the World Anti-Doping Agency. Testosterone is also produced endogenously in the human organism.
Endogenous and exogenous testosterone together with their metabolites can be unambiguously distinguished by carbon isotope ratios if compared to endogenous reference compounds.

Established isotope ratio mass spectrometry methods for analyzing urinary steroids for doping control purposes consist of two time-consuming HPLC purification steps to achieve the required purity of all analytes. In order to accelerate the sample preparation, multidimensional gas chromatography was applied. This technique is known to be suitable for the separation of complex matrices. Multidimensional gas chromatography consists of two gas chromatographs connected by a pressure-controlled heart-cutting device. In the first dimension, a less polar column was installed for peak purification. In the second dimension, separation was obtained employing a column of medium polarity. Retention time stability and cutting windows were monitored by a flame ionization detector. Detection was performed simultaneously by isotope ratio mass spectrometry and a single quadrupole mass spectrometer for structural confirmation and assessment of peak purity.
The method was validated according to the guidelines of the World Anti-Doping Agency and by means of linear mixing model considering the parameters linear range, limit of quantification, intra- and interday precision, accuracy and specificity. Additionally, a reference population (n=74) was investigated together with an excretion study as proof-of-concept. The results demonstrate that the method is fit for purpose for an application in routine doping control analysis.

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# [O1] Detection and Identification of Performance Additives in Fuels for Forensic Applications

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The identification and comparison of ignitable liquids are the main tasks of forensic fire debris analysis. Gasoline and to a lesser extend diesel are most commonly used for arson. Modern fuels derive a significant part of their performance from the addition of small amounts of chemicals, also referred to as performance additives. In addition identification of fuel performance additives gives a new valuable parameter for forensic fuel identification and sample comparison, especially in complex case situations such as highly burnt samples, as well as additional forensic intelligence by potentially establishing correlations between crime scenes, filling stations and suspects.

In order to detect these additives a simple sample preparation method and new analytical methods based on LC-MS were developed. 93 gasolines from Germany, 33 gasolines from UK and 126 gasolines from 15 different European countries were analyzed and the corresponding mass spectrometric data evaluated. Only a small number of diesel fuels have been investigated.

The results show that characteristic polymeric distributions of performance additives are observed between fuels of various brands, qualities and areas. Obtained results demonstrate that, brand identification of the globally operating oil companies is possible in Europe. Only a limited identification of the gasoline quality is possible if no quality specific characteristics can be identified.

Due to the high complexity of the recorded data sets not only four different databases have been created, but in addition a concept of data analysis has been developed.

Fuel additive analysis promises new forensic intelligence and can provide additional information for casework.

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# [O2] Quantitation of Acetaminophen Covalent Protein Binding in Vivo by LC-MS/MS

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**Background.** Acetaminophen (APAP) is one of the most commonly used analgesic and antipyretic compounds worldwide. However, it is linked to hepatic necrosis, leading to acute liver failure (ALF) and, in extreme cases, death. APAP is metabolized into a reactive metabolite, *N*-acetyl *p*-benzoquinone imine (NAPQI), which covalently binds to proteins. We have developed a fast and sensitive assay to accurately quantify NAPQI-modified human serum albumin (HSA) as a biomarker for APAP-related hepatotoxicity.
**Methods.** This newly developed assay employs peptic digestion of serum, isotope dilution and solid phase extraction, coupled to LC-MS/MS analysis in MRM mode. The calibration curve is based on a surrogate protein standard, which after digestion yields a positional isomer to the target modified analyte peptide. A deuterated isotope labeled internal standard was used. Finally, absolute quantitation was performed by LC-MS/MS, and the final method was applied to evaluate calibration standards, quality control samples and patient samples.

**Results.** The assay yielded high accuracies and precisions in the linear range of 0.11-50.13 nmol/mL for serum NAPQI-HSA. The validated LC-MS/MS assay was successfully applied to serum samples of patients suffering from APAP-induced ALF. Cohorts of non-spontaneous survivors and individuals with irreversible liver damage showed statistical significant difference in assessed NAPQI-HSA serum levels ($p$-value = 0.028), demonstrating the power of this assay.
**Conclusions.** We have developed a fast and sensitive assay to accurately monitor APAP-related covalent binding in human serum. This study represents the first absolute quantitation of a modified protein in human patient samples.

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# [O3] Quantitative Lipidomic Fingerprinting for Antiepileptic Drug Monitoring

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*BACKGROUND.* Quantitative LC/MS methods are increasingly used to identify disease-related changes of the lipidome. Imbalanced lipid metabolism is associated with neuropathological conditions, including epilepsy. The lack of effective anti-epileptic therapies targeting molecular underlying mechanisms and multidrug-resistance highlight the need to discover new therapeutic targets and improve disease management. Recent research evidenced antiepileptic and neuroprotective properties of Palmitoyelthanolamide (PEA). Discovery of epilepsy-type specific lipid fingerprints gives opportunity to define markers for prediction, follow-up monitoring, and distinction of multi-drug resistant patients. Therefore, we aim at spatiotemporal assessment of lipid level alterations in brain and periphery of i) PEA-treated, ii) untreated epileptic mice and iii) controls.
*METHODS.* Co-extraction and multiplex quantification using LC/MRM with polarity switching of endocannabinoids and eicosanoids in hippocampus and plasma of treated, untreated epileptic mice and controls.

*OUTCOME.* Profiling of 10 lipid mediators at 4 time points of acute epilepsy revealed distinct lipid level alterations associated with seizure progression of epileptic mice with vs. without PEA treatment.

*PERSPECTIVES.* Lipids undergoing significant temporal changes serve as set of potential lipid markers for perspective studies aiming at antiepileptic strategies improvement.

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Lerner R, Post JM, Ellis SR, Vos DRN, Heeren RMA, Lutz B, Bindila L (2017) Simultaneous lipidomic and transcriptomic profiling in mouse brain punches of acute epileptic seizure model compared to controls. *J Lipid Res.*

*Speaker

# Petroleomics and synthetic polymers

# [Keynote] How primary amine commonly used as dopant can be useful analytical tool for the semi-targeted analysis of complex matrixes by ESI FT-ICR MS

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Ammonia is a commonly used deprotonation agent in negative-ion electrospray ionization mass spectrometry (ESI-MS). But this primary amine is also reactive towards carbonyl compounds (aldehyde, ketone) to form imine. As part of sample study, it may skew its composition description. This is of significant importance in the study of complex mixture such as oil or bio-oil. To assess the ability of primary amines to form imines with carbonyl compounds during the ESI-MS process, different carbonyl standards (vanillin, cinnamaldehyde, butyrophenone, and trihydroxyacetophenone) have been infused in an ESI source with ammonia or different amines (aniline and 3-chloronaniline). The (+) ESI-MS analyses have demonstrated the formation of imine, whatever the considered carbonyl compound and the used primary amine, which structure was extensively studied by tandem mass spectrometry. Thus, it has been established that the addition of ammonia, in the infused solution, may alter the composition description of a complex mixture and leads to misinterpretations due to the formation of imines. Nevertheless, this experimental bias can be used to identify the carbonyl compounds in complex matrixes such as pyrolysis bio-oil. Indeed, infusion of the bio-oil with 3-chloroaniline in ESI source leads to specifically derivatized carbonyl compounds. Thanks to their chlorine isotopic pattern and the high mass measurement accuracy, (+) ESI Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) unambiguously highlighted them from the numerous CxHyOz bio-oil components. These results offer a new perspective into the detailed molecular structure of complex mixtures such as bio-oils.

*Speaker

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# [O1] Comprehensive mass spectrometric evolved gas analysis in the context of Petroleomics

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Petroleomic materials remain to be one of the greatest challenges in chemical analysis. The conversion of heavy oils or exploration of alternative sources raises new challenging issues, such as formation of deposits or efficient processing of residues. Thermal analysis using evolved gas analysis is one powerful approach for deciphering these materials.
For this purpose, several thermal analysis systems coupled to different mass spectrometric analysers, such as ultra-high resolution FT-MS and TOF-MS, were applied to study low-viscous and solid petroleomic materials. The setups varied in ionisation techniques covering soft vacuum and atmospheric pressure approaches, and hard electron impact allowing in combination an in depth-chemical description of the desorbed and pyrolysed material.

Besides the description of heavy oils and bitumen, asphaltenes were in the primary focus of the study. Generally, for the intended pyrolysis of the asphaltenes, an ultra-complex molecular pattern is revealed. This molecular space could be comprehensively analysed by ultra-high resolution mass spectrometry equipped with atmospheric pressure chemical and photo ionisation revealing the highly aromatic and sulphur-containing core structures, whereas vacuum single photon ionisation was able to analyse the thermally fragmented side chain distribution. Moreover, a vacuum direct inlet probe equipped with electron ionisation was able to broaden the picture by evaporating larger components prior thermal decomposition and thereby allow for additional structural information.
Summarising, mass spectrometric evolved gas analysis has shown to be an efficient technique for Petroleomics. Most importantly the data combination of various setups enabled an in-depth chemical and structural analysis and hypothesising of macromolecular structural features.

*Speaker
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# [O2] Graphical Decoding of Messages Stored in Sequence-Controlled Synthetic Polymers using Kendrick Mass Defect Analysis

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Kendrick mass defect (KMD) plots are used to allow fast and extremely useful visualization of many structural features in complex mass spectra. Mostly used in petroleomics, this methodology was extended to polymer mass data by using the repeating unit of polymers as the base unit for the calculation of the Kendrick mass[1]. Accordingly, a blend of homopolymers containing the same monomer but different terminations can be readily deciphered in KMD plots displaying as many horizontal lines as species in the sample. In contrast, MS data of polymers composed of different units compared to that used as the base unit line up in an oblique direction.
The assets of these horizontal/oblique lines can be extended to decrypt MS/MS data aimed at reading messages encoded in sequence-controlled synthetic polymers[2]. These macromolecules are composed of uniform chains with a precise location of co-monomers units, intentionally defined as 0-bit and 1-bit to store information at the molecular level. As co-monomers have different masses, so-encoded messages can be readily deciphered by MS/MS sequencing[3].

In this study, performance of KMD analysis were hence evaluated to improve the decoding step of messages encrypted in two families of sequence-controlled synthetic polymers, namely, polyurethanes and poly(alkoxyamine phosphodiester)s. Using fractional base units [4] to improve data point resolution, KMD allowed automatic and graphical decoding of digital messages stored in these polymeric media.

*J. Am. Soc. Mass Spectrom.* 25 (2014), 1346–1355.

*Nat. Rev. Mater.* 1 (2016), 16024.

*Macromolecules* 48 (2015), 4319-4328.

*Mass Spectrom.* 6 (2017), A0055.

*Speaker

# [O3] Characterization of a complex polymer mixture by Atmospheric Solid Analysis Probe (ASAP) MS combined with Kendrick mass defect analysis

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Fluorinated polymers are a diverse and important class of polymers with unique applications. However, characterization of fluorinated polymers by conventional mass spectrometric methods is challenging because: i) the high fluorine content makes them insoluble, or only sparingly soluble, in most common solvents; and ii) common matrices used in MALDI do not desorb/ionize them efficiently. In this work, Atmospheric Solid Analysis Probe (ASAP) high resolution OrbitrapTM mass spectrometry (HRMS) was used as a new tool for the molecular characterization of various fluorinated polymers including polyvinylidene fluoride (PVDF), and fluorinated copolymers containing PVDF and chlorotrifluoroethylene (KEL-F 800), or hexafluoropropylene (Viton A, Tecnoflon). In order to display graphically the distribution of the fluorinated polymers, we used a modified Kendrick mass defect (KMD) analysis. The mass scale of the KMD was set on the mass of the common vinylidene difluoride repeating unit of all fluorinated polymers under investigation. Oligomers with common repeat units lined up in the horizontal direction on the KMD plot, whereas oligomers with different structures were shifted vertically. The Kendrick maps thereby simplified mass spectral interpretation and provided confident peak assignments through homologous series recognition. A specific fingerprint for each polymer has been identified and the ability to discern the four species in a blend through KMD analysis was demonstrated. This novel combination of ASAP/Orbitrap analysis combined with KMD analysis enabled the successful discrimination of the various fluorinated polymers in a blend. We will discuss the application of this high throughput yet comprehensive method in the context of forensic chemistry.

*Speaker
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# Thermofisher Scientific Workshop

# Tuesday 12:00 – 13:00

# Room 0.06

# Shimadzu Workshop
# Tuesday 13:00 – 14:00
# Room 0.23

# Tuesday Afternoon
# Plenary Lectures

# Coupling mass spectrometry with ion mobility spectrometry, helium droplet isolation and infrared spectroscopy

Gert von Helden

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Gert von Helden is research group leader at the Fritz-Haber-Institut der Max-Planck-Gesellschaft in Berlin and a professor by special appointment at the Faculty of Science of the Radboud University, Nijmegen, the Netherlands. His research focuses on biomolecules and biomolecular complexes in the gas phase and their characterization using a variety of techniques that use and combine mass spectrometry, ion mobility spectrometry and infrared spectroscopy. For the latter, his group makes use of the tunable infrared free-electron-lasers available in Berlin and Nijmegen.

**Abstract:**

Mass-spectrometry can be coupled to infrared-spectroscopy to obtain additional information on ion structure and dynamics. In the frequently used IR multiple photon dissociation (IRMPD) approach, ions are m/z selected, irradiated by intense and tunable IR light and fragmentation is monitored as a function of IR wavelength. IRMPD spectroscopy has been proven to be a powerful, robust and fast method, however, two complications that can arise in IRMPD spectroscopy. First, different isomers or conformers may be present and the resulting spectra represent the sum of the spectra of the individual components. Second, intrinsic in the IRMPD process are line shifts and spectral broadening.

The address the question of different isomers and conformers, we constructed a setup, in which ion mobility methods are used to obtain m/z selected ions of defined shape, which are then further investigated by IR spectroscopy. The approach has been applied to a variety of biomolecules, ranging from amino acids to proteins as well as clusters, ranging from inorganic clusters to peptide aggregates.

To avoid line shifts and broadening, IR spectroscopy on cold ions can be performed. To do so, m/z selected ions are captured in liquid helium droplets prior to IR spectroscopic investigation. The conditions inside a helium droplet are isothermal at 0.38 K and the interaction between the helium matrix and the molecules are weak so that only small perturbations on the ion are expected. IR spectra for m/z small molecules as well as proteins containing more than 100 amino acids have been measured. The spectra of the smaller species show very narrow lines. For the larger species, band envelopes are obtained and for the case of highly charged proteins, a transition from helical to extended structures is observed.

# Glycan Analysis by IR Spectroscopy Integrated into Mass Spectrometry

Baptiste Schindler[1], Loïc Barnes[1], Gina Renois Predelus[1], Stéphane Chambert[2], Abdul-Rahman Allouche[1], Isabelle Compagnon[1,3]

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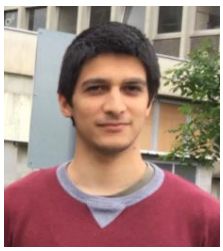

Baptiste Schindler obtained his PhD in Physics at the University of Lyon in 2016. This concluded a strongly interdisciplinary academic training, which started in 2007 at the Pharmacy school of Nancy, followed by a Master degree in Analytical Sciences at the Chemistry Dpt. of the University of Lyon. During his PhD, Baptiste Schindler specialized in laser spectroscopy. By combining his skills in Infrared ion spectroscopy with his background in analytical chemistry, he developed an entirely new approach to tackle a major challenge in analytical sciences: carbohydrate sequencing. In 2017, with the support of CNRS and University of Lyon, he set up the IROGLYPH facility which provides expertise in carbohydrate analysis to research teams from academia and industry.

**Abstract:**

Sequencing techniques have been established for proteins and DNA and have revolutionised modern biology but similar technique do not exist for glycans. In particular, top down analysis of glycans by mass spectrometry is often ambiguous due to the presence of various isomerisms. To fully characterize a glycan, one must describe:

1. The nature of the monosaccharide content
2. The regiochemistry of the glycosidic bond linking two carbohydrate moieties
3. The anomer stereochemistry (α or β)
4. The structure of the monomeric ring (pyranose or furanose)
5. The identification of the position of commonly observed modifications such as sulfation or acetylation

While MS readily offers valuable information on the size distribution of glycans in a complex sample, a full characterization requires additional structural detail, which calls for hyphenated approaches. In our group at Institut Lumière Matière, we have built an instrument coupling Mass Spectrometry and Vibrational Spectroscopy (InfraRed Multiple Photon Dissociation), dedicated to the structural characterization of glycans. This instrument offers the structural resolution of spectroscopic techniques for mass-selected glycan ions and operates at typical MS conditions, i.e. it requires significantly less sample than traditional spectroscopy.

First, we have shown that the IR signature in the 3 µm range obtained with our instrument is a powerful metric which is able to resolve simultaneously all these isomerisms. Then the conservation of the molecular structure of a precursor ion within MS fragments has been revealed on disaccharides, opening the way to top-down, *de novo* MS analysis. Following this demonstration we have established a set of carbohydrate sequencing rules using a combination of Mass Spectrometry and IRMPD Spectroscopy. Finally, we have applied our approach to the determination of the sequence of different oligosaccharides.

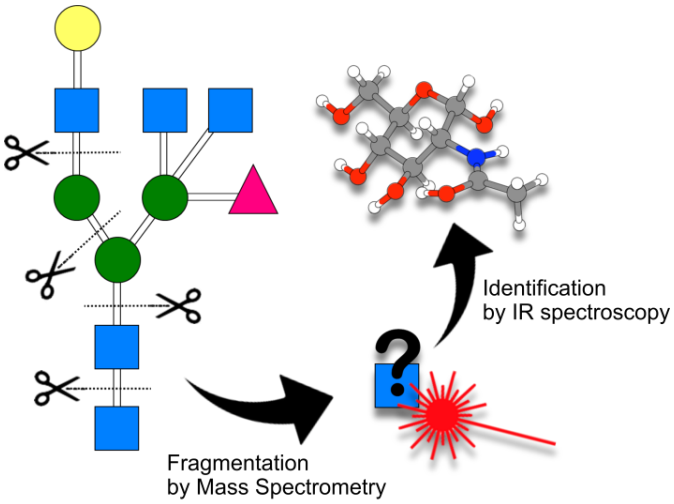


**Figure.** Concept of top-down analysis of glycans using IR spectroscopy integrated into mass spectrometry

[1] B. Schindler, L. Barnes, G. Renois, C. Gray, S. Chambert, S. Fort, S. Flitsch, C. Loison, A.-R. Allouche, I. Compagnon, Anomeric memory of the glycosidic bond upon fragmentation and its consequences for carbohydrate sequencing. *Nature Communications* **2017**, 8 (1), 973.

[2] B. Schindler, G. Renois-Predelus, N. Bagdadi, S. Melizi, L. Barnes, S. Chambert, A.-R. Allouche, I. Compagnon, MS/IR, a new MS-based hyphenated method for analysis of hexuronic acid epimers in glycosaminoglycans. *Glycoconj J* **2017**, *34* (3), 421.

# Proteomic, PTM and structural biology 2

# [Keynote] The forgotten proteome – proteomics approaches for the identification of short open reading frame encoded peptides

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The recent discovery of an increasing number of small open reading frames (sORF) creates the need for suitable analytical technologies for the identification of the corresponding gene products (sORF encoded peptides, SEP). Using the models *Methanosarcina mazei* and *C. elegans*, we evaluated different analytical approaches that allow for the simultaneous analysis of widest parts of the proteome together with the predicted SEP.
Isolation of SEP using different extraction conditions, the depletion of higher mass proteins ($>$ 10kDa) by means of ultrafiltration or GelFree-open tube electrophoresis and the separation by 2D-LC MS utilizing a high/low pH reversed phase LC separation scheme were tested.

The combination of a semi-top down approach and a 2D-LC-MS based bottom up analysis provided the highest proteome coverage for *M. mazei* reached so far. More than 30 formerly unidentified SEP could be identified, which was possible by adaptation of the criteria for single peptide MS/MS identifications. Novel extraction procedures and the avoidance of ultrafiltration steps were shown to improve detection of SEP. Interaction partners of several SEP could be detected, providing hints for the function of these peptides.
Due to neglecting sORF during genome annotation and the bias of classical proteomics approaches towards proteins larger than 10 kDa, SEP represent a yet forgotten part of the proteome. However, the first important biological functions of SEP have been elucidated, even in human cells, showing that the adaption of analytical approaches for their identification, quantification and characterization bears a high potential to shed light on this "dark" proteome.

*Speaker

# [O1] Structural analysis of peroxisomal translocon complexes using cross-linking and native mass spectrometry

Friedel Drepper *† [1], Daniel Wendscheck [1], Julian Bender [1], Andreas Schummer [1], Sven Fischer [1], Silke Oeljeklaus [1], Tobias Hansen [2], Wolfgang Girzalsky [2], Ralf Erdmann [2], Bettina Warscheid‡ [1]

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Import of peroxisomal matrix proteins into the organelle involves a highly dynamic multiprotein importomer. We have analysed protein interactions of yeast Pex14p, the central component of its membrane docking complex. Chemical cross-linking combined with mass spectrometry analysis (XL-MS) of affinity-purified complexes was used to identify homo- and heteromultimeric interactions of Pex14p and its core interaction partners Pex17p and Dyn2p. XL-MS and native MS of a C-terminal soluble part of Pex14p revealed a homo-trimeric organisation made up by contact sites in the putative coiled-coil regions of the protein. After recombinant co-expression of Pex14p and Pex17p, a high-molecular weight complex was isolated by size-exclusion chromatography. Analysis by native MS suggests the composition and stoichiometry of the predominant complex. The results further give evidence for some heterogeneity or dynamic changes of complex composition. XL-MS was used to identify molecular contacts leading to a detailed map of interactions. Possible structural arrangement of domains and docking sites at the membrane will be discussed.

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# [O2] In-depth glycoproteomics analysis of human C1-inhibitor using C18-PGC-LC-QTOF-MS/MS

Kathrin Stavenhagen *† [1,2], H. Mehmet Kayili [2,3,4,5], Stephanie Holst [1], Carolien A. M. Koeleman [1], Ruchira Engel [6,7], Diana Wouters [6,7], Sacha Zeerleder [6,7], Bekir Salih [5], Manfred Wuhrer [1,2]

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[4] Department of Nutrition and Dietetics, Karabuk University – Turkey
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Human C1-inhibitor is a serine protease inhibitor and the major regulator of the contact activation pathway as well as the classical and lectin complement pathways. Although it is one of the most heavily glycosylated plasma glycoproteins, only little is known about the structural features and biological role of C1-Inhibitor glycosylation. Here, we present an in-depth site-specific *N*- and *O*-glycosylation characterization of C1-Inhibitor using a panel of MS-based approaches. The purified glycoprotein was treated with different proteases, partly in combination with PNGase F and exoglycosidases, to generate a variety of *N*- and *O*-glycopeptides. Subsequent MS analysis included the application of an integrated C18-porous graphitized carbon (PGC)-LC-ESI-QTOF-MS/MS approach operated in stepping-energy CID mode. This approach facilitated enhanced glycosylation site coverage of Pronase-treated glycopeptides within a single analysis run. Our study revealed extensive mucin-type *O*-glycosylation of C1-Inhibitor with up to 26 occupied O-glycosylation sites, mainly carrying core1-type *O*-glycans. In addition, all known six *N*-glycosylation sites of C1-Inhibitor were confirmed and characterized in a site-specific manner. This in-depth investigation of C1-Inhibitor glycosylation will form the basis for further functional studies on its biological role.

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# [O3] Characterization of the virulome of Staphylococcus aureus by a highly multiplex approach with Scout-MRM

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The emergence of antibiotic-resistant bacteria strains is seriously threatening human life, as recently underlined by the World Health Organization. The case of *Staphylococcus aureus, S.a.* is of particular concern, whose forms resisting to vancomycin or methicillin caused numerous patients death in Hospitals. For patient treatment, the selection of the right antibiotic treatment still requires time-consuming antibiotic susceptibility testing. Indeed, it still relies on conventional microbial growth monitoring in the presence of antibiotics within 48 hours. As a result, one to three days of delay occur between the initiation of the empirical antimicrobial therapy and the antibiotic susceptibility testing result.

In complement with the development of alternative drugs, it is also crucial to better understand how mutations of the bacterial genome induce the emergence of new resistant strains. The sensitivity and multiplexing possibilities of mass spectrometric (MS) approaches appears particularly relevant to tackle such problems. We thus developed a targeted assay covering 80 key proteins involved in *S.a. virulence and antibiotic resistance* using a novel and a highly multiplexed targeted-MS approach, Scout-MRM. The virulome assay has been implemented for deciphering the proteogenomic links across a clinical collection of 300 *S.a.* strains. In parallel, we also drastically simplified the sample preparation and analysis time for shortening the delay to one hour between the positive detection of a blood culture and *S.a.* virulome characterization. Since this assay integrates peptides used for *(SA)* identification, the data dependent Scout-MRM technique opens the route toward rapid *S.a.* identification and virulence typing on a single instrument.

*Speaker

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# Instrumentation 1

# [Keynote] Chemical and topographical 3D-surface imaging of non-planar objects on the micrometer scale using AP-SMALDI MSI

Mario Kompauer * [1], Sven Heiles [1], Bernhard Spengler† [1]

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High-lateral-resolution mass spectrometry imaging (MSI) systems suffer from a shallow depth of field and thus generate geometry-related artifacts when imaging samples of insufficient flatness. To analyze non-flat tissue sections or three-dimensional (3D) sample surfaces, it is essential to constantly refocus the desorption/ionization probe. We therefore developed an autofocusing MSI system which allows to describe the topographical surface of objects on the micrometer scale, regarding both, geometry and molecular composition. Autofocusing was achieved by triangulation, using a focused continuous-wave diode laser, and by subsequently adjusting the desorption/ionization laser focal plane to the sample spot surface. This allowed to simultaneously obtain topographic and mass spectrometric data from 3D surfaces with high lateral and depth resolution. The MSI system was controlled and the data was visualized by a home-built MATLAB (R2016a, The MathWorks GmbH) application. The autofocusing system was used for plant, insect, polymer and metal samples, resulting in combined 3D surface MS images. A lateral resolution of 10 $\mu$m, a depth resolution of 1.5 $\mu$m and steep sample inclinations with pixel-to-pixel height differences up to 960 $\mu$m will be demonstrated. MSI and MS2I experiments with high mass resolution ($R$=240,000 at $m/z$ 200) and high mass accuracy (in vivo and also to improve common 2D tissue section MSI analysis by avoiding ion signal intensity variations across large samples of imperfect flatness.

*Speaker

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# [O1] Hybrid SIMS: A new instrument for high resolution organic imaging with high mass-resolving power and MS/MS

Matthias Kleine-Boymann * [1], Alexander Pirkl [1], Rudolf Möllers [1], Henrik Arlinghaus [1], Niehuis Ewald [1], Nichola Starr [2], David Scurr [2]

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SIMS offers the possibility to acquire chemical information from submicron regions on inorganic and organic samples. This capability has been especially intriguing for researchers with life science applications. In recent years, the vision to image and unambiguously identify molecules on a sub-cellular level has been driving instrumental and application development. While new ion sources expanded the usability of SIMS instruments for biological applications, SIMS analysers lacked the required mass resolution, mass accuracy and MS/MS capabilities required for the thorough investigation of these materials.
To specifically address the imaging requirements in the life science field we developed a powerful new Hybrid SIMS instrument. The instrument combines an Orbitrap-based Thermo Scientific Q Exactive HF mass analyser with a high-end ToF-SIMS system. The instrument provides highest mass resolution ($>$ 240,000) and highest mass accuracy ($<$ 1 ppm) with high lateral resolution cluster SIMS imaging for the first time.
In this presentation we will introduce this new instrument and present first application data including high resolution SIMS spectrometry, MS/MS analyses, high resolution imaging of tissues and 3D organic depth profiles of biological samples.

*Speaker

# [O2] Development and optimization of a membrane-inlet-photoionization mass spectrometer for real-time analysis of (poly)aromatic compounds in aquatic systems

Christian Gehm * [1], Thorsten Streibel [3,2,1], Ralf Zimmermann [1,2,3], Detlef Schulz-Bull [4]

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[3] Helmholtz Virtual Institute of Complex Molecular Systems in Environmental Health (HICE) – Germany
[2] Helmholtz Zentrum München – German Research Centre for Environmental Health (HMGU) – Cooperation Group "Comprehensive Molecular Analytics" (CMA), Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany, Germany
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Fast and sensitive analysis of (poly)aromatic hydrocarbons (PAH) is of high importance due to their large impact on environmental and human health. However, very low concentrations of PAHs in marine environments complicate their detection and monitoring. An outstanding analytical technique, regarding to online determination of PAHs in gaseous mixtures, is resonance-enhanced multiphoton ionization (REMPI) coupled to time-of-flight mass spectrometry. The unique ionization scheme of REMPI provides highly selective and sensitive detection of (poly)aromatic species. In contrast, the high substance specific selectivity impedes the quantification. One way to overcome this drawback is the usage of relative photoionization cross sections to quantify the desired analytes. For the application of REMPI in marine systems a promising approach constitutes the utilization of membrane-inlet mass spectrometry (MIMS). In MIMS dissolved analytes are transported selectively from the water phase through a semipermeable membrane into the vacuum of the mass spectrometer. Additionally, the analytes are enriched on the membrane surface. As a consequence, no laborious enrichment techniques (such as purge-and-trap or extraction) are needed and the time of analysis is considerably reduced. As a proof of principle, in this work we present the first laboratory measurements of water samples with a homebuilt REMPI-MIMS system, including two different external inlet designs for sheet as well as for hollow fiber membranes. With this early-stage system concentrations down to the mid-ppb range for selected small (poly)aromatic compounds are easily accessible in minutes without any sample preparation. Additionally, results of relative photoionization cross sections for selected (poly)aromatic species are presented.

*Speaker

# [O3] Ion mobility mass spectrometry of phosphoric acid cluster ions

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Because ion mobility depends on the collision cross section (CCS) of the ions that is related to their conformations in the gas phase, the coupling of ion mobility and mass spectrometry is spreading as a supplemental method of structural characterization to mass spectrometry. Several ion mobility techniques, such as travelling wave ion mobility (TWIMS), necessitate the use of reference ions and calibration to measure the CCS. However, the pool of existing reference ions is small for negative ions, especially for multiply charged ions and with nitrogen as drift gas.
Since a first study in 2014, we have started studying negative phosphoric acid cluster ions in view of investigating their potential as reference for the calibration of the TWIMS.

Here, we measured the CCS values of twenty four negatively charged phosphoric acid cluster ions, ranging from 140 to 530 Åˆ2, singly, doubly and triply charged with a drift-tube ion mobility-time-of-flight mass spectrometer (IMS-TOF, TOFWERK), at 30∘C and nitrogen as the drift gas.
These measured CCS values were used as reference values for calibrating a Waters SYNAPT G2 instrument (TWIMS).The resulting correlations were excellent for singly (Rˆ2 > 0.999) and doubly (Rˆ2 = 0.992) charged cluster ions, but lower for triply charged ions (Rˆ2 = 0.902). Accuracy, tested using singly charged ions from dextran and the Agilent tune mix, led to relative differences of 2 to 8%. TWIMS calibration with phosphoric acid cluster ions therefore allowed estimation of the CCS within less than a 10% error.

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# Lipidomics

# [Keynote] Study of acetogenins in a natural extract using SFC-HRMS/MS and post-column metal cationisation

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Acetogenins, lipids from the polyketide class and specifically found in plants from the Annonaceae family, are food toxins suspected to be responsible of atypical Parkinsonian syndromes without known therapy. Acting as an inhibitor of mitochondrial complex I, acetogenins are also known to have cytotoxic properties and are proposed as potential anti-cancer molecules. Previous dereplication studies highlighted some limitations of the HPLC-MS approach including long analysis time and low chromatographic resolution.
Nowadays, supercritical fluid chromatography (SFC) can be considered as an alternative to LC for the study of hydrophobic compounds. Indeed, physicochemical properties of supercritical CO2 allow excellent solubility of such analytes and give analytical performances above that of LC. However, examples using SFC-HRMS/MS to analyze natural extracts are poorly developed in the literature.

Due to the rarity of standards, a SFC-HRMS/MS method was directly developed using a crude seed extract of *Annona muricata* L. with a reduced analysis time by a factor of 4. Nevertheless, the fragmentation of protonated or sodium/potassium cationized species only led to limited structural information. For the first time, post-column lithium cationisation was fully optimized after SFC in order to form ion fragments of structural interest but of very low intensity. Finally, copper salt was employed allowing the detection of intense odd and even ion fragments related to unique radical-driven fragmentation pathways.
The final SFC-HRMS/MS method significantly improved the structural characterization of acetogenins highlighting the power of SFC-HRMS/MS in the field of natural products.

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# [O1] In-depth Lipidomics using Laser Capture Microdissection

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Histologically liver has a robust architecture, which defines metabolic zonation of this organ. The liver lobule contains periportal and pericentral hepatocytes, which, under normal conditions, show different metabolic behaviour. This behaviour is determined by the blood flow direction and oxygen gradient.
The zonation is typically characterized at the protein and transcriptional level, however lipid profiles remain elusive. While lipidomics profiles of different hepatocytes in healthy are partially studied, lipid liver zonation knowledge at pathological conditions is missing.

In our work we aim to develop a method, which allows to perform a deep characterization of lipids on various zonated hepatocytes. We present a method combining laser capture microdissection (LCM) coupled to top-down shotgun lipidomics. By analyzing an area of 0.5 mm2 of 20 $\mu$m thick liver sections we could determine molar quantities of 233 species from 16 major lipid classes.

We collected specific ROIs (hepatocytes next to pericentral and periportal regions) to investigate differences in spatial lipid distribution. Preliminary data showed only marginal differences in lipid class composition between pericentral and periportal regions. However, we observed differences in lipid species distribution within certain lipid classes (ceramides and sphingomyelin) .

Shotgun analysis determined the molar abundance of major lipid classes from the same amount of liver tissues that is typically used for transcriptomics. Further method optimization is required to minimize lipid degradation and to down-scale the amount needed for analysis.

Further application of this approach will help us to investigate NAFLD, HCC and other liver diseases.

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# [O2] An adaptive and dynamic approach to generate unique SMILES for lipidome homology

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The Simplified-Molecular-Input-Line-Entry-System (SMILES) is a line notation to describe chemical structures using ASCII strings. SMILES are essential to represent lipidomes in chemical space to determine lipidome homology [Marella 2015].
SMILES are not unique by nature, because valid SMILES can be written starting from any position in a chemical structure. Our current approach using LIPIDMAPS tools enabled only limited control over the starting position. In case of **Phosphatidylcholines (**PC), SMILES are written starting from the glycerol while for **Phosphatidylserine** (PS), the starting point is the head group. Consequently, all similarities between PC and PS are systematically too low.

We generated Template SMILES following a dedicated database model, which contains building blocks for each lipid class like fatty acids, head groups and sphingosines. All SMILES starts from a structural position, which is most conserved and can be considered as a reference point for related lipid classes. Because mass spectrometric identification cannot deliver information down to the molecular species level, all isomers are automatically generated by the lipid name. As representative SMILES the centroid of all isomers is selected.

The approach generates Template SMILES for all glycerophospholipids, their lysoforms and sphingomyelin and ceramides. As a results, all Template SMILES are generated according to a well-controlled principle. The chemical relationship between PC and PS are now corrected and the accuracy of lipidome models is improved compared to other generators.
This prototype SMILES generator will be integrated within the LUX score browser, which offers an interactive and a user friendly plattform to determine lipidome homologies.

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# [O3] The effect of double bond functionalization for their localization in lipids by means of MS/MS

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Lipids are a group of biomolecules with a broad variety of chemical structures. This renders them suitable for various kinds of tasks in a number of different organisms. Double bonds have an influence on lipids' chemical, biochemical and biophysical properties. Since localization of double bonds in lipids is still a difficult task regarding their structural diversity and possible complexities of available mixtures, the development of a new method addressing those challenges is required. The photochemical Paternò B́uchi (PB) reaction, based on the binding of acetone to the double bonds, was used. Precise localization was achieved due to diagnostic fragments observed in MS/MS experiments.A combination of a HPLC separation with an online PB reaction was developed to address complex lipid mixtures.
Online PB reaction was performed using post column derivatization by UV irradiation and an additional constant acetone flow. After UV light (254 nm) irradiation, a signal with a mass increase of 58 Da was detected in addition to the deprotonated lipid. The acetone adduct was formed due to the reaction of acetone with the double bond resulting in an oxetane. MS/MS experiments generated diagnostic fragments allowing localization of the double bond position.
The successful hyphenation of HPLC with the online reaction enables utilization for lipids with more complex structures and lipid mixtures. Matrix effects can be reduced and further structural information, for example in case of constitutional isomers, can be obtained.

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# DGMS Awards Session

# Tuesday Posters

# [P001] Characterizing structural isomerism of liganded gold nanoclusters using ion mobility and mass spectrometry.

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Liganded nanoclusters are promising as potential biomedical probes. However, their physico-chemical properties are highly size- and structure-dependent.(1,2) As consequence, precise characterization of the composition and structure of those clusters at the atomic level is a crucial towards the rationalization of their synthesis and the design of new clusters with tailored properties.

Glutathione-liganded gold nanoclusters (glutathione : $\gamma$-L-Glutamyl-L-cysteinylglycine) were formed by reducing gold salt in the presence of excess glutathione, using a method similar to that previously reported.(2) We studied a series of as-synthesized glutathione-liganded gold nanoclusters using ion mobility spectrometry and mass spectrometry. Our results demonstrate the interest of such combined approach to gain detailed insight in the dispersity of the clusters both in terms of size and conformation. In particular, we show that samples which appear as monodisperse based on their mass spectrum may display structural dispersity. On the other hand, different schemes for the distribution of charges between the ligands and the cluster core could be identified and interpreted in terms of the oxidation state of the core.(3)

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# [P002] Vacuum Photoionisation TOF-MS as technique to analyse complex gas mixtures

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There are two general ionisation techniques that are covered by vacuum Photo-Ionisation Time-of-Flight Mass-Spectrometry (PI-TOFMS). On the one hand there is SPI (Single Photon Ionisation) ionising a wide range of organic molecules and on the other hand there is REMPI (Resonance Enhanced Multi Photon Ionisation) focussing primarily on aromatic structures. Especially the complementary use of SPI and REMPI can access new information.
SPI is using varying ionisation light sources based on lamps or lasers. The respective wavelengths limit the range of ionisable organic compounds. The ionisation energy of most organic compounds is in a range of 7 to 11 eV (177 nm to 112 nm). Due to the fact that most matrix gases such as oxygen, nitrogen, carbon dioxide and especially water vapour have higher ionisation energies of 12 eV and more, they will be suppressed efficiently. Depending on the specifically used REMPI method at least two photons are used for ionisation. This ionisation mechanism requires a stable excitable intermediate state that is primarily present and accessible in aromatic structures. Accordingly, REMPI is selective for aromatic chemical structures while it suppresses non aromatics and matrix gases.
PI-TOFMS is well suited for a wide range of applications focussing on on-line characterisation of complex gas mixtures such as coffee roast gases, biomass pyrolysis products, polymers, oil and crude oil investigation using an additional TG (Thermo-gravimetric unit), cigarette smoke and new smocking products and many more.

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# [P003] Between coffee and cigarettes - Photoionisation MS as a tool for analysis of complex gas mixtures

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Photo-Ionisation Time-of-Flight Mass-Spectrometry (PI-TOFMS) is well suited for an on-line characterisation of complex gas mixtures such as coffee roast gases, cigarette smoke and new smocking products. With the increasing legal availability not only for medical purposes of marihuana/cannabis and THC (Tetrahydrocannabinol) containing smoking products in various countries the interest in understanding the release processes of the active components is increasing as well.
PI-TOFMS enables an on-line process control in a sub second range as it is needed to resolve a single puff of a smoke product or even observe the time resolved coffee roast progress in time scale of 3 to 12 minutes per roast. Two general soft PI methods in vacuum can be distinguished. On the one hand there is SPI (Single Photon Ionisation) ionising a wide range of organic molecules and on the other hand there is REMPI (Resonance Enhanced Multi Photon Ionisation) focussing primarily on aromatic structures. Both can be uses as complementary techniques for complex organic gas mixtures in on-line process monitoring and investigation.
Regarding smoke products the puff by puff nicotine release is of special interest for consumers, industry and science. There are significant differences in nicotine release between conventional cigarettes compared to new smoking products such as e-cigarettes or tobacco heating products, but also the release of HPHCs (Harmful and potentially Harmful Compounds) is varying. Focussing on more or less legal THC containing smoke products the scientific question moves to observing active compounds such as $\Delta$9-THC and cannabinol due to their different effects.

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# [P004] Differences in observable charge states of intact biomolecules: comparing nESI-MS, LILBD-ToF-MS and LILBID-qToF-MS

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Many applications used in mass spectrometers are highly charge state dependent processes. This includes CID (collision induced dissociation), CIU (collision induced unfolding) as well as IM-MS (ion mobility MS). Different ionization methods result in different detectable charge state distributions. In addition, the type of instrument in use has an impact on the observed charge state distribution too. Depending on the application highly and lowly charged species both have their advantages and disadvantages respectively.

Here we compare two different ionization methods with respect to the influence on the observed charge state distribution of intact biomolecules. In detail we compare a commercially available nanoESI-MS instrument (Synapt G2s; Waters) as a representative of the electrospray-type of instruments with a home-build LILBID-ToF-MS with its IR-laser desorption ion source. Typically the actively ionizing nanoESI results in highly charged species which is in contrast to the non-actively ionizing / desorbing LILBID-ToF where mostly lowly charged species are detected. Moreover, we compare LILBID-MS on two different types of instrument: the home-build ToF and the LILBID source adapted to a commercially available, modified qToF (QTOF I; MS Vision). With the same ion source we observe slightly higher charged species on the qToF than on the ToF.
In order to investigate the observed charge state distribution in more detail we examined native hen-egg lysozyme and compared it with chemically modified lysozyme. By acetylation we can modify the surface charges of the protein. Here we can monitor charge state differences between the native intact biomolecule and the modified species.

*Speaker

# [P005] Improving the chromatographic capabilities of an atmospheric pressure chemical ionisation source coupled to a gas chromatograph

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Coupling a gas chromatograph to an atmospheric ionisation source mass spectrometer presents a number of challenges when compared to a traditional vacuum ionisation source MS system. The reduced analyte exit velocity and thermal discontinuities from convective heat loss, leads to a loss in chromatographic resolution for higher boiling point analytes. A computational fluid dynamics modelling program was used to characterise an APCI GC source and to improve its thermal continuity. The new source design was then tested with a range of high boiling point compounds. CFD modelling identified areas of the source design where significant thermal discontinuities were present, both above and below the regulation temperature of the interface between the ionisation source and the gas chromatograph. A range of modifications to the design were modelled, including changes in materials and key dimensions, resulting in a final design which was predicted to have superior thermal continuity. The new design was manufactured and preliminarily tested using a Polywax 655 sample. This sample contains a distribution of even number linear alkanes with carbon chain lengths from C20 to C80. The sample was used for characterising chromatographic performance by determining the first alkane where chromatographic peak distortions are observed and hence indicating the temperature limits of the interface where discontinuities are limiting the analysis.

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# [P006] Moving toward quantitative assessment of binding affinities with LILBID-MS

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LILBID-MS is a native mass spectrometry ionization method, in which IR laser light irradiates aqueous droplets containing sample biomolecules. This leads to explosive expansion of the droplets and release of sample molecules as ions. To date, the method is used to determine stoichiometry and nearest-neighbor relationships and to qualitatively assess binding. We seek to extend LILBID-MS to quantitatively assess binding affinities of biomolecular complexes.
In initial studies, we showed that increasing laser harshness leads to more dissociation during desorption and that, at constant laser settings, complexes with stronger binding affinities show less dissociation. Laser settings can be scanned from mild to harsh and the increasing amount of dissociation recorded. We are developing a method to assign quantitative binding affinities based on this dissociation curve.
To do this, we have to overcome the following challenges. Small changes in laser power or in droplet position within the laser beam mean that the droplet receives a different amount of energy, leading to a different explosive expansion, different amount of energy transfer to biomolecules, and different amount of dissociation. We developed a method to image the explosive expansions of individual droplets and correlate these to the corresponding spectra. Explosive expansions with similar shapes resulted in similar spectra. Thus, the shape of the droplet's explosive expansion can be used as a reliable parameter to track laser energy transferred. By plotting this parameter against dissociation it should be possible to obtain dissociation curves comparable to UV-vis melting curves or titration curves to yield binding affinities.

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# [P007] Electron impact ionization source using homemade electron gun for Micro-Time-Of-Flight mass spectrometer

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**M**ass **S**pectrometry (MS) has become a classical tool widely used in analytical chemistry. Reliable, fast, and powerful, MS apparati are still heavy and expensive laboratory equipments rather kept in laboratories than taken on the field.
The development of a portable gas chromatography-MS is therefore of great interest, especially for *in situ* gas analysis. To be efficient on the field, the device should be small, light and low power consuming. In order to address the size constraints, we used the **M**icro **E**lectro **M**echanical **S**ystem technology (MEMS). This technology has been developed by the microelectronic industry and has tremendous applications in the sensors area.
Our new generation of microfabricated time-of-flight mass spectrometer ($\mu$-TOF) uses a homemade thermoionic electron gun as electrons source (EI 70 eV) to achieve electron impact ionization, allowing NIST comparison. A 2.5 mm diameter electrons beam is applied on the 3 x 3 mm ionization chamber grid drilled with 120 square holes, resulting in 2.2 $\mu$A electron current inside the ionization chamber. Using electron impact ionization, ionization efficiencies in the range of 1.10-6 ion / neutral are shown. A generated ion current in the range of a few tens of nanoamperes lead to an ionization yield around 0.1 %, in the range of the best microfabricated ionizers.

*Speaker

# [P008] How hot is a MALDI plume? Probing the effect of excitation laser fluence and wavelength with thermometer molecules and via Substance P peptide fragmentation

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Although MALDI-MS is a particular "soft" desorption/ionization technique, that is well suited for the analysis of a large class of thermally labile biomolecules, some fragmentation of the generated gas phase ions generally occurs. The most relevant, thermal fragmentation mechanism is due to the heat imparted on the analyte ions during the MALDI event. Numerous studies have shown that fragmentation rates depend on used matrix (i.e., its physiochemical properties) and irradiation conditions (e.g., laser fluence and wavelength). One common approach to study the "temperature" in a laser-generated plume is via thermometer molecules that fragment with temperature-depending rates along a distinct chemical bond. However, the typically used benzylpyridinium salts constitute "preformed" ions which may underlie different ionization pathways than typical biomolecules.
Here we compared dissociation of *p*-methoxybenzylpyridinium chloride and substance P, a small peptide with well-characterized fragmentation characteristics. 2,5-Dihydroxybenzoic acid (DHB) and 4-hydroxycinnamic acid (CHCA) were investigated as common, well-studied MALDI-matrices.

A tunable OPO laser (220-350 nm), coupled to an axial-TOF mass spectrometer (4800 TOF/TOF), was used to obtain comprehensive insight into the effect of laser wavelength and fluence on the internal energies of the generated TM and peptide ions. The rate constants *k(E)* for the matrix-, fluence-, and wavelength-dependent fragmentation reactions were calculated using the delayed extraction time of the TOF-MS as time base.
Whereas our results corroborate the experience that internal energies are generally increasing with laser fluence, we show that with regard to the interplay of laser wavelength and matrix absorption profiles a more complex behavior is found.

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# [P009] Implementation of a DESI sampling probe into an autarkic ion source for ambient in-field MS applications

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The ambient ionization method desorption electrospray ionization (DESI) combined with mass spectrometry (MS) is a powerful tool for the analysis of various samples. It allows direct surface sampling under atmospheric pressure conditions, making it especially useful for applications in which a fast measurement is needed, directly at the sampling site. In this work, we present an autarkic DESI source in which a DESI sprayer is implemented in a handheld sampling probe. This setup simplifies the measurement procedure, allows DESI-MS analysis from uneven sample surfaces and reduce sample preparation efforts. The important geometrical parameters regarding sample surface, DESI spray and MS transfer capillary, are fixed within the probe head and are widely independent of the sample shape. A sample object does not have to be placed below a DESI spray or touched by an operator, because the flexible probe can be directed to the sample surface. A connection to any portable or stationary mass spectrometer is possible as long as it is equipped with an atmospheric pressure interface. In case of discontinuous pressure interfaces, the ion flow through the one meter long probe tubing to the MS inlet can be pressurized by the autarkic source. Performance studies will be presented for different types of consumer goods and target compounds like plasticizers, drugs and pesticides.

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# [P010] Airborne laser-spark for ambient desorption/ionization of liquids

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The development and enhancement of new ionization techniques for mass spectrometry often needs to be custom-tailored for specific sampling approaches. Here, a direct sampling ionization technique is presented for ambient mass spectrometry. Ambient mass spectrometry based techniques are typically used to analyze samples in their native states without sample pretreatment. This new design is based on a quasi-continuous airborne plasma which is ignited inside the particulate air via a focused laser irradiation. Desorption and ionization of the analyte molecules are achieved by the laser plasma without reaching the plasma. The ionization process is induced by interaction with nascent ionic fragments, electrons and ultraviolet photons in the plasma vicinity. Previously, this method was solely used for the characterization of solid and gaseous analytes. The sample introduction was occurred via thermal desorption and headspace analysis. This study focuses on the potential applicability of liquid samples. In comparison to previous approaches, the usage of liquid samples has an impact on the stability of typically used plasma of 532 nm. It was necessary to realize an alternative plasma using light of the fundamental wavelength of 1064 nm. That new plasma resulted in a significant more stable and bright plasma and the first laser plasma ionization spectrum was recorded for an analyte in the condensed phase with a mass spectrometer of type LCQ DecaXP.

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# [P011] Benchmarking of monoclonal antibodies using size exclusion chromatography hyphenated to native ion mobility mass spectrometry (SEC-native IM-MS)

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Monoclonal antibodies (mAbs) have emerged as a very promising class of therapeutic proteins for clinical use against a large number of diseases such as cancer, infection and immune disorders1. The development of therapeutic mAbs progress considerably, the FDA has already approved 75 therapeutic mAbs to date2. Native MS is the most direct approach and now well established to characterize mAbs and their related compounds under non-denaturing conditions according to its high mass accuracy3. Moreover, native IM-MS provides a simple and direct size/shape measurement for global mAbs conformation assessment. Nevertheless, the sample preparation based on buffer exchange is manual and time consuming which is the only specific requirement for native MS analysis.
In this work, several mAbs were analyzed by SEC hyphenated to native IM-MS. SEC affords an on-line and fast desalting while IM-MS allows straightforward identification and conformational characterization of mAbs. Furthermore, several SEC columns were evaluated in terms of desalting efficiency. Ways to provide improved data treatment will also be presented.

Our results demonstrate that SEC-native IM-MS provides well-resolved native mass spectra with very high mass accuracy (80 to 6.7 ppm). Thanks to automated SEC-native IM-MS, we believe that native MS has now reached a level where it can be implemented in biopharma companies for routine intact mAb analysis in non-denaturing conditions.

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# [P012] High Resolution Mass Spectrometry hyphenated to different Inlet Techniques for the Characterisation of high boiling Petroleum fractions

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Comprehensive gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOF-MS) has established itself as a powerful technique for detailed characterisation of petroleum samples. However, conventional GC×GC-TOF-MS with nominal mass resolution has limitations for high boiling fractions due to the higher complexity and low volatility of the matrix.
In this study, we will show the advantages of GC×GC-high-resolution-mass-spectrometry (GC×GC-HRMS) for the analysis of petrochemical samples and expand the applicability of TOF-MS by hyphenation to thermo analytical techniques. For the characterisation of non-volatile matrices like vacuum residues, a thermo balance was coupled to the same mass analyser (TGA-HRMS) via a transfer capillary to identify evolved gases by mass spectrometry. Moreover, evolved gases could be separated with online gas chromatography before mass spectrometric analysis (TGA×GC-HRMS). Direct Inlet Probe (DIP-HRMS) was applied as a third inlet technique for the introduction of high boiling components. Controlled heating under reduced pressures in the ion source enabled the vaporisation of low-volatile molecules. The applied temperature gradient allowed a rough separation of the sample matrix according to the components volatility. Beside electron impact ionisation (EI), two soft ionisation techniques, namely single photon ionisation (SPI) and chemical ionisation (CI) were utilised on the same system in order to obtain complementary information.
Summarized, the application of GC×GC-HRMS, TGA(×GC)-HRMS and DIP-HRMS has shown to be a versatile approach for the detailed characterisation of a broad spectrum of crude oil derived fractions.

*Speaker

# [P013] Continuous post-column standard substance infusion for LC-ESI-MS/MS analysis – An effective quantification approach

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While liquid chromatography tandem-mass spectrometry with electrospray ionization (LC-ESI-MS/MS) analysis is gaining high traction as method of choice for multi-sample analysis, it is strongly susceptible to matrix components.
Matrix effects (ME) are the main source for substantial losses in detection sensitivity and had to be compensated via extensive sample purification and correction methods. The continuous post-column infusion of internal standards (PCI-IS) is a sophisticated ME correction and quantification method for the LC-ESI-MS/MS analysis.

The performance of the PCI-IS approach was tested in various urine specimens for 16 pharmaceutical compounds at ng/L concentration and was compared to quantification by isotopically labelled internal standards. The quantification results by PCI-IS methodology were within +/- 20% accuracy and low relative standard deviation of 15%. The results demonstrate that one post-column infused internal standard suffices to analyze multiple target analytes in complex matrices.

The introduced method is a simple quantification approach and a potent alternative to classic internal standard methodology. In combination to extensive sample dilution and high sensitivity, the PCI-IS technique could avoid elaborate sample preparation and replace current quantification methods. The proposed technique reduces the required steps for sample preparation, costs of additional stable isotopically-labelled internal standards and sample manipulation. The PCI-IS method has been successful applied by other scientists for stable specific sample matrices as blood, plasma, cerebrospinal fluid and urine.

**New Aspects**
Quantification using the continuous response of post-column infused standard substances instead of usual retention time dependent internal standards.

*Speaker

# [P014] Ultra-High Resolution Ion Mobility Spectrometry for Separation of Isotopologues

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In drift time ion mobility spectrometry (IMS), ions are driven through a drift tube by a homogenous electric field and collide with neutral drift gas molecules. The separation mainly depends on the ion-molecule collision cross section (CCS). Therefore, in more and more applications IMS are used for pre-separation of isomers that cannot be distinguished by their mass-to-charge ratio (m/z) in mass spectrometers (MS). One major benefit of IMS is the fast separation within several milliseconds revealing the CCS. However, usually IMS exhibit rather poor resolving power (defined as FWHM divided by the drift time). Here, we present measurements using a compact ultra-high resolution IMS with resolving power of 250, corresponding to a resolvable $\Delta$CCS of 0.8 %. To achieve such resolving power a drift voltage of 25 kV is used. Nevertheless, the length of the drift tube is only 15 cm. Although the mass has only a minor influence on the ion mobility compared to the CCS, the system is capable of separating the isotopologues of acetone and perdeuterated acetone, corresponding to a mass difference of 6 u. Acetonitrile and perdeuterated acetonitrile having a mass difference of 3 u are also well resolved. For ionization, atmospheric pressure photo ionization (APPI) with 10.6 eV can be used as well as atmospheric pressure chemical ionization (APCI) with a miniature soft x-ray source. Thus, improved IMS-MS systems with ultra-high resolution IMS are possible. This work is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – ZI 1288/4-1.

*Speaker

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# [P015] Different Selectivity in C–C-Coupling Reactions of Ag(III) and Cu(III) Ate Complexes

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The central role of organocopper(III) intermediates for synthetically valuable C–C-coupling reactions of organocopper(I) reagents and alkyl halides has been highlighted by various mechanistic studies [1,2]. On the contrary, the mechanistic details of related silver-mediated C–C-coupling reactions are poorly investigated.

By means of ESI mass spectrometric measurements of solutions of LiAgMe2LiCN and 0.5 equiv. RI (R = Me, allyl) in THF, we detected Ag(III) ate complexes [RAgMe3]– analogous to the results of a similar study on organocuprates [2]. Gas-phase fragmentation experiments on [RAgMe3]– revealed their ability to undergo C–C-coupling reactions, again, similar to their copper-containing counterparts. However, while [(allyl)AgMe3]– mainly reacted via cross-coupling, the homo-coupling channel was predominant for [(allyl)CuMe3]–.

To understand the different reactivity of [(allyl)AgMe3]– and [(allyl)CuMe3]–, C–C-coupling pathways for these species were investigated with hybrid DFT and DLPNO-CCSD(T) calculations. For both complexes, one homo-coupling and three cross-coupling mechanisms could be identified. A multi-configurational diagnostic revealed that for both [(allyl)AgMe3]– and [(allyl)CuMe3]– one cross-coupling transition structure features significant multi-configurational character. Indeed, only additionally performed multi-reference calculations predicted the experimental observations for both complexes correctly.

In conclusion, we have shown that markedly different mechanisms operate for the C–C-coupling reactions of isolated [(allyl)AgMe3]– and [(allyl)CuMe3]–, which explains their opposed selectivity. Moreover, our results emphasize the importance of mass-spectrometric reactivity studies on well-defined transition metal systems for benchmarking electronic structure methods.

E. R. Bartholomew et al., *J. Am. Chem. Soc.* **2008**, *130*, 11244.

A. Putau et al., *J. Am. Chem. Soc.* **2012**, *134*, 613.

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# [P016] Unraveling the Microscopic Reactivity of Organocuprates

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Organometallic reagents are indispensable tools in modern organic synthesis. For the rational development and optimization of these reagents, knowledge about their reactivity at a molecular level is crucial. However, in solution, organometallic compounds typically feature fast equilibria between different aggregation, association and coordination states [1]. Thus, conventional methods cannot monitor the microscopic reactivity of individual species, which would offer invaluable insights.

Here, we demonstrate that the combination of ESI MS with gas-phase experiments is a powerful approach to overcome the difficulties associated with the determination of the microscopic reactivity of organometallic compounds. In particular, we focus on highly reactive solutions of organocuprates LiCuR2Li(CN) as prototypical organometallic reagents.

We have previously reported that several aggregation and association states of organocuprates can be transferred to the gas phase directly from solution by ESI [2]. Proceeding from these results, we measured the reactivity of various mass-selected organocuprates towards water with tandem MS. For the interpretation of the experimental results, we identified energy profiles for the hydrolysis of the investigated species by quantum chemical calculations. Moreover, we calculated bimolecular hydrolysis rates by master equation calculations to directly compare experiment with theory.

Our results highlight that lithium-containing organocuprates react several orders of magnitude faster than their lithium-free counterparts. Altogether, the outstanding role of lithium for the enhanced reactivity of organocuprates towards oxygen-containing electrophiles could be proven at the microscopic level for the first time.

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A. Putau et al., *Organometallics* **2010**, *29*, 3593.

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# [P017] Large Halogen-Bonded Capsules: Structure and Reactivity Elucidated by Mass Spectrometry and Ion Mobility

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Robust and linear [N···I+···N] halogen bonds have recently gained interest for the assembly of large supramolecular capsules.[1] Mass spectrometric and ion mobility experiments, as well as theoretical calculations on dimeric and hexameric [N···I+···N] halogen-bonded supramolecular capsules reveal their well-defined gas-phase structures and give information on their anion-binding properties. Depending on the solvent, the hexamer can rearrange into an unprecedented pentameric [N···I+···N] halogen-bonded capsule. Its rather unusual trigonal bipyramidal structure is elucidated by a combination of experiment and theory. Furthermore, the combination of collision-induced activation with ion mobility mass spectrometry resolves the opening process of these large capsules that precedes fragmentation.

L. Turunen, U. Warzok, R. Puttreddy, N. K. Beyeh, C. A.Schalley, K. Rissanen, Angew. Chem. Int. Ed. **2016**, *55*, 14033–14036; L. Turunen, U. Warzok, C. A.Schalley, K. Rissanen, *Chem* **2017**, *3*, 861–869.

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# [P018] Studies of a L-proline catalyzed inverse electron demand Diels-Alder reaction by ESI MS

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The synthetic scope of the *L*-proline catalyzed inverse electron demand Diels-Alder reaction between tetrazines and ketones has been published by *Xie et al.*[1] in 2008 alongside a postulated catalytic cycle. They hypothesized that the enamine **I** derived from *L*-proline and a ketone acts as an electron rich dienophile which undergoes a [4+2] cycloaddition with the electron poor aryl substituted tetrazine in an inverse electron demand Diels-Alder reaction forming a Diels-Alder adduct **II**.[1] The Diels-Alder adduct **II** then undergoes a retro Diels-Alder reaction by eliminating nitrogen and forming intermediate **III**, which in turn eliminates *L*-proline and yields the pyridazine product.[1]
We set out to study the reaction with ESI-MS as to investigate the catalytic cycle. While studying the reaction without implementing a charge tag into any of the participating species the third intermediate **III** could be detected and kinetic studies could be obtained. A charge tagged tetrazine was synthesized as to be able to detect the second intermediate **II** as well, but to no avail; however again kinetic studies could be obtained. By using the charge tagged *L*-proline derivative from our group[2] as a catalyst all three intermediates could be detected and characterized by CID experiments. Thereby it was possible to thoroughly verify the catalytic cycle for the inverse electron demand Diels-Alder reaction.

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# [P019] Class Selective Ion-Molecule Reactions in a Differential Ion Mobility Cell

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Gas phase ion-molecule reactions frequently occur in rf-only collision cells and ion traps. However, until now, no defined reactions have been described for differential ion mobility spectrometry (DMS) devices except hydrogen exchange and adduct and cluster formation. In this study, we report the first delberate ion-molecule reactions in a DMS cell. A class selective reaction for 4-quinolone antibiotics was utilized to demonstrate the ability of performing chemical reactions in a differential ion mobility cell. Seven auxiliary compounds as chemical reagents in the DMS cell and four 4-quinolone antibiotics were used in this study. Dehydrated reactive precursor ions were formed and annulation reactions with the reagents occurred during the residence time within the DMS. Charge isomer separation by DMS and collision energy differences of adducts and newly formed compounds clearly demonstrated formation of covalent bonds.

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# [P020] Ion formation of saturated hydrocarbons in 3H-Ion Mobility Spectrometry is comparable to Atmospheric Pressure Chemical Ionization Mass Spectrometry

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[2] Institute of Food Chemistry, University of Hamburg – Germany

In the literature, ion formation in 3H or 63Ni ionization based ion mobility spectrometry (IMS) is commonly postulated as a purely proton transfer mechanism that occurs only with sufficiently high proton affinity. As a consequence, saturated alkanes are considered not to be amenable by such systems. Nevertheless, we were able to generate ions and fragments from alkanes, which however seem to follow a charge-transfer type of ionization mechanism. The drift time IMS (DTIMS) system used in this study employs a tritium source with nitrogen as drift gas, in which we could demonstrate that these substances feature a specific pattern of ions and respective fragments. Ions with reduced mobilities of 5.4, 5.1, 4.8, 4.5, 4.3 and 4.1 cm2/Vs could be observed for alkanes of different chain lengths. To prove the hypothesis of a potentially charge transfer followed by fragmentation, the mobility spectra were compared to mass spectra obtained by solvent-free atmospheric pressure chemical ionization mass spectrometry (APCI-MS) with a corona discharge ion source operated with nitrogen. Under these conditions, mostly charge-transfer reactions occur, which follow an EI-like fragmentation behavior. The observed ion pattern was similar to that obtained by the DTIMS system, which indicates at least a comparable ionization and fragmentation pathway.

*Speaker

# [P021] Self-Sorting Systems in Ion Mobility Mass Spectrometry

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In my Master thesis, I have found out, that IMS is fit to investigate self-sorting systems. I examined a system of a divalent axle and a divalent crown ether, which come together to form a self-sorted rectangle. We would like to further study different kind of self-sorting systems, which would ideally be switchable. For this, we have thought of a system of a divalent axle which would thread two different divalent crown ethers with a tetrathiafulvalen (TTF) spacer. With the two TTF units being in close proximity to each other, effects upon oxidation can be investigated. We propose the following possible oxidation steps: 1. One TTF unit is oxidized. This would stabilize the complex due to charge transfer interactions. 2. Both TTF units are once oxidized. A pi-complex could emerge, changing the geometry. 3. Both TTF units are doubly oxidized and the complex falls apart. 4. A third, electron-rich, divalent crown ether is introduced to the solution which could thread instead of a TTF-bearing one. Hence, through oxidation, the complex is switchable and two different crown ethers can be exchanged via oxidation. On my poster I would like to present these ideas and discuss possible outcomes with the participants of the congress.

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# [P022] IR Ion Spectroscopy of Key Intermediates of Carbene-Catalyzed Umpolung: Breslow Intermediates vs. Keto Tautomers

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[2] University Cologne, Germany – Germany
[3] Radboud university [Nijmegen] – Comeniuslaan 4, 6525 HP Nijmegen, Netherlands
[4] The University of Sheffield [Sheffield] – Western Bank Sheffield S10 2TN, United Kingdom
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Breslow intermediates, formed by the addition of a *N*-heterocyclic carbene (NHC) to a carbonyl functionality, are of fundamental importance for enzymology and for NHC derived organocatalysis. The (di)amino enol structure of Breslow intermediates formed of aldehydes and imidazolidinylidenes was first identified by Berkessel *et al.* by *in situ* NMR.1 However, the nature of the aldehyde and of the NHC influences whether an aminoenole, or its keto tautomer is formed.2,3

Additionally, Berkessel *et al.* reported that the reaction of benzaldehyde with a triazolin-5-ylidene NHC delivers the keto tautomer, whereas the analogous reaction with a saturated imidazolidin-2-ylidene NHC leads to the exclusive formation the diaminoenol tautomer.3 We now present first gas-phase spectroscopic data of ionic Breslow intermediates resulting from the reaction of a charge- tagged benzaldehyde with aromatic or saturated NHCs probed by infrared ion spectroscopy and theory (DFT). We demonstrate that the charge tag allows tandem MS and IRMPD-spectroscopy analysis of proto-typical Breslow intermediates. We investigated the ion structures by comparison of their IR spectra with computed ones, in particular on the basis of characteristic fingerprint IR absorptions bands. The IR structure assignments are consistent with 2D-NMR-spectroscopy and X-ray crystallography results.

A. Berkessel, S. Elfert, V.R. Yatham, J.-M. Neud´orfl, N.E. Schl´orer, J.H. Teles, *Angew. Chem.* **51** (2012), 12370-12374.

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# [P023] Identification and localization of neuropeptides in the brain of Cataglyphis desert ants using imaging mass spectrometry

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[3] SCiLS, Zweigniederlassung Bremen der Bruker Daltonik GmbH – Germany
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Desert ants of the genus *Cataglyphis* undergo an age-related polyethism with very distinct and brief behavioral stages. The internal regulation of this age-related behavioral plasticity is largely unknown. Recent studies associated neuropeptides with the underlying neuromodulation of the behavioral stage transitions in social Hymenoptera, including *Cataglyphis* ants. In insects, neuropeptides are known to modulate a wide range of physiological and behavioral processes including locomotor activity, feeding, and learning and memory; processes which likely change with the behavioral transitions in *Cataglyphis* ants. However, comprehensive neuropeptidomic studies on *Cataglyphis* ants are missing so far. We therefore characterized neuropeptides in the brain of *C. noda.* By using direct matrix assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS), we were able to identify 36 neuropeptides encoded by 19 different protein precursors. To study the spatial distribution of neuropeptides throughout the ant brain, we applied for the first time MALDI imaging MS on brain cryosections of *C. noda.* We were able to describe the distribution of 29 neuropeptides at a spatial resolution of up to 15 $\mu$m in 14 $\mu$m brain cryosections. To verify our generated IMS results, immunostainings were performed. We conclude that MALDI-IMS represents an appropriate and efficient tool to investigate the distribution of a wide number of insect neuropeptides, including multiple precursor products, with high spatial resolution in a single tissue section. Thus, MALDI IMS open up new possibilities for investigating insect neurobiology such as the different behavioral stages in ants.

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# [P024] IMS-MS of Responsive Crown Ether Ammonium Complexes

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The ever increasing interest in supramolecular functional materials, also highlighted by the noble prize of 2016, brings with it an increasing demand for new analytical approaches. Nowadays, a major focus lies on mechanically interlocked molecules (MIMs), which are hold together by their interlocked three-dimensional arrangement. This mechanical bonding allows the design of responsive materials that are not accessible via conventional covalent strategies. However, the analytic methodology necessary in this rapidly evolving field is still lacking. While NMR is the established analytical method, it is very limited with regard to complex mixtures and scaling over system size. Mass spectrometry is advantageous as multi-component mixtures are easily analyzed by their m/z. However, the m/z information alone is often insufficient to assess the dynamic response behavior. Here, we show that travelling wave ion mobility mass spectrometry can be a valuable alternative tool to study responsive supramolecular materials, and showcase advantages and limitations with several examples of crown ether ammonium chemistry.

*Speaker

# [P025] Anionic Palladium(0) and Palladium(II) Ate Complexes

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Anionic palladium(0) complexes are often suggested to be important intermediates in palladium-catalyzed transformations, but have up to now been only scarcely characterized at the molecular level [1-3]. Herein, we have examined the formation of anionic palladium(0) complexes from the electron-poor palladium catalyst [L3Pd] (L = tris[3,5-bis(trifluoromethyl)-phenyl]phosphine) and a lithium salt LiX (X = Br, I, OAc) with a variety of analytical techniques, including electrospray-ionization mass spectrometry, electrical conductivity measurements, and 31P NMR studies [4-5].
It was found that [L3Pd] readily reacts with the lithium salt to form [L$n$PdX]-Li+ ($n$ = 2,3). Ligand exchange experiments show that more electron-rich phosphine ligands do not tend to be incorporated in anionic Pd0 complexes.

Gas-phase fragmentation of [L3PdX]- yields [L2PdX]- in a ligand dissociation reaction, whereby the less-coordinated palladate complex [L2PdX]- is presumed to be a reactive intermediate in cross-coupling reactions. This ligand dissociation equilibrium is also present in solution according to the 31P NMR experiments.

Furthermore, the addition of an aryl iodide ArI to the palladate complex results in the formation of [L2PdArIX]- *via* oxidative addition.

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# [P026] Reactivity Revealed - Reductive Elimination from Ag(III) Ate Complexes

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[2] University of Melbourne – Australia

Anionic cuprates are an important class of organometallic compounds with numerous applications in synthetic organic chemistry and, thus, have been extensively studied.[1] In contrast, the chemistry of ate complexes of its heavier homologue silver has not been explored in detail. Electrospray-ionization mass spectrometry (ESI MS) has proven to be a valuable tool for the analysis of charged organo-copper species.[2-5] Here, we use a combination of ESI MS, gas-phase fragmentation experiments, and quantum chemical calculations to study the formation and unimolecular gas-phase reactivity of Ag(I) and, in particular, Ag(III) organometallates.

The reaction of AgCN with MeLi affords dimethylargentate(I) complexes, which form [RAgMe3]– species upon addition of organic iodides RI (R = methyl, butyl, allyl, phenyl). In collision-induced dissociation experiments, these silver(III) intermediates undergo reductive elimination reactions. For the cases of R = butyl and allyl, we observe a selectivity in favor of the cross coupling products RMe, which is in contrast to the gas-phase reactivity of the corresponding Cu(III) species.[5]

These results illustrate the distinct reactivity of silver and point to its potential to mediate synthetically valuable cross-coupling reactions.

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# [P027] Influence de la composition d'un peptide (encombrement stérique et rigidité) sur sa dynamique conformationnelle

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La dynamique conformationnelle associée à des transferts de charge (électrons ou protons) au sein de molécules biologiques font partie des mécanismes impliqués dans des processus fonctionnels tels que la photosynthèse ou la respiration cellulaire. Ces mécanismes sont difficiles à étudier expérimentalement en raison de la complexité des systèmes et de leur environnement. Grâce à un dispositif " pompe-sonde " laser à deux couleurs couplé à un spectromètre de masse, nous pouvons analyser la dynamique d'un de ces mécanismes, le transfert de proton, dans un système biologique simple – un peptide – et isolé – piégé en phase gazeuse. Nous avons montré, grâce à ce dispositif, que le transfert de proton entre le tryptophane et l'histidine, dans le penta-peptide HGGGW, nécessitait de l'ordre de quelques centaines de microsecondes et était limité par la dynamique conformationnelle du peptide. Il semble donc possible d'utiliser la signature du transfert de charge pour suivre la dynamique conformationnelle des peptides. Nous nous proposons donc d'utiliser ce dispositif pour apporter des éléments de compréhension sur l'influence de la composition en acides aminés des peptides en termes d'encombrement stérique et de rigidité, sur la dynamique conformationnelle. Pour ce faire, nous sonderons la dynamique du transfert de proton au sein d'une série de peptides de taille et de composition variable. Ainsi, on comparera plusieurs longueurs de chaines, mais aussi des chaines à glycine *vs.* proline, et enfin des chaines à glycine *vs.* alanines et isoleucines. Sur ce dernier point, des résultats préliminaires semblent indiquer que l'encombrement stérique joue un rôle limité.

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# [P028] Combining Comprehensive Two-Dimensional GC with Ultra High Resolution Time-of-Flight Mass Spectrometry (GCxGC-HRT+) for Non-Targeted Analysis of Complex Environmental Samples

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Gas chromatography coupled to mass spectrometry provides the best analytical tools, combining selectivity, sensitivity, reliability, and information capacity for targeted or non-targeted methods of environmental analysis. GC-MS identification of knowns and structural elucidation of unknowns becomes considerably more reliable, if complemented by accurate mass measurements using HRMS. However, when analyzing real-world samples containing a very high number of compounds of interest, a significant increase of chromatographic peak capacity is required, which can be provided only by using multidimensional GC. Therefore, GCxGC coupled to a high-resolution mass spectrometer is becoming a powerful tool for comprehensive analysis of complex samples.

A novel GCxGC-HRMS with enhanced sensitivity via Encoded Frequent Pushing was used for the measurements. The Duty Cycle is a critical parameter affecting the sensitivity of analysis by Time-of-Flight Mass Spectrometry with an Orthogonal Accelerator (OA-TOFMS). The Duty Cycle for an OA-TOFMS is defined as the ratio between the time to fill the acceleration region with the largest mass/charge ratio ions and the time-of-flight (TOF) through the mass analyzer of these ions.

LECO has implemented Encoded Frequent Pushing$^{TM}$ (EFP$^{TM}$), a method of pulsing an orthogonal accelerator multiple times per transient with unique time intervals between each push pulse. Environmental samples were analyzed in three different modes: 1D GC runs with EFP; GCxGC runs with EFP and GCxGC runs without EFP. More than 300 related analytes were detected in the samples while using GCxGC-HRMS, while only 70 analytes were detected using GC-HRMS. Examples of improved identification reliability using GCxGC-HRMS are presented.

*Speaker

# [P029] New application of direct analysis in real time high-resolution mass spectrometry (DART-HRMS) for the untargeted analysis of fresh and aged secondary organic aerosols (SOAs)

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Secondary organic aerosols (SOAs) represent a significant portion of total atmospheric aerosols. They are generated by the oxidation of volatile organic compounds (VOCs) and particularly biogenic VOCs (BVOCs). The analysis of such samples is usually performed by targeted methods that often require time-consuming preparation steps that can induce loss of compounds and/or sample contaminations. Recently, untargeted methods using high-resolution mass spectrometry (HRMS) have been successfully employed for a broad characterization of chemicals in SOAs. Herein we propose a new application of the direct analysis in real time ionization method (DART) combined with HRMS to quickly detect several hundred chemicals collected on a quartz filter without sample preparation or separation techniques.

The measurements were reproducible, with several hundred elemental compositions common to three different replicates. The relative standard deviations of the intensities of the chemical families ranged from 6% to 35%, with sufficient sensitivity to allow the unambiguous detection of 4 ng/mm$^2$ of pinic acid. Utilizing DART for samples collected at the atmosphere simulation chamber SAPHIR during an SOA experiment, the presence of oligomers and specific tracers was highlighted by MSn (n$\leq$4) experiments, an achievement that is difficult to attain with other ultrahigh-resolution mass spectrometers.

The applicability of this untargeted analysis by DART-HRMS was then tested by the study of the aging of several SOA obtained from limonene, from a mixture of b-pinene and limonene, and from scots pines emissions. The method has shown the apparition or the disappearance of specific classes of compounds after aging.

*Speaker

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# [P030] Use of bio-analytical approach as a strategy to identify compounds of interest in environmental complex mixture

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All compounds used in everyday life and endogen molecules are collected in wastewaters which release them after treatment in natural waters. These pollutants may cause environmental problems due to their adverse effects on wildlife; among them endocrine disrupting compounds (EDCs) are particularly important to study. The emission of these contaminants is of concern because some of them as well as their effect are unknown. Current challenges in analytical chemistry are to identify these EDCs in effluents of sewage facilities in order to assess their occurrence in natural waters. However, effluents contain thousands of compounds and the identification of all the molecules is challenging. Thus, it is necessary to develop specific strategies to identify EDCs. For this purpose, we developed a systematic approach combining effect-directed analysis (EDA) and high resolution mass spectrometry (HRMS) in order to target only biological active compounds. EDA aims at the establishment of cause-effect relationships by sequential reduction of the complexity of environmental mixtures, eventually to individual toxicants. Firstly, wastewater extracts were characterized for estrogenic, androgenic and glucocorticoid activity using *in vitro* bioassays. Then, the complexity of the samples was reduced by a finest fractionation by High-Performance Liquid Chromatography. Biologically active fractions were isolated for chemical identification performed using target analysis (LC-MS/MS-QQQ) and/or non target methodology involving HRMS (LC-QTOF). A confirmation step was then performed to prove the identified chemicals are those responsible for the observed effects. This approach allowed identifying several compounds including drugs, their metabolites, and EDCs such as plasticizers and phytoestrogens in wastewaters.

*Speaker

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# [P031] The Analysis of Environmental Samples Using High Resolution Mass Spectrometry to Identify Novel PFAS Compounds

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There are a very wide number of PFAS compounds that are actually present in environmental samples that are not part of the current EPA methods for PFAS analysis. The goal of this work was to use a QTOF mass spectrometer with Non Target Screening workflow to locate and identify unique PFAS compounds that were not known to be in the sample.
Water samples were analyzed using a Sciex X500R QTOF system set up to acquire data in a Non Target Screening workflow. The instrument first performed a TOF scan from 100-1500. This scan was followed by a data dependent scan where ions above a set threshold were selected for fragmentation and acquisition of a full scan MS/MS spectrum. The resulting MS/MS spectra were searched against a high resolution library to identify potential PFAS of compounds present in the samples.

Perfluoroalkyl substances (PFASs) encompass a range of fully fluorinated alkyl compounds and are prevalent in Aqueous Film Fire Foam (AFFF). In addition, PFASs are ubiquitous as they are used in many household goods and have been found in various environmental and biological samples. Here, we demonstrate the use of QTOF technology to exploit the power of high resolution mass spectrometry and the use of product ion spectra to identify novel compounds in AFFF contaminated samples.

*Speaker

# [P032] A fast and simple method for the quantification of iodinated X-Ray contrast media by LC-MS/MS analysis in HILIC mode

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The occurrence of emerging micro-pollutants, as pharmaceuticals, in aquatic environment became a major concern. Among these, iodinated X-Ray contrast media (ICM) are widely used in hospitals to visualize organs and soft tissues in order to perform diagnostic examinations. They are administered in high doses (60-200 g) and are eliminated in urine without metabolisation in 24h due to their high hydrophilic properties (log D between -4.2 and 0.45 at pH 7). They are then released in hospital effluents and have been reported to be removed with poor efficiency by conventional wastewater treatment plants. Therefore, they are frequently detected in surface water, groundwater and even drinking water. In this context, a fast LC-MS/MS analytical method, without sample preparation, except a dilution step, was developed to quantify seven ICMs, namely iohexol, iomeprol, iopromide, ioversol, iopamidol, diatrizoic acid, iopromide and iopentol in raw waters. Several chromatographic columns and mobile phase conditions were investigated and a good separation of the ICMs was obtained on a mixed-mode column used in HILIC conditions. A comparison between MRM and MRM3 ionization modes was performed for the mass spectrometric detection. The best results in terms of sensitivity, dynamic range and reproducibility were obtained with the MRM mode. The validation was performed using a synthetic matrix: the limits of quantification (LOQ) were comprised between 0.5 and 1 $\mu$g/L and linearity within the [LOQ-500LOQ] range. The method was then applied to various raw waters and allowed the quantification of ICMs at concentrations comprised between 1 and 400 $\mu$g/L.

*Speaker

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# [P033] Novel method for the sensitive quantification of Glyphosate, AMPA, Glufosinate and MPPA in water without derivatization.

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Glyphosate and glufosinate are two broad-spectrum systemic herbicides. AMPA and MPPA are their two major metabolites, respectively. Glyphosate is the most frequently used herbicide both worldwide and in the EU. Glufosinate is registered for use in Europe, but has been withdrawn from the French market since 2017 by the ANSES, due to its classification as a possible reprotoxic chemical. Due to their wide use and the restrictive regulations in the EU, very sensitive methods for their determination are required. As an example, the needed LLOQ for glyphosate and AMPA in water is 0.1 ppb (ng/mL) in the EU guidelines, and 0.03 ppb to get an agreement in France. Reaching such low LLOQs is very challenging due to their ionic character, low volatility, low mass and high polarity. Derivatization quickly become a standard procedure for their determination, but is exhaustive and time-consuming. We here report a 20 min HILIC method for the direct analysis of these pesticides in water. The samples were monitored using a high sensitivity UHPLC-MS/MS system (Nexera X2 and LCMS-8060, Shimadzu). Linearity in water was confirmed in the range 0.02-10 ppb for glyphosate, AMPA and glufosinate, and 0.05-10 ppb for MPPA, with $r^2$ of linearity models above 0.99, and S/N above 10 for LLOQ levels. Accuracies (and RSD) were calculated in mineral water at 0.1ppb (n=5) : 98.6 to 113.9% for glyphosate (RSD 5.5%), 97.2 to 115.2% for AMPA (RSD 6.8%), 97.8 to 100.8% for glufosinate (RSD 1.2%) and 92.5 to 108.9% for MMPA (RSD 5.6%).

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# [P034] Automatic, simultaneous and rapid analysis of pesticides in surface and underground water by on line SPE and UHPLC-MS/MS.

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Water contamination is a global problem caused by agricultural, nonagricultural and industrial pollution. Pesticides are chemo-synthetic products used in agriculture, for the maintenance of roads or by hobby gardeners to fight against organisms considered harmful to crops. Pesticides are carried by the wind at shorter or longer distances or they are leached by the rains and accumulated directly in the soil and water. Pesticides have a particularly slow natural degradation which makes them serious contaminants from surface water and underground water. Even if they initially have a role in crop protection, they end up to be dangers more or less important for animals, humans and ecosystems, with an immediate or long-term impact. These compounds cause health problems such as alterations of the nervous system, immune system diseases, fertility and development problems as well as cancers. Reference separation techniques were proved to be weakly sensitive or require tedious pretreatment protocol to reach thresholds set by environmental standards. Here is proposed a new on line SPE-LC-MS/MS method for simultaneous high sensitive quantification of 250 pesticides in surface water and groundwater with a total analysis time of 30 minutes. The lower limits of quantification (LLOQ) were between 1 and 10 ng/L depending on the compounds. Good repeatability was obtained (RSD$<$ 20% on 3 injections). This method is robust, automatic and allows to analyze lot of compounds in different waters matrix, on a single run, so it generates a huge saving of time.

*Speaker

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# [P035] Development of an on-line SPE-LC-MS/MS-method for the detection of estrogen trace levels in environmental waters and wastewaters

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Endocrine Disruptive Compounds include a wide range of substances such as natural and synthetic hormones, pesticides, phyto-hormones as well as industrial chemicals. Hormones such as estradiol and ethinylestradiol are reported to induce adverse effects on aquatic organisms below the nanogram per liter. The main source of these contaminants including steroids is wastewater treatment plants (WWTP) that release treated effluents in water compartments. In order to assess the presence of such substances, analytical strategies must be optimized to obtain very high level of sensitivity (< 0,5 ng.L-1). In this study, we focused on a list of compounds of interest including estriol, estradiol, estrone and ethinylestradiol. We developed a method based on large volume on-line Solid Phase Extraction coupled to Liquid Chromatography and tandem mass spectrometry (SPE-LC-MS/MS) to obtain quantification limits around Predicted No Effect Concentration.

On-line SPE in terms of loading speed, cartridge cleaning, and volume with regards to leak volume, as well as mass spectrometry parameters (electrospray source parameters, MRM transitions, acquisition parameters (dwell times, ...)) were optimized. Quantification limits (S/N = 9) obtained with the developed method ranged from 0.2 and 0.5 ng.L-1.

The methodology was tested on several types of water such as wastewaters, surface waters and drinking waters. Estrone was quantified in surface waters and WWTP effluents; no target steroids were detected in drinking waters.

*Speaker

# [P036] Study of the particle from the mainstream smoke of French and Indonesian cigarette by LDI-FTICRMS

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A non-targeted analytical approach was conducted by laser desorption/ionization high resolution mass spectrometry (LDI–FTICRMS) to highlight the specific composition of the particulate matter produced when cigarettes from the same manufacturer, but coming from France (regular and mentholated cigarette) or Indonesia (regular and kretek –i.e containing cloves– cigarette) are smoked. The influence of the filter (regular and "O-ring") was also investigated. The cigarette is smoked by a home-made smoking machine, which also allows the mainstream smoke particles to be collected on a quartz filter. After introduction in the ion source, laser desorption/ionization process (LDI) at 355 nm is performed and ions are investigated in positive and negative detection modes. After internal mass calibration, a mass measurement accuracy better than 1 ppm is obtained.
The detected ions are mainly protonated or deprotonated and to a lesser extent, radicals. They mostly come from the combustion or pyrolysis of the tobacco (CxHxN1-4 species). Whatever the ion detection mode, the main part (70 to 75%) of the detected species is common for the four samples. The base peak of the (+) LDI mass spectra is always the protonated nicotine. The CxHxO2-6 species, which come from the pyrolysis of the tobacco lignocellulosic material, significantly contribute to the TIC in (–) LDI. Significant differences are observed in respect which the smoked cigarette. For example, some CxHyO3-4 and CxHyN1-2O1-3 species are specific of the French regular cigarette and CxHyO5-6 compounds of the Indonesian kretek.

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# [P037] Fenton versus Ozone: Oxidation of Polyaromatic Hydrocarbons in contaminated Soil as clean-up procedure

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With ongoing industrialization and technological progress, more and more anthropogenic chemical pollutants were released into the environment. Polyaromatic hydrocarbons (PAHs) comprise one of the most occurring classes of contaminants in soil and have been identified as hazardous, because of their toxic, mutagenic and carcinogenic effects. PAHs are divided into low (LMW, 1-3 rings) and high (HMW, $\geq$ 4 rings) molecular weight compounds. LMW are more easily biodegradable than HMW, because of their better solubility and lower toxicity. In order to make HMW bioavailable, chemical oxidation can be used for transformation into biodegradable products. Ultrahigh-resolution mass spectrometry is a powerful method for the analysis of the various oxidized products obtained during the reaction.
The aim of our current study is to compare the efficiency of PAH oxidation using Fenton reaction or ozonation. In addition to the reaction conditions, different reaction setups were investigated. One difficulty when oxidizing HMW PAHs is the strong adsorbance of the pollutants on soil and thus the limited accessibility for the oxidizing agent.
Results from the mass spectrometric analysis using different ionization methods such as ESI(-), ESI(+) and APPI(+) show a more efficient oxidation of soil organic matter (SOM) and heteroatom containing PAHs compared to pure PAHs

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# [P038] Quantitative and qualitative analysis of three classes of sulfur compounds in crude oil

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Awareness of the environmental hazards and health related risks accompanied by the utilization of fossil fuels has lead to strict legislative rules. Among the most challenging rules is the limitation of sulfur content within transportation fuels to as little as 10 ppm for land based vehicles or 1000 ppm for ships within Sulfur Emission Controled Areas (SECA).
Such low limits require increased technological efforts as more and more heavy fuels with sulfur contents exceeding previously typical boundaries of 1-5 % are now being processed. The removal of sulfur from a given crude oil feedstock is typically performed by hydrodesulfurization (HDS) on a heterogenous catalyst. However, certain compounds such as alkylated dibenzothiophenes commonly withstand the process, while less condensed thiophenes and especially sulfidic compounds are desulfurized with relative ease.

A major problem for the evaluation of the quality of a given feedstock as well as for HDS process optimization is lacking knowledge of the quantitative distribution of sulfur containing compounds into the respective compound classes.

Here we introduce a new approach, that combines separation of sulfur containing compounds into different subgroups by HPLC with mass spectrometric analysis and uniform response quantification by ICP-MS/MS.
The combination of online-coupling of the separation method to both high resolving FTMS for qualitative analysis as well as to ICP-MS/MS for sulfur selective detection and quantification allows for the first time the simple and fast quantification of three different groups of sulfur containing compounds within a crude oil without any sample pre-treatment.

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# [P039] Analysis of microcystins in lake water by online Solid-Phase Extraction coupled to ESI-LC-MS-MS

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Microcystins, which are part of cyanotoxins, toxins produced by cyanobacteria, are considered as very harmful for health. They are hepatotoxic and potentially carcinogenic. Cyanobacteria, also called "blue-green algae", are observed as blooms since many years on the lake of Haute-Sûre (Luxembourg), which is an important recreational area and drinking water reservoir. In order to ensure that there is no risk in practicing activities such as bathing, water sport, fishing etc., the concentration of microcystins -LR, -RR and -YR in the lake was monitored during the algae proliferation season from May to November 2017.
As the formation of cyanobacterial blooms is dependent on factors such as weather, wind conditions and nutrients availability, the concentration of microcystins in water can increase in a sudden and rapid way, thus the analytical procedure should be fast and reliable. The biomass was separated from the water by filtration, before being extracted by ultrasonication. Filtered water and extracted biomass were then analysed by online Solid-Phase Extraction (SPE)-ESI-LC-MS-MS. This technique offers high sensitivity and specificity, requires minimum sample preparation and ensures maximal safety for the operator.
In recreational water, the World Health Organisation (WHO) indicates that an average value of 4 $\mu$g/L of microcystins corresponds to a low probability of adverse health effects. As soon as this concentration was reached, the bathing and water activities were forbidden in the monitored areas by the authorities. In 2017, two alerts were emitted, in August and October, confirming the seasonal public health risk due to contact with harmful cyanobacterial blooms.

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# [P040] Multi-residue analysis of pesticides in bee bread and pollen from Luxembourg

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Since the last decade, honey bees are subject to high losses in developed countries. Parasites and viruses, weather, changes in land use and exposure to pesticides have been identified as the main factors impacting bee health. Pesticides applied on crops can potentially contaminate pollen collected by bees. A part of the pollen is directly consumed, while another part is fermented and stored in the hives as bee bread. Among the objectives of the present study were the development of an analytical method for the quantification of pesticides in pollen and bee bread, and the investigation of a potential relationship between the occurrence of pesticides in these matrices and the mortality of honey bee colonies in winter. The main challenges of the analytical development were the complexity of the investigated matrices and to target the relevant pesticides in pollen and bee bread. A priority list of pesticides was established, based on scientific literature and authorised active substances in Luxemburgish crops. On the basis of this list of target pesticides, three complementary analytical methods were developed using Triple-Quadrupole LC-MS/MS (positive and negative modes) and GC-MS/MS. In addition, an extraction protocol, based on the QuEChERS method, has been optimized considering the matrices and pesticides of interest. The final methods allow the detection of 112 pesticides at the ppb level and have been applied on 85 bee bread and 154 pollen samples. Data were analysed statistically to investigate potential correlations between the colony mortality and the presence of individual or combined pesticides.

*Speaker

# [P041] Investigating molecular interactions of organotin compounds with proteins by means of LC-ESI-MS and LC-ICP-MS

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The application of organotin compounds (OTCs) as biocides in antifouling paints in the late 20th century has led to their wide distribution in the marine environment, especially in costal regions and harbors. Alongside with the first toxicological studies, OTCs were observed to cause toxic effects to living beings and further accumulate in the food chain. Despite their subsequent ban in most of Europe and North America in the 2000s, these xenobiotic pollutants are still present in the environment today due to their persistence in sediments and soils. Since the molecular mechanisms of OTC toxicity are still not fully understood, further research on their interactions with different biomolecules is necessary.
In this study, molecular interactions of di- and triorganotins with different proteins, including the model protein $\beta$-Lactoglobulin A (LGA), were investigated by means of liquid chromatography (LC) coupled to electrospray ionization-mass spectrometry (ESI-MS) and inductively coupled plasma-mass spectrometry (ICP-MS). To simulate physiological conditions, OTCs and proteins were incubated at distinct molar ratios in phosphate buffered saline (pH 7.4) at 37 ∘C prior to the analysis.
The complementary approach using LC-ICP-MS and LC-ESI-MS enabled the identification and characterization of several protein-OTC adducts from the incubated solutions. Moreover, the adducts reveal different binding characteristics for di- and triorganotin compounds and free thiol groups of the examined proteins might be involved. Under acidic conditions, the adducts were observed to be unstable, which impedes the sample work-up and requires the development of mild chromatographic conditions regarding pH, mobile phase additives and ionic strength.

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# [P042] Cluster Analysis of Tandem Mass Spectra

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In LC-MS/MS experiments peptides are often chosen for fragmentation more than once, resulting in multiple $MS^2$-spectra that originate from the same amino acid sequence. These spectra are not identical because the corresponding peptides may be decomposed at different bonds and produce different fragmentation patterns, but they are similar. Cluster analysis groups these spectra by similarity and finds sets of spectra that are likely to belong to the same peptide. Choosing one representative spectrum per cluster reduces the amount of data and can for example be helpful for the construction of spectral libraries. It also results in an improvement of peptide identifications and can be a part of quality control. Furthermore, cluster analysis can shed light on the "dark matter of proteomics", $MS^2$-spectra that remain unidentified after database searches. These spectra, which amount to a high percentage of measured spectra, might originate from peptide variants or post-translational modifications that are especially interesting in biomarker research.

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# [P043] Protein ANOVA and Background based t-test inference in proteomic analyses

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Expression change inference in mass spectrometry-based proteomics faces several challenges. Material and acquisition time restrict noticeably the number of measured biological and technical replicates reducing the statistical power of protein ANOVA-based analyses. In order to circumvent this issue, proteins may be compared to a background of non-changing proteins assuming that quantification variability is strongly related to detected signal to noise. Comparing each protein to several proteins is also advantageous since it allows taking into account technical and biological variability. While this method works reasonably well in most of proteomics profiling experiments, it requires quantification of a substantial number of background proteins. Many experimental approaches like immunoprecipitations or subcellular enrichments would not provide background enough for a correct expression change inference. Additionally, a higher number of replicates make protein ANOVA-based inference over perform inference precision of background-based t-test. We are interested on exploring the limitations of each methodology.
Two samples were prepared by mixing three different proteomes in known ratio. Cell cultures were first digested with trypsin, mixed, labelled with Thermo TMT-10plex reagents , and then fractionated by high pH reversed-phase peptide fractionation resulting in 10 fractions for each sample. Each fraction was acquired twice in a Thermo LTQ-Orbitrap Elite instrument.

We analyzed different fraction mixes (modifying the number of quantified proteins) and different number of replicates (by using different numbers of TMT channels) in Proteome Discoverer 2.2.

Data analysis allowed us to find advantages and limitations of both strategies to better define which method should be applied.

*Speaker

# [P044] Data fusion of orthogonal mass spectrometric and spectroscopic data obtained from food analysis – is it worth the effort?

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The increasing consumers' interest in the safety, quality and authenticity of food has also driven forward the complexity of analytical techniques. Fast sensors, spectroscopic and hyphenated techniques have emerged and together with chemometric workflows, data analysis has been improved significantly by reducing analysis time and providing more informative results. Nevertheless, selectivity and sensitivity issues of the individual techniques still remain, therefore, the fusion of data of different techniques has become more and more a means for increasing the reliability of classification or prediction of foodstuff specifications as compared to using a single analytical technique. Although promising results have been obtained in a number of application fields, e.g. food and beverage authentication, the combination of data from several techniques is challenging.
This study compares the potential of data fusion approaches on data obtained from the analysis of authentic honey samples from different botanical origins by FT-IR, MALDI-TOF-MS and headspace GC-IMS. In the first step, the data were preprocessed accordingly and then analyzed by PCA-LDA, $k$NN and SVM algorithms. The classification error rates were determined by 10-fold cross validation. Secondly, data from MALDI-TOF-MS and GC-IMS or respectively FT-IR and GC-IMS were combined into a common data matrix after appropriate weighting. Finally, a PCA-LDA, kNN and SVM analysis was performed and the error rates were determined again.
In all cases, the classification error rate was significantly reduced and the predictive quality was improved. This demonstrates that data fusion of multidimensional data from orthogonal sources may significantly increase reliability of chemometric workflows.

*Speaker

# [P045] Feature detection pipeline for UDMSE data sets in Apache Spark

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Data-independent acquisition (DIA) strategies overcome typical limitations arising from data-dependent acquisition (DDA), such as unbalanced precursor ion selection, and thus are increasingly used in quantitative proteomic studies. In combination with the integration of traveling wave-based ion mobility separation (TW-IMS) in commercial instruments, e.g., SYNAPT G2-S, DIA workflows improve both reproducibility and proteome coverage. However, the integration of TW-IMS causes a dramatic increase of complexity of resulting raw data and puts challenges on existing analysis tools as well as storage routines. Furthermore, the acquired raw data are stored in proprietary formats and the provided software packages for handling these formats are usually closed-source, hampering the development of open-source processing tools. Here, we present a prototypical pipeline for feature detection of ultradefinition (UD)MSE raw data based on our open-source file format triMS5 (currently prepared for publication). With regard to continuously growing raw file sizes, we developed the pipeline in the well-established Big Data framework Apache Spark to allow efficient analysis in a reasonable amount of computing time. In a first step, peak picking is performed by means of the convolution of the signal with a wavelet. These detected peaks are then grouped to form isotopic patterns. Finally, characteristics of the features are extracted as fitted model parameters. The entire pipeline was successfully implemented and tested on a small scenario with promising results.

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# [P046] Isotopic pattern analysis based on a new Fourier deconvolution approach

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With the presence of isotopic pattern of different peptides in various charge-states, the efficient analysis of MS spectra is still an open question, specially when MS spectra present an important spectral overlap with a poor resolution. The large size of MS spectra and the complexity of the pattern models increase the difficulty of their analysis. These constraints require the usage of efficient data processing algorithms, able to resolve this problem.
In our work, we propose to formulate this problem as a convolution problem of isotopic pattern with different charge-states.
To solve this problem, an estimation of sparse signal using a dictionary-based approach relying on the protein averagine model will be applied with a new convex optimisation based on proximity operator of the l1 regularization function. A proximal primal-dual splitting convex algorithm is used in this case.
Unlike the peak-picking strategy, our method can be considered as a general approach to analyse the isotopic pattern even with high spectral overlap.
The quality and robustness of this approach is tested on synthetic data-sets, and examples of use are shown on bottom-up, top-down proteomics and on native MS.
Our approach is dedicated to proteins model based on averagine model on which an application from FTICR spectra will be shown. Moreover, an extention of this method to 2D-FTICR MS experiments will be presented.

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# [P047] Complex two-dimensional matrix plots for mapping genealogical links between products in complex mixtures of lignin degradation products

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Today, waste has become an important resource. Lignin is a complex phenolic biopolymer and provides a useful biomass for obtaining a wide range of aromatic molecules, resulting in highly complex mixtures of thousands of different molecules. Typical linkages between monomers include various phenyl linkages and bonds originating from $\beta$-carbon. Here, ESI and high resolution FTICR-MS afforded the necessary performance characteristics to provide detailed insight into the complex mixtures, exhibiting $>$ 1000 assigned products within a single full scan spectrum. Lignin mixtures generated ultra-complex mass spectra with a density of over 20 compounds within a 0.1 u wide window, with peak abundances differing over several orders of magnitudes. And therefore, many of the low abundant compounds were impossible to be pre-isolated for MS/MS analysis. The two-dimensional fractional mass filtering was utilized as a visualization tool to allow meaningful interrogation of the complex data sets. The plots provided systematic line ups of the different lignin linkages using structure-specific metrics, which greatly simplified data interpretation, as theoretical m/z values and chemical structures were readily deduced from the genealogical links using specific metrics, rather than unsystematically assigning elemental compositions. Starting from the low m/z region, consisting mainly of monomers; the core structure of lignin can be easily identified via MS/MS. The higher oligomers originated from the same linkages and were quickly visualized using the repeating units; structures of higher molecular weight but lower abundance in the samples can be predicted based on this information. The proposed structures were all confirmed by detailed CID experiments.

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# [P048] BioInfra.Prot: Bioinformatics Services for Proteomics

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The service center BioInfra.Prot, which is part of the German Network for Bioinformatics Infrastructure (de.NBI) and ELIXIR, provides a comprehensive portfolio of bioinformatics services for proteomics. This includes data standardization, data conversion and data publication services as well as computational and statistical consultancy and data analysis (esp. protein inference and expression analysis), software tools, hardware sharing and training courses. Altogether a comprehensive proteomics workflow is offered. The relatively high usage of these free and high-quality services confirms the strong need for bioinformatics infrastructure in proteomics research.

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# [P049] Pharmacometabolomics reveal side effects: Dexamethasone application in inner ear cell model

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Cochlear implants are hearing aids that provide a sense of sound for patients with impaired hearing. The auditory nerve innervating the cochlear is stimulated by electric signals. It has been reported that scarring of tissue around the implant due to inflammation increases impedance and thus decreases efficacy of the hearing aid. Hence, the impedance is increased and subsequently the efficacy of the hearing aid is decreased. Matrix embedded Dexamethasone has shown to inhibit fibroblast proliferation. To the best of our knowledge, no data has been collected on the influence of glucocorticoids to the cellular homeostasis of inner ear cells. This was investigated in a cellular model using House Ear Institute-Organ of Corti 1 cells (HEI-OC1) cells. HEI-OC1-cells have been incubated with Dexamethasone for 1, 2 or 3 days. After harvesting, cells were lysed and metabolites were extracted. GC-MS-profiling and subsequent annotation using Nist14-library search yielded 100 metabolites. Statistical analysis using MetaboAnalyst revealed characteristic metabolic signatures in DEX-treated HEI-OC1-cells. This is the first step for a deeper pharmacometabolomic understanding of effect and side effect of DEX in inner-ear-application.

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# [P050] Improved riboflavin production with Ashbya gossypii from vegetable oil based on 13C metabolic network analysis with combined labeling analysis by GC/MS, LC/MS, 1D, and 2D NMR

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The fungus *Ashbya gossypii* is an important industrial producer of riboflavin, i.e. vitamin B2. In order to meet the constantly increasing demands for improved production processes, it appears essential to better understand the underlying metabolic pathways of the vitamin. Here, we used a highly sophisticated set-up of parallel 13C tracer studies with labeling analysis by GC/MS, LC/MS, 1D, and 2D NMR to resolve carbon fluxes in the overproducing strain *A. gossypii* B2 during growth and subsequent riboflavin production from vegetable oil as carbon source, yeast extract and supplemented glycine. The studies provided a detailed picture of the underlying metabolism. Glycine was exclusively used as carbon-two donor of the vitamin's pyrimidine ring, which is part of its isoalloxazine ring structure, but did not contribute to the carbon-one metabolism due to the proven absence of a functional glycine cleavage system. The pools of serine and glycine were closely connected due to a highly reversible serine hydroxymethyltransferase. Transmembrane formate flux simulations revealed that the one-carbon metabolism displayed a severe bottleneck during initial riboflavin production, which was overcome in later phases of the cultivation by intrinsic formate accumulation. The transiently limiting carbon-one pool was successfully replenished by time-resolved feeding of small amounts of formate and serine, respectively. This increased the intracellular availability of glycine, serine, and formate and resulted in a final riboflavin titer increase of 45 %.

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# [P051] Metabolic flux analysis of Pseudomonads - resolution of parallel and cyclic pathways of glucose catabolism using parallel 13C tracer experiments and GC-MS labeling information of various biomass constituents

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*Pseudomonads* are microbes of high relevance. *Pseudomonas putida* is a widely used workhorse in industrial biotechnology [1], whereas *Pseudomonas aeruginosa* is receiving increasing interest as human pathogen [2]. *Pseudomonads* are known to assimilate glucose via three peripheral pathways converging at the level of 6-phosphogluconate, which is cleaved into glyceraldehyde 3-phosphate and pyruvate via the Entner-Doudoroff pathway [3]. In addition, triose phosphates might be recycled back to glucose 6-phosphate.
Unfortunately, these features could not be resolved in previous studies, which relied on the most routinely applied approach for microbial fluxomics, the measurement of proteinogenic amino acids whose 13C labeling is used to derive the flux information. Here we present a GC-MS-based approach to resolve the complex metabolic architecture of *Pseudomonads* combining parallel labeling experiments using different tracer mixtures with the measurement of labeling obtained from amino acids, storage carbohydrates and cell wall constituents. The additional information resulted in an extensive data set with sufficient power to resolve all relevant fluxome features in the investigated bacteria. Excellent fits between experimental and simulated data were obtained. Using the novel approach, studies for *P. putida* on glucose revealed distinct flux adjustments, when the strain is stressed by toxic aromatic compounds. Finally, the new technique was successfully applied to *P. aeruginosa*, yielding for the first time highly defined metabolic flux distributions for this human pathogen.

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# [P052] Metabolite profiling, antibacterial and antioxidant activity of leaf extracts from three Myrtaceae plants grown in Vietnam.

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*Cleistocalyx operculatus*, *Psidium guajava* and *Rhodomyrtus tomentosa*, three popular plants in Vietnam belonging to Myrtaceae family have been used for medicinal purposes since the dawn of time. In this study, we evaluated the potential of the leaf extracts of these three plants to be used as antimicrobial and antioxidant agent in the food industry. Interestingly, all three extracts showed high inhibitive activity on the growth of 7 bacteria isolated from spoiled cold-stored white-leg shrimps (*Penaeus vannamei*). The antimicrobial activity was dependent on the concentration of the extract and on the bacterial strains considered. The extract powder of *R. tomentosa* leaves had the highest antioxidant capacity (4.00 mmol Trolox equivalent (TE)/g) followed by the one of *P. guajava* leaves (3.99 mmol TE/g) and that of *C. operculatus* leaves (2.85 mmol TE/g). In order to better understand these activities, a metabolite profiling of the three extracts was carried out by UPLC-TripleTOF. High resolution mass spectrometry data combined with fragmentation data allowed us to identify around 30 predominant components in each of the three species, all belonging to the phenolic or triterpene family of compounds. The findings of this study suggested the potential application of three Myrtaceae plants as sources of antimicrobial agent in cold storage of shrimps.

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# [P053] Utilizing very high resolution for selective component separation in complex matrices

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The analysis of complex sample matrices is a common challenge in many metabolomics applications and identifying specific compounds of interest is an important step. Here, we demonstrate the utility of applying a mechanism for selective component detection utilizing very high resolution analysis of complex plant matrix samples to allow direct detection of compounds by detection and identification of the resolved hyperfine 18O / 13C isotopic signature on a chromatographic timescale. Samples of various plant matrices were chromatographically separated on a simple reverse phase linear gradient with subsequent mass spectrometric analysis by electrospray ionization in the positive mode (Orbitrap$^{TM}$ Fusion Lumos$^{TM}$). Full scan MS1 data acquisition was performed at a resolution of 1,000,000 (FWHM) at m/z 200. The data was processed to identify all peaks in the data with isotopes and adducts assembled to create components. Components which were flavonoid conjugate candidates were identified in matrices containing 4,000-6,000 compounds by using the fine isotopic pattern of the resolved signal for 18O and 2X13C in the A2 isotope to flag peaks which contained more than 8 oxygen atoms. Subsequent confirmation was performed by interpretation of acquired MSn fragmentation spectra. This technique can be applied to many different natural isotopic patterns as a means to provide additional selective detection when very high resolution analysis is applied.

*Speaker

# [P054] Metabolic Characterization of Anks3 Depletion in Murine Collecting Duct Cells by GC-MS and LC-MS/MS coupling

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Nephronophthisis (NPH) is an autosomal recessive variant of cystic kidney disease and the most frequent cause of hereditary kidney failure in childhood and young adults. Like NPH proteins, the NPHP16/Anks6-interacting protein Anks3 has been identified to cause laterality defects in humans. However, the cellular functions of Anks3 remain enigmatic. Combination of different mass spectrometric enable a deeper understanding of metabolic dysregulation in disease context. Therefore, we investigated the metabolic impact of Anks3 depletion in cultured murine inner medullary collecting duct cells via GC-MS profiling and LC-MS/MS analysis. Out of 155 successfully identified metabolites, 48 metabolites were identified to be differentially regulated by decreasing Anks3 levels. Especially, amino acid and purine/pyrimidine metabolism were affected by loss of Anks3. Branched-chain amino acids were identified to be significantly downregulated suggesting disrupted nutrient signalling. Tryptophan and 1-ribosyl-imidazolenicotinamide accumulated whereas NAD+ and NADP+ concentrations were diminished indicating disturbances within the tryptophan-niacin pathway. Most strikingly, nucleosides were reduced upon Anks3 depletion, while 5-methyluridine and 6-methyladenosine accumulated over time. These results suggest that Anks3 may be involved in DNA damage responses by balancing the intracellular nucleoside pool.

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# [P055] Mapping of Greek olive oil using FTMS FIA and multivariate data analysis

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The increasing popularity of virgin olive oil (VOO) has provided the need for quality and authenticity control. Its chemical complexity impedes the transaction of the typical analytical methods. In this study, a holistic approach to map Greek VOO is presented. The workflow takes advantage of the rapid, LC free, FIA based data acquisition by ultra-high resolution FTMS. The obtained mass spectra were evaluated using a novel software for deisotoping, as well as for identifying the most significant metabolites. Additionally, the data was subjected to multivariate data analysis which revealed clusters and trends according to significant discriminating factors of VOO, such as geographical origin, cultivation practice and production procedures.
Samples were collected from the main Greek olive oil producing regions and stored at room temperature, in darkness, under nitrogen. Stock solutions were prepared by dissolving 10uL of samples in 500 mL MeOH. The stock solutions were then diluted 1:20 in 50% MeOH + 10 mM Ammonium Acetate. VOO samples and their biophenolic extracts were analyzed using a Bruker solariX-XR 7T mass spectrometer using ESI (-) mode by FIA.

C free, FIA-MRMS based profiling enabled to distinguish different Greek virgin olive oils (VOO). FIA facilitates VOO analysis, requiring limited sample preparation. Interesting clusters were revealed according to geographical origin of VOO and the used cultivation practice. The responsible metabolite biomarkers were identified. The novel software provides an intuitive and powerful new workflow which enabled the profiling of FIA-MRMS data and confident assignment of molecular formulas for metabolite markers.

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# [P056] Targeted and non-targeted metabolomics to study developmental neurotoxicity of biocides - Metabolomic pathway analysis reveals developmental neurotoxic effects of pesticides

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Serious concern has arisen worldwide regarding the dramatic increase in incidences of learning and developmental disorders in children. Recently, various epidemiological studies have indicated that during childhood, exposure to low doses of biologically active contaminants in the environment can have deleterious effects on cognitive and behavioral development. The aim of the current study was to investigate the behavioral and cognitive effects of pesticide exposure in mice and to study the underlying molecular mechanisms of the observed associations using metabolomics. This study was part of the European DENAMIC project.
Mice were exposed to a single dose at PND10 of the pesticides chlorpyrifos, carbaryl, cypermethrin, PFHxS or endosuldfan (0.5 to 20 mg/kg bw). Metabolomic analysis was carried out using methanolic:chloroform extracts of mouse brain tissues (cerebral cortex and hippocampus). Data for targeted and non-targeted metabolite analysis was acquired by HILIC-QTOF-MS/MS (Compact, Bruker Daltonics). MetaboScape 3.0 (Bruker Daltonics) was used for data processing.
The applied software provides a fully integrated workflow for discovery metabolomics and enabled a higher throughput for profiling of complex tissue extracts. Data analysis time was reduced due to the simultaneous and confident assignment of known targets and tentative annotation of unknown peaks. Early-life exposure of pesticides can result in persistent effects on behavior and cognition later in life.

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# [P057] Mitochondrial metabolomics reveals compartment-specific metabolic response in yeast cells

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**Introduction**: Subcellular compartmentalization enables eukaryotic cells to carry out different reactions at the same time, resulting in different metabolite pools in the subcellular compartments. Thus, mutations affecting the mitochondrial energy metabolism could cause different metabolic alterations in mitochondria compared to the cytoplasm. Given that the metabolite pool in the cytosol is larger than that of other subcellular compartments, metabolic profiling of total cells could miss these compartment-specific metabolic alterations.
**Objectives**: To reveal compartment-specific metabolic differences, mitochondria and the cytoplasmic fraction of baker's yeast *S. cerevisiae* were isolated and subjected to metabolic profiling.

**Methods**: Mitochondria were isolated through differential centrifugation and were analyzed together with the remaining cytoplasm by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) based metabolic profiling.

**Results**: Seventy-two metabolites were identified, of which eight were found exclusively in mitochondria and sixteen exclusively in cytoplasm. Based on the metabolic signature of mitochondria and of the cytoplasm, mutants of the succinate dehydrogenase (respiratory chain complex II) and of the FOF1-ATP-synthase (complex V) can be discriminated in both compartments by principal component analysis from wild-type and each other. These mitochondrial oxidative phosphorylation machinery mutants altered not only citric acid cycle related metabolites but also amino acids, fatty acids, purine and pyrimidine intermediates and others.
**Conclusion**: By applying metabolomics to isolated mitochondria and the corresponding cytoplasm, compartment-specific metabolic signatures can be identified. This subcellular metabolomics analysis is a powerful tool to study the molecular mechanism of compartment-specific metabolic homeostasis in response to mutations affecting the mitochondrial metabolism.

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# [P058] Non-targeted High Resolution Profiling to Characterize Metabolic Responses of Nicotiana attenuata on the Infection with a Mycorrhizal Fungus

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Exploring the chemical composition of plants is challenging due to high dynamics and modification by environmental factors. Knowledge of these modulations is necessary to decode the underlying mechanisms. Metabolomes as mediators of biological functions are important for understanding the plant's ecological interaction. The interaction between plants and root colonizing fungi was shown to result in extensive reprogramming of plant metabolism. However, efficient workflows are required to rapidly analyze metabolite profiles and to discriminate known compounds from novel ones. With a software-based metabolite identification method we completely assessed the metabolic responses of *Nicotiana attenuata* during an infection with the mycorrhizal fungus Rhizophagus irregularis.
Nicotiana plants were grown in dual-communities, with either living or dead inoculum of *Rhizophagus*. Tissue of Nicotiana was harvested 6 weeks after inoculation and extracted with 80% methanol. Chromatographic separation of extracts was done using an UltiMate 3000 RSLC (Thermo Fisher) on an Acclaim RSLC 120 C18 2.2$\mu$ column. MS analysis was performed using a high-resolution QTOF (impact II, Bruker Daltonics) in ESI positive ion mode. MetaboScape 3.0 (Bruker Daltonics) was used for multi-pass, region complete, feature extraction and alignment, dereplication of known compounds, the identification of novel metabolites and pathway mapping.

This workflow enabled us to detected and link diverse effects in the phytometabolome of *N. attenuata* during R. irregularis infection. E.g., AMF infection strongly influences malonylation of HGL-DTGs. A novel phenolamide was identified by comparing MS/MS spectral similarities. The workflow and results will be presented, also as an example for similar metabolic studies.

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# [P059] Untargeted serum metabolite profiling of colorectal cancer using GC-Orbitrap technology

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Globally affecting more than one million new persons each year, and killing more than 700,000, colorectal cancer is the second leading cause of cancer-related deaths in women and the third in men. Nevertheless, diagnosis is still largely based on invasive tissue sampling, while gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics is one way to address these issues. Through metabolite profiling, it provides a picture of the outcome of the disease. To do so, significant variations between pathological and healthy phenotypes have to be found, and the responsible metabolites must be confidently identified. In this study, the ability of the Q Exactive GC-MS Orbitrap system to detect and identify metabolites related to colorectal cancer in an untargeted manner was assessed. The workflow uses the advantages of high peak capacity and chromatographic resolution of gas chromatography with the high resolution and sub-ppm mass accuracy of the Orbitrap mass spectrometer. The samples analyzed belonged to two populations linked to colorectal adenocarcinoma (active and remission, 12 samples each) along with two controls cohorts of the same size specifically matched for possible biases (gender, age, BMI, smoking status etc.), and pooled QC samples. Analytical raw data files were automatically processed through two software platforms specifically designed for the Orbitrap technology (TraceFinder™ and Compound Discoverer™). Compound identification was made using existing commercial libraries as well as an in-house developed high resolution Orbitrap metabolomics library.

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# [P060] Mass spectrometrical investigation of the Fulvuthiacene biosynthesis

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The discovery and characterization of novel scaffolds from bacterial sources is becoming more and more challenging because low hanging fruits such as highly abundant metabolites and those featuring strong MS or UV signals have already been characterized in the past. Therefore innovative approaches are necessary to guide the choice of producers and to mine their metabolomes in order to discover novel natural product scaffolds exhibiting biological activities. In this work, a myxobacterial strain belonging to the species *Myxococcus fulvus* was subjected to a comprehensive dereplication and statistical metabolome analysis workflow, comprising high resolution MS and MS/MS data evaluation to reveal the presence of novel compound families. A putatively novel family of PKS-NRPS hybrid compounds was identified and selected for further investigation. Subsequent upscaling afforded sufficient amounts of natural product to enable structure elucidation using 1D and 2D NMR spectroscopy, revealing the structures of two novel myxobacterial secondary metabolites named Fulvuthiacene A and B. A candidate secondary metabolite biosynthesis gene cluster responsible for the compound family's biosynthesis was identified in the producer strain's genome. By analyzing candidate tailoring enzymes encoded within the cluster with respect to the secondary metabolite structure, an intriguing post-PKS modification cascade putatively responsible for the synthesis of the Fulvuthiacenes' terminal methoxymethacrylate moiety was identified. Heterologous transfer experiments of this biosynthetic machinery to the Myxothiazol biosynthetic gene cluster were performed to shed light on the underlying enzymology and to enable the production of new hybrid compounds.

*Speaker

# [P061] An isotope-dilution mass spectrometry based metabolomics workflow to study bacterial cell cycle dynamics

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The output of chemical processes summarized as metabolism most directly reflects the cellular phenotype. However, biological processes and regulation is often dynamic, and reliable metabolome-profiling in a time-resolved manner remains challenging. Here, we introduce a dynamic isotope-dilution based untargeted metabolomics approach using liquid chromatography high-resolution mass spectrometry (LC-HRMS) and apply our workflow to study cell-cycle dependency in the metabolome of a model bacterium. The basic principle of our method is the untargeted assembly of extraction windows to build an organism-specific metabolite table for isotope-dilution based LC-HRMS data. To this end, we use non-targeted extraction of metabolite features from U-12C and U-13C-labelled bacterial extracts and map respective m/z features to a corresponding isotope-dilution sample. Predicted 13C distances allow matching the corresponding U-12C/U-13C mass traces to individual metabolites. This yields the respective extraction windows for a subsequent, highly quantitative, IDMS-based metabolomics approach. Concomitantly, isotope matching helps reducing the noise of assembled metabolite-features and improves their identification by constraining possible number of carbons. To exemplify our workflow, we performed dynamic metabolomics on a cell-cycle synchronized, bacterial culture. Overall, we were able to follow quantitative changes of hundreds of metabolites during a bacterial cell cycle, to identify global changes of metabolite pools, and to describe a discrete metabolic footprint that accompanies the G1 to S phase transition.

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# [P062] Simultaneous untargeted and targeted profiling of underivatized primary metabolites on sulfur-deficient barley

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Metabolomics based-mass spectrometry are increasingly applied in diverse scientific domains, notably agronomy and plant biology, to understand plants' behaviors under biotic/abiotic stress conditions. In fact, these stress conditions are able to disrupt many biosynthetic pathways that include mainly primary metabolites. Profiling and quantifying primary metabolites remain a challenging task because they are poorly retained in reverse phase columns due to their high polarity. The aim of our method which is to simultaneously perform an untargeted/targeted metabolite profiling in order to understand the nutrient deficiency effect on plants. Two fast and accurate methods were developed to detect and quantify amino acids, organic acids, sulfur metabolites, and secondary metabolites using an UPLC coupled to a QTOF mass spectrometer. An HSS T3 column was used to analyze amino acids and sulfur containing metabolites in positive ionization mode, and a Luna® Omega PS C18 column was used for organic acids profiling in negative mode. Ionization was achieved using an electrospray ion source (ESI). Methods were successfully applied allowing to detect, quantify and discriminate primary metabolites in short-runs without any additional sampling step such as derivatization or ion pairing. On the other hand, untargeted analysis was conducted using Progenesis QI performing alignment, peak picking, normalization, metabolite identifications and multivariate analysis. The simultaneous analysis provided cumulative information allowing to discriminate between two plant batches. Thus, discriminant biomarkers were identified and validated. A fast and innovated simultaneous untargeted/targeted method has successfully been developed and applied to sulfur deficiency on Barley.

*Speaker

# [P063] Effect of sample preparation on the hydrophilic metabolites of human plasma samples

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Introduction:
The aim of this study was to analyse the effects of a filtration step on the hydrophilic metabolites of human blood samples.

The sample preparation can be seen as a crucial step. Filtration is often part of the sample preparation. Experiments showed a high impact of filtration on the metabolite spectra, in particular on the intensity of metabolites or the occurrence itself.

Material and Methods:

Human plasma samples were extracted by a modified SIMPLEX approach, i.e. the ratio of sample to solvent volume was changed in comparison to the original protocol. Before the measurement by FT-ICR-MS the hydrophilic phase was either centrifuged or filtered or treated in both ways in order to remove protein residues. The protein content was analysed by Bradford protein assay.

Data validation was conducted by the quality control (QC) approach. Data were evaluated by non-targeted (non-supervised) and semi-targeted approaches using MetaboScape 3.0 (Bruker, Germany).

Results:
Results from the non-supervised approach indicate the impact of the sample preparation. The PCA-models showed tight clusters for each treatment. It was possible to detect different metabolites that showed a higher intensity in comparison to the filtered samples or are only present in the centrifuged samples. Out of 10.000 detected metabolites more than 700 were influenced by the filtration step.

*Speaker

# [P064] Non-targeted metabolic profiling of the downy mildew attack on V. vinifera

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Downy mildew caused by *Plasmopara viticola*, is a highly destructive disease of grapevines in all grape-growing areas of the world. Although all green parts of the grapevine are susceptible, the first symptoms are usually seen on the leaves as soon as 5 to 7 days after infection. In the present study, the two grapevine cultivars "Regent" and "Ḿuller-Thurgau" were infected with two different isolates of *P. viticola.* It was seen that the isolates produced different reactions on the tolerant grapevine cultivar "Regent". However, in "Ḿuller-Thurgau" (a sensitive cultivar) both isolates behaved very similarly. We attempt to interpret the chemical communication between the plant and the pathogen that allows one isolate to produce unrestricted infection and the other to just manage to survive. In order to examine the metabolic mechanisms behind these different reactions, UPLC-ESI-HRMS was done on infected and non-infected sample leaf extracts. The secondary metabolites were studied since they are key to understanding how plants respond to their environment, to stress, and what mechanisms are involved. In particular, phytoalexins like stilbenes are important for plant defense. Stilbenes are produced in the phenylpropanoid pathway and are synthesized under biotic stress. In this study, we address additional compounds to phytoalexins that are responsible for the growth arrest of the pathogen. Since all cultivars of *V. vinifera* (the Eurasian species) are considered susceptible to downy mildew, it is imperative to dig deeper into the defense mechanisms of resistant cultivars.

*Speaker

# [P065] Bacterial versus chemical diversity: the taxonomy paradigm in microbial natural product discovery

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A number of bacterial clades are recognized as important sources for the discovery of novel bioactive natural products. Estimating the magnitude of chemical diversity available from such a resource is complicated by issues including cultivability, isolation bias and limited analytical datasets. In this work, the order *Myxococcales*, a well-established source of natural products, was used for a systematic metabolite survey of the entire clade using mass spectrometric data from _˜2,300 myxobacterial extracts. The comprehensive approach taken here encompasses both known and previously unidentified metabolites detected under laboratory cultivation conditions, thereby enabling large-scale comparison of production profiles in relation to myxobacterial phylogeny. Our findings substantiate a correlation between taxonomic distance and the production of distinct secondary metabolite families. Furthermore, this study clearly shows and exemplifies improved prospects for discovering novel metabolites through examination of strains from new genera in contrast to analyzing additional representatives within the same genus. This paradigm underpins an important strategic focus in natural product discovery subsumed as "diverse taxonomy for chemical diversity" and as such encourages continuing isolation efforts aiming to further extend taxonomical coverage of – presumably not only – the myxobacteria clade. We complement our study by reporting the discovery and structure elucidation of rowithocin, a new myxobacterial secondary metabolite featuring an uncommon phosphorylated polyketide scaffold.

*Speaker

# [P066] The GC-MS based intra-thallus profiling of primary and secondary metabolites of Fucus vesiculosus L. (Phaeophyceae)

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Macrophytic brown algae have a complex thallus structure with tissues differing in their anatomy and physiology. Thallus zones are physiologically specialized and connected with each other by a long distance transport system. Here we used a GC-MS-based metabolomics approach to explore the biochemical network underneath the known pattern of physiological processes specific for each thallus zone.
Samples from four thallus zones of *Fucus vesiculosus* L. – thallus base, middle zone, vegetative apices and receptacles - were ground and extracted with methanol. GC-MS analysis of the extracts was carried out on a Trace GC coupled with a MAT95XP double focusing sector field mass spectrometer with electron impact ionization. Peak deconvolution was accomplished using AMDIS; GMD and NIST14 libraries were used for peak identification.

Among ˜100 detected compounds including 26 secondary metabolites, most abundant were mannitol, maltitol, fucosterol, citric acid and several polyols and sugars, presumably derived from hydrolysis of the long-term storage compound laminaran. The thallus base contained the highest amount of TCA-cycle intermediates, except for citric acid, while sugars and polyols were concentrated in the middle zone. Actively growing vegetative apices were characterized with a relatively high level of free amino acids and compounds associated with respiration. Our data exhibits one of the still rare metabolomic studies on brown algae. As such, for the first time, it provides a comprehensive snapshot of metabolite localization/accumulation in different thallus zones to understand the transport network between source and sink tissues in this species.
The research was supported by the RFBR (project 17-04-01331).

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# [P067] SWATH® Acquisition Improves Metabolite Coverage over Traditional DDA for Untargeted Metabolomics

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SWATH® acquisition, a data independent acquisition (DIA) workflow is well adopted in quantitative discovery proteomics, but not commonly used in discovery metabolomics. SWATH® acquisition combines the benefits of quantitation at the MS2-level of targeted MRM- based workflows with MS2- level based untargeted identification for metabolite identification of DDA workflows with the comprehensive nature of the MSMSall workflow. Because of the comprehensive, non-stochastic nature of the fragmentation in SWATH® acquisition, more fragmentation and thus structural information of the analytes compared to the DDA approach is achievable. Reproducibility and coverage is lower for DDA approaches compared to DIA workflows. Here we describe the improvements in metabolite coverage using SWATH® acquisition without sacrificing quantitation. Results obtained demonstrated a significant improvement of metabolites identified at the MS2 level by using SWATH® with variable windows in comparison with fixed windows in all analyzed matrices. We compared the ID rate from SWATH® acquisition to standard DDA. Here we were able to identify up to 45% more metabolites from the spectral library by SWATH® acquisition using 30 variable windows than by DDA. More confident MS2 based identifications then lead to more quantifiable metabolites in a metabolite expression experiment, which at the end allows better understanding of the biology. Spiked experiments into matrix samples of heavy labeled metabolites highlighted ten times higher sensitivity (signal-to-noise) using the MS2 ion to quantitate versus the traditional MS1 approach thus demonstrating the specificity nature of SWATH acquisition to more traditional data dependent approaches.

*Speaker

# [P068] Lipid- and Metabolomics analyses of two epileptic cohorts: searching for new seizure biomarkers

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Epilepsy is one of the most common disorders of the nervous system. There are a number of epilepsy syndromes that are defined based on a unique combination of symptoms. Keeping track of any factors that may precipitate a seizure can help to predict the event of a seizure. For some forms of epilepsy metabolites that functions as triggers are known. Such an example is pipecolic acid and alpha-aminoadipic semialdehyde for the pyridoxine-dependent epilepsy (Plecko, 2005; Vezzani, 2011). However, for most forms biomarkers or patterns of biomarkers have not been identified, yet.

The aim of this study is the identification of metabolites or patterns of metabolites as biomarker candidates for the epileptic seizures like absences, Rolandic, febrile and focal seizures due to focal cortical dysplasia. In order to identify characteristic variation of metabolites in bio-fluids, samples were obtained from patients (62 children and 37 adults) with epileptic symptoms directly after the event of a seizure and several hours thereafter. Further, the samples were compared with those of healthy volunteers in the FoCus cohort.
The sample preparation was conducted according to a modified SIMPLEX protocol and subjected to FT-ICR-MS (Bruker) in the shotgun mode. For mass calibration, synthetic lipids and C13-amino acids were used. The data validation was conducted with the QC-approach and further evaluated with MetaboScape 3.0 (Bruker), LipidXplorer (Herzog, 2012) and R. PCA models were used for the identification of changed metabolites during & after the seizure, identified with the semi-targeted approach by means of local and web-based databases.

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# [P069] Electrochemistry coupled online to mass spectrometry for biotransformation and metabolite elucidation of pesticides

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Online coupling of electrochemistry with mass spectrometry (EC/MS) is highly promising for prediction and simulation of metabolic processes of xenobiotics in living organisms [1]. Less time and cost of analysis, matrix free detection, and automation make EC/MS-based metabolomics superior over traditional *in-vivo* and *in-vitro* methods. Furthermore, EC/MS has a special feature to identify reactive intermediates and reaction mechanisms.
The main objective of this work was to simulate biotransformation processes of pesticides by EC/MS and to elucidate the transformation products (TPs). We have studied the oxidative phase I metabolism processes of selected pesticides by EC/MS or with liquid chromatography (EC/LC/MS) and compared the derived TPs with cytochrome based metabolites. The electrochemical TPs were produced by boron-doped diamond electrode, separated by LC, and detected by single quadrupole ESI-MS online. Structural identification of both electrochemical oxidation and liver microsome metabolites were based on accurate mass measurements by FT-ICR high-resolution mass spectrometry, isotopic pattern, MS/MS fragmentation, and retention time alignments.

Main phase I oxidative metabolites by P-oxidation, N- & O- dealkylation, dechlorination, hydroxylation, and -OH- oxidation have been identified [2]. Many targeted and untargeted metabolites have been identified by EC/(LC)/MS. Additionally, reactive species have been trapped online by biomolecules to study phase II conjugative reactions. Furthermore, we synthesized TP standards by EC/MS and applied them for pesticide's TPs occurrence investigation in foodstuff matrices.

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# [P070] Determination of pesticide residues in an upper Elbe River floodplain by LC-MS/MS

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**Abstract**: While monitoring studies focusing on pesticide contamination in soils at Elbe River floodplains mainly addressed legacy pollutants, such as DDT or lindane, little attention was given to occurrence of modern pesticides in Elbe floodplain soils. In order to assess the soil pollution state, it is vital to be able to measure different classes of pesticides simultaneously.
**Materials and Methods**: A multi-compound pesticide extraction method using accelerated solvent extraction and associated substance determination with UHPLC-MS/MS was developed. Method development processes revealed best extraction results for an acetonitrile/water mixture (2:1, v/v) at 80∘C or 100∘C and 1500 psi. Pesticide screening was performed by LC-MS in the MRM mode using a mass-spectra library containing MRM data of 401 pesticides provided by Waters (Waters Technologies Corp., MA, USA).
**Results**: One fungicide (azoxystrobin) and two herbicides as well as two herbicide transformation products (ethofumesate, simazine, 2-hydroxy-atrazine, 2-hydroxy-terbuthylazine) were detected in the floodplain as well as backwater inflow samples of an Upper Elbe River floodplain. Ethofumesate as well as azoxystrobin were detected primarily in backwater inflow samples, whereas simazine and both transformation products were detected in all sampling points of the area. The results indicate that there is a link between pollutant detection frequency in water samples and occurrence of these pesticides in adjacent terrestrial areas.

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# [P071] A chemometric investigation of aromatic emission profiles from a marine engine in comparison with residential wood combustion and road traffic: Implications for source apportionment inside and outside sulphur emission control areas

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Ship traffic is known to globally affect climate and human health. Since the introduction of sulphur emission control areas, the marine fuel consumption shifted from heavy fuel oil (HFO) to low-sulphur marine gas oil (MGO). However, marker substances for ship emission are exclusively related to HFO and show lower abundances and ratios for MGO. Therefore, the identification of ship emissions in emission source apportionment studies has been complicated.
The primary aromatic volatile organic emissions (VOCs) of a marine engine of 80 kW, operated on HFO (1.6-2.7% S) and MGO (0.08% S) at different representative engine settings were studied by laser-based time-of-flight mass spectrometry with resonance-enhanced multi-photon ionisation (REMPI) at 266 nm. REMPI refers to a soft ionisation technique with high selectivity for aromatic compounds. Furthermore, the obtained spectra were combined with REMPI spectra from previously published studies of wood stoves, diesel cars, gasoline cars and scooters to derive markers or latent variables suitable for ship emission identification and quantification by chemometric approaches.
Ship and land-based emissions were investigated for possible markers or discriminative m/z values by Kruskal-Wallis test and principal component analysis. Both techniques revealed that PAH with higher degree of alkylation are characteristic for ship emission of both fuels and less abundant in the land-based emissions. With a simulation of ambient air REMPI spectra includ-

*Speaker

ing available single sources, a model based on partial least-square (PLS) regression for each fuel was created to predict the relative contribution of ship emissions to ambient aromatic VOCs.

# [P072] Investigation on the chemical changes of acidic compounds in crude oil by fouling using high resolution mass spectrometry

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Reducing the deposition of solid material in upstream operations is the biggest challenge in petroleum industry. The presence of organic material has raised a number of problems such as loss of thermal efficiency on the heat transfer equipment, catalyst poisoning, plugged pipelines and others which result in various environmental and economic impacts. The formation of organic solid deposits is a highly complex phenomenon involving not only different processes (crystallization, corrosion) but also different chemical reactions (polymerization, oxidation). To face these problems characterization of crude oil with emphasis on heteroatom containing compounds (N, O, S) is necessary. Among them, oxygen containing compounds are of great interest due to their corrosive character. Organic acids in the form of carboxylic acids and phenols cause liquid-phase corrosion at elevated temperatures. Naphthenic acids are responsible for the corrosion of the inner surfaces of distillation columns and pipelines. In this study, simulation of an industrial reactor was achieved by the use of a laboratory built reactor. Electrospray in negative ion mode was used as ionization method for the investigation of the acidic compounds. An overall increase of the Ox containing compounds is observed with the increase of the temperature. Different organic acids were detected as a result of decomposition of peroxides and hydroperoxides which can be formed by oxidation mechanism. In addition, OxS species were detected at temperatures of 150 ∘C and 250 ∘C. Under inert conditions the trend stays similar but to a lower extend. The absence of oxygen limits the reactivity of the mixture.

*Speaker

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# [P073] Effect of different organic solvents for the extraction of compounds from asphaltenes

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Fossil materials, such as crude oil, are still the inevitable energy resource in our industrialized world. Due to their high complexity, there is no sound knowledge about their detailed chemical composition. Asphaltenes are among the most problematic fractions of crude oil during extraction, transportation and processing which are even more complicated, thus less understood in details. They contains highly aromatic systems, usually incorporating high ratio of heteroatoms and there are reports of their molecular size reaching up to 10,000 Da. Considering all these features, asphaltenes are known as the most complex fraction of crude oil, therefore the most challenging fraction to be analyzed. Simplification of asphaltenes can make this challenge easier and helps to get more detailed information about their chemical composition.
This study investigates the effect of using different organic solvents for the extraction of compounds from asphaltene fraction on the results of HRMS analysis by ESI-(+)-FTMS.
Measurement results of extracted compounds and the residual apshaltenes are compared to the results from analysis of untreated aphaltene sample. The simplification method enables us to get more detailed information on the composition of the sample. This effect is mostly observed around the edges of homologous series (e.g. showing species above 100 carbon atoms with equally high double bond equivalents).

*Speaker

# [P074] New methodology for the analysis of highly hydrophobic calixarenes by MALDI-TOF mass spectrometry

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**Introduction**

Calixarenes have received much attention during the last decades due to the tremendous possibilities offered by these easily accessible macrocycles. In that context, the new family p-(benzyloxy)calix[n]arenes are offering novel possibilities for the synthesis of functionalized calixarenic platforms. In most cases, the MS characterization of these new calixarene molecules with usual electrospray technique is difficult due to poor solubility in solvents compatible with ESI or complexity of the direct analysis of crude mixtures. In order to overcome these limitations, MALDI-TOF was used for the detection and the fast screening of calixarene synthesis.

**Methods**

MS experiments were performed using an UltrafleXtreme mass spectrometer (Bruker Daltonics, Bremen). Different matrices (DHB, DCTB, DIT) were compared in terms of robustness and sensitivity. Matrix solutions were freshly prepared in tetrahydrofuran (THF) at a concentration of 20 mg/ml. In order to increase the ionization yield, addition of several alkali metal ions (Na+, K+, Cs+) was also tested. We also showed that suitable functionalizations also considerably increase the sensitivity of the analysis, both in terms of signal-to-noise (S/N) ratio and highest detected $m/z$.

**Preliminary data**

In terms of sensitivity DCTB gives better results than the other tested matrices. This matrix produces spectra with high S/N ratio and high resolution which allows accurate mass measurement. To increase the sensitivity, alkali metal salts were added to the samples. Cesium was giving the best results according to the fact that calixarene exhibits a high affinity for this metal. This effect is reinforced by the use of N,N (diethyl)acetamide-functionalised calixarenes.

*Speaker

# [P075] Non-Targeted Screening of Extractables and Leachables in E-cigarettes using a Single Platform UPLC-APGC-QTOF-MS

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Characterization of extractables and leachables is essential for ensuring the safety, quality and efficacy of inhalation tobacco products such as e-cigarettes. The characterization of extractables from e-cigarettes, e-liquids, refill cartridges and e-cigarette aerosol involves both targeted screening (i.e. testing the extracts for known impurities) and non-targeted screening to look for unknown impurities that may potentially migrate from the starting materials and other packaging and device components. FDA Deeming Regulation (May 2016) and EU Tobacco Product Directive (2014/40/EU) require manufacturers and importers to conduct full scientific evaluation of e-cigarette products including disclosure of ingredient listing, harmful and potentially harmful constituents, labeling requirements, demonstration of good manufacturing practices, product registration and premarket approval required in the US. Regulatory submissions must demonstrate that products meet the product safety and quality requirements and are appropriate for the protection of public health.

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# [P076] ESI MS of highly sulfated oligosaccharides

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Glycosaminoglycans as long, unbranched polysaccharides are major components of the extracellular matrix. They do not only define structure and biophysical properties of the respective tissue, but also affect the embedded cells via their surface receptors and the interaction with different signaling molecules.
GAG from natural origin are very inhomogeneous regarding their monosaccharide composition and the sulfation pattern and there is a high risk of contamination. This results in a growing interest in reliably characterizing native GAG and synthesizing artificial GAG derivatives.

The relevance of quality control, and thus the importance of fast and reliable analytical methods, emerges from the growing number of potential applications of the native or artificially modified GAG.

In the case of defined or fully sulfated oligosaccharides with varying anomeric ligations a fast and reliable MS characterization is desirable in parallel to the standard NMR analysis, because the MS spectrum alone gives accurate information about the number of sulfate residues and the outcome of other chemical modifications. The sensitivity of the sulfate residues towards MS ionization is the main critical issue here.

Fondaparinux – a sulfated pentasaccharide clinically used as anticoagulant – was used as a model substance for the optimization of the MS investigations and the improved sample preparation. The insights gained enabled the characterization of different HA oligosaccharides with defined sulfation patterns and with varying anomeric ligations by means of ESI MS. Such derivatives with up to 18 sulfate residues became detectable without significant sulfate loss in the positive and negative ion mode.

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# [P077] Adduction of ammonium to polylactides to modify their dissociation behavior in CID

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Characterization of end-groups in synthetic polymers can be efficiently achieved by tandem mass spectrometry (MS/MS). While both end-groups contribute to the molecular mass measured for polymer ions in MS, each termination can be individually determined from fragments generated upon cleavage of backbone bonds in MS/MS. Detailed knowledge of dissociation mechanisms is first required to accurately describe the structure of fragments and establish fragmentation rules to be used for MS/MS data analysis of the polymer family.
Here, we report a case where this MS/MS approach failed due to mass coincidence of fragments. The studied polylactide (PLA) hold an $\alpha$ benzyl moiety and a $\omega$ hydroxyl group. These chains were readily ionized as sodium adducts, and their dissociation lead to the two product ion series predicted by the rules established for PLA [1-2]. Unfortunately, fragments of these series were isobaric, hence preventing reliable characterization of each end-group. In order to remove the mass degeneracy induced by the competing charge-remote reactions experienced by sodiated PLA, we used ammonium adduction as an alternative ionization mode. This allowed different, charge-assisted, mechanisms to proceed upon collisional activation, leading to three fragment series that could usefully be employed for end-group analysis of this PLA [3].

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# [P078] Characterization for Physical and Chemical Properties of Synthetic Polymer using Ion Mobility-Mass Spectrometry

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**Abstract**

Only since very recently, ion-mobility mass spectrometry (IM-MS) starts to attract attention as a very powerful tool for obtaining microscopic physical and chemical properties of polymers. IM-MS simultaneously provides molecular weight and collision cross section (*CCS*), which corresponds to the spacious size of a polymer chain. The contribution of each individual monomer unit to the increment of *CCS*, which depends on the type of the monomer unit, can then be used to obtain information about the polymer coil. Following this line, we have recently proposed a new method for evaluating the stiffness, i.e., the characteristic ratio (*Cn*) of the polymer [1]. The presented method is easy to perform and provides stunningly rapid access to *Cn* of synthetic polymers. Besides that, IM-MS provides the intrinsic *Cn* of polymers, since the experiment is carried out under vacuum condition without the disturbances of solvent and neighboring polymers. IM-MS has the potential for providing many other innovative strategies that may be important in polymer science and technology. Our group is thus working on the development of new IM-MS methods that e.g. are addressing branching of polymers or dielectric properties [2]. The presentation will give an overview of several methods being currently established in our group.

**Reference**

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# [P079] A covalent coating method for CE-MS capillary for the analysis of intact proteins

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In a context of quality control and/or fast screening of biological analytes, analysis of intact proteins in a complex sample would be the first information in the analytical strategy. Utilization of a single method to separate and identify the proteins can be necessary meanwhile can also be challenging.
The most popular method would be liquid chromatography based on SEC separation coupled to mass spectrometry (LC-MS), however, capillary electrophoresis - mass spectrometry (CE-MS) will appear as a better solution due to its great power of separation. Nevertheless, studying intact proteins by capillary electrophoresis is not so usual due to the potential adsorption on the capillary walls which can induce loss of the material and poor reproducibility. Moreover, in combination with mass spectrometry, which does not tolerate salts and usual CE solvents, analyses need some researches and adaptation.
Another way to work with CE-MS is to modify covalently the surface of the capillary to prevent loss of proteins. This involves, most of the time, tedious protocols or expensive already made material. However, an overnight method based on covalent modification of the silanol groups of the capillary with polyethyleneimine (PEI), developed by Sciex application department was tested in the laboratory to analyse an artificial mix of proteins and monoclonal antibodies. The protocol was easy, simple and effective. A mixture of Cytochrome C, Ribonuclease and Myoglobin was separated at first glance in a simple way. An antibody, infliximab was also analysed with satisfying identification. Reproducibility was also tested with good results.

*Speaker

# [P080] Fluorescently Labeled Glycans Analyzed by Ion Mobility-Mass Spectrometry

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Complex carbohydrates, also referred to as glycans, are ubiquitous in nature and form one of the major classes of biopolymers. Glycans can adopt highly branched structures with a complex regio- and stereochemistry, resulting in isomers that are often indistinguishable by available analytical approaches.
LC-MS analysis of fluorescently labeled glycans – one of the most widespread methods in analytical glycosciences – often struggles with the aforementioned problem: the in-depth characterization of very similar isomeric species. The hyphenation of ion mobility spectrometry (IMS) with liquid chromatography (LC) and mass spectrometry (MS) offers the possibility to overcome this limitation. To exploit the full potential of the resulting three-dimensional coupling, a comprehensive investigation of the influence of fluorescent labels on the mobility behaviour of glycans is essential. Therefore, we present a systematic study on the effects of various reducing end modifications on the mobility separation of glycans.
For this purpose, the Lewis antigen tetra- and trisaccharide system was chosen as a representative set of model oligosaccharide structures. Ions of native and derivatized species labeled with procainamide, 2-aminobenzamide and 2-aminobenzoic acid were generated by electrospray ionization, and analyzed in a drift tube IM-MS instrument. Our data show that ion mobility separation is strongly dependent on the specific labels and on the nature of adduct ions. While it is often not feasible to differentiate glycan isomers in their underivatized form, we demonstrate that certain fluorescent labels can lead to significantly enhanced separation of isomeric species.

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# [P081] Evaluation of plant extract composition by statistical analysis and molecular networks

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Cosmetic field is in perpetual evolution and needs innovation to discover new cosmetic ingredients. Extracts from Lamiaceae plant family, such as Lavandula or Rosmarinus gender for example [1], are interesting candidates showing antioxidant activities. As the protection and the environmental respect became a major subject of concern nowadays, eco-extraction techniques such as Accelerated Solvent Extraction (ASE) are mandatory. In order to obtain the largest view of all secondary metabolites of the selected plants, different solvent polarities from water (relative polarity 1.000) to cyclohexane (relative polarity 0.006) and different temperatures (20 to 100∘C) were tested. Extracts were then analyzed with an HPLC-QTOF instrument using both HRMS and HRMS/MS mode. Major components were identified by exact mass comparing to literature data and by the calculation of molecular networks allowing the classification of MS/MS spectra by similarity. A complementary statistical analysis (PCA) permitted us to determine discriminating factors of each extract. In the future, extraction and analysis of identified lipophilic or amphiphilic molecules of interest for cosmetics will be optimized by a green analytical approach using supercritical CO2. [1] http://www.clarins.ch/fr/la-marque-recherche-%26-d%C3%A9veloppement/ingredients.html

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# [P082] The true complexity of carrageenans of marine red algae as revealed by LC/MS

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The determination of the structure of natural polysaccharides challenges analytical chemistry, although this remains the key to better understanding and controlling their function. Moreover, these biomolecules are the main components of the cell walls of terrestrial and marine plants and, as such, they represent abundant and potentially interesting bioresources. In marine macroalgae, cell wall polysaccharides are frequently sulfated, which has no equivalence in land plants while sulfated polysaccharides are also found in animals. In our study, Chondrus crispus was used as a model to reveal the true complexity and heterogeneity of the cell wall components in red macroalgae. Carrageenans are the main structures composing the walls of Chondrus crispus. If the ideal motifs of these polymers are well described, unexpected physico-mechanical properties of the walls suggested that other motifs exist which justified the need for a deeper knowledge of carrageenans' structures. To discard the predominance of ideal motifs and thus reveal minor motifs, mild depolymerization of carrageenans was performed in order to produce longer structures, which presumably better reflect the original polymer. However, these longer and highly sulfated oligosaccharides were difficult to ionize with a good efficiency into the mass spec while maintaining their integrity. Furthermore, they were poorly discriminated by LC-MS. We will thus present the development of an optimized method of IP-RP chromatography, which achieved excellent separation up to high degrees of polymerization and sulfation. By this method, minor and so far undescribed structures have been revealed.

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# [P083] Semi-preparative electrochemical drug metabolite generation and identification by means of LC/ESI-MS and LC/ESI-MS/MS

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During the development of new molecular entities (NME) the elucidation of oxidative metabolic drug transformation plays an important role in order to eliminate negative side effects of promising drug candidates as early as possible. For full structure elucidation of possible metabolites, milligram amounts are required for nuclear magnetic resonance (NMR) analysis. In contrast to conventional organic synthesis, preparative electrochemistry (EC) represents an alternative technique for metabolite generation at the milligram scale. Electrosynthesis is frequently applied for the formation of new molecules, whereas the top-down degradation of drug metabolites has been reported as a technique of poor conversion efficiencies. Hence there is a need for careful experimental optimization. In this study, the betablocker metoprolol was used as a model compound to optimize and evaluate several parameters like applied potential, pH value, solvent composition, duration of oxidation, substrate concentration and temperature on the electrochemical conversion. The top-down metabolite synthesis was carried out in a self-made electrochemical batch cell under potentiostatic control. Samples were obtained within defined time sections in order to realize a time-resolved overview about the observed and varied experimental conditions. Sample analysis and metabolite identification was done by LC/ESI-MS/MS. Each of above mentioned parameters showed a dependency on the conversion of metoprolol to its oxidative metabolites. Remarkable is the influence of the pH value on degradation of metoprolol and generation of metabolites and their consecutive reactions. Additionally, variation of the substrate concentration had a high impact on the conversion rate and time-efficiency.

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# [P084] Investigation of halide and formate adducts on coinage-metal phosphine complexes in isolation

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Coinage-metal complexes exhibit interesting photophysical properties resulting in potential application, e.g. photocatalysis [1]. We investigated phosphine complexes of the type [M1M2(LCy)2]2+ (M1,2=Cu, Ag, Au, LCy=bis(dicyclohexylphosphino)methane) and their adducts X (Cl, Br, I) as model systems in order to explore metalliophilic d10-d10 interactions [2,3]. Therefore, the influence of the halide anions X (Cl, Br, I) of the isolated [M1M2(LCy)2X]+ species are elucidated via collision-induced dissociation (CID). We found that the relative gas phase stabilities are in accordance with Pearson's HSAB-concept for homometallic complexes, and we observed metal/halide dependent fragmentation pathways. Formate adducts [M1M2(LCy)2(CHOO)]+ were investigated via infrared multiple-photon dissociation (IRMPD) spectroscopy in the C-O- and C-H-stretching region, in conjunction with density functional theory (DFT) calculations. We identified coinage metals dependent shifts in the antisymmetric C-O and C-H-stretching modes.
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# [P085] Peptoids as chemokine capture agents

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Chemokines are small secreted proteins that direct the migration of leukocytes in immune surveillance and inflammation. As excessive invasion of immune cells correlates with the severity of several inflammatory diseases, such as rheumatoid arthritis and asthma, the inhibition of chemokine interaction with their receptors is a means to treat these medical conditions. We are searching for peptoids that bind to the inflammatory chemokine CXCL8 with sufficient affinity to inhibit receptor binding and cellular responses.
To generate random libraries we used the sub-monomer methods in a mix-and-split approach. We developed a semi-automated procedure based on two-channel fluorescence microscopy and fluorescently labeled CXCL8 to screen this library for CXCL8-ligands. Candidate sequences were identified by MALDI-MS/MS. These sequences were resynthesized and re-screened. Affinity to CXCL8 was measured by fluorescence polarization to be in the range of 10-100 $\mu$M. [1] Peptoids are mostly unstructured in solution leading to a large entropic penalty upon interaction with a protein binding site. Cyclization leads to a more rigid structure. We could increase affinity by a factor of 10-100 by cyclization of the hexapeptoids. In ongoing work we are elucidating the chemokine-peptoid binding site and developing methods for the screening of cyclic peptoids.
Helmer, D., Brahm, K. Helmer, C., Wack, J.S., Brenner-Weiss, G., Schmitz, K. (2016) Two-channel image analysis method for the screening of OBOC libraries, Anal. Meth. 8, 4142. doi: 10.1039/C5AY02981C

*Speaker

# [P086] The molecular organization of Salmonella T3SS sorting platform unraveled by native MS, SAXS and computational modeling

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The Type III secretion system (T3SS) is a macromolecular protein complex utilized by many pathogenic Gram-negative bacteria to initiate infection by injecting virulence factors from the bacterial cytoplasm directly into eukaryotic host cells. Acting as a molecular syringe the T3SS needle complex spans both bacterial membranes. On the cytoplasmic side of the T3SS there is another multi-protein complex that is proposed to function as a sorting platform, which selects and targets substrates for translocation through the needle. Thus far, the precise molecular organization of the T3SS sorting platform is unknown.

Here, we studied protein complexes, containing two C-ring proteins, a regulator protein and an ATPase by native mass spectrometry (MS). In-depth native MS and collision-induced (CID) MS/MS analysis of different combinations of proteins and different protein domains revealed protein stoichiometries and a protein interaction network. For different subcomplexes the overall complex shapes were obtained by small angle X-ray scattering (SAXS). Results from native MS and SAXS were combined into structural models of the T3SS sorting platform using fragment-based structural modeling and molecular dynamics (MD) simulations.
Our results show how native MS can be used in conjunction with SAXS and computational modeling to get insights into the molecular organization of a multi-protein complex. The largest characterized protein complex consists of 6 proteins that adopt an extended L-shaped conformation in solution, which can be assumed to be the minimal building block for the assembly of the fully functional T3SS sorting platform.

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# [P087] MALDI-Supported Screening of Large Peptide Libraries for Identification of Protein Binders

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Screening of one-bead-one-peptide libraries is a powerful analytical tool for the identification of protein ligands. The demand for binders with high affinity and high specificity towards various target proteins has surged in the biomedical field in the recent years. However, the traditional peptide screening procedure involves tedious steps such as manual selection, sequencing, and characterization. We present a high-throughput "all in one chip" system, allowing the screening of a high number of resin beads in short time. Here, beads of a combinatorial one-bead one compound peptide library are immobilized on an in-house produced chip, on which every bead has a well-defined position. The chip is then incubated with a fluorophore-labeled protein, identifying suitable peptides by a high-resolution fluorescence scan. The screening is followed by MALDI-MS experiments directly on the respective glass chip. To circumvent the need for peptide fragmentation normally used for peptide de novo sequencing, which can result in incomplete sequence information, an approach based on termination synthesis has been used. This allows the peptide sequence identification by fragmentation-free MS with almost 100 % accuracy. For this purpose, a software tool was developed automatically translating MALDI-MS spectra into the corresponding peptide sequences.

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# [P088] Carboxyl-Photo-Reactive MS-Cleavable Cross-Linkers: Unveiling a Hidden Aspect of Diazirine-Based Reagents

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Cross-linking/MS is a powerful tool to elucidate the 3D-structure of protein and protein complexes. Protein cross-linkers serve as "molecular rulers" imposing geometrical constrains for modeling protein structures. The most promising approach for a reliable, automated identification of cross-linked products relies on CID-MS/MS-cleavable cross-linkers. Upon collisional activation, they generate diagnostic product ions for improved data analysis. A major challenge in cross-linking/MS is efficiently targeting carboxyl functions in proteins under physiological conditions. We discovered that carboxyl-reactive cross-linkers have already been employed for many years in cross-linking/MS studies - yet in a different context. Diazirine-based cross-linkers, such as photo-methionine and succinimidyl 4,4'-azipentanoate (SDA) cross-linkers, are currently considered to react non-specifically upon UV-A photoactivation with all 20 proteinogenic amino acids through a reactive carbene that inserts mainly into C-H bonds. We discovered that the cross-linking capability of diazirines based on X-H (X= C, N, O) insertion is in fact only the tip of the iceberg. Diazirines mainly isomerize to linear diazo compounds that can react with carboxylic acids to yield esters in ~75% of cases. The resulting cross-linked products are CID-MS/MS-cleavable requiring customized software tools for automated analysis of cross-links. Therefore, diazirines open an entirely new route for photo-cross-linking of carboxylic acids. All previous cross-linking studies using diazirine-based reagents have been based on conventional software designed for non-MS-cleavable cross-linkers. In the light of our new findings, they are not sufficient for a correct identification of diazirine cross-links and relevant structural proteomics studies may deserve to be revisited.

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# [P089] Identification and Affinity Determination of Antibody Epitopes against the Chemokine CXCL8/Interleukin-8 by Proteolytic Excision Mass Spectrometry and Biosensor Analysis

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**Introduction**

The chemokine CXCL8 (Interleukin-8) is a biomarker for inflammation and involved in lung diseases, Rheumatoid arthritis, Psoriasis and cancer. Antibodies against IL-8 and its receptors CXCR1 and CXCR2 have been used to identify its role in several diseases. Humanized monoclonal anti-CXCL8 antibodies have been tested as potential therapeutics, but the epitope binding site(s) of inhibitory antibodies are unknown. Knowledge of the IL-8 antibody epitope could give insight to the inhibition mechanism and contribute to the development of new drugs. Here, a commercial monoclonal anti-IL8 antibody has been investigated, with the major aim to develop a combination of biosensor and affinity- MS analysis for epitope elucidation.

**Experimental**

Epitope analyses were performed by digestion of IL8 and proteolytic excision-MS on an affinity column with anti-IL8 antibody functionalized sepharose beads. To digest IL8 with Trypsin and LysC, it is reduced with DTT and alkylated with IAM. The anti-IL8 antibody is bound to CNBr-activated sepharose 4B beads via antibody lysine residues. Anti-IL8 binders captured by the affinity micro column were studied by MALDI-TOF-MS.

**Results and Discussion**

The results revealed a short linear epitope sequence (61-64) in the C-terminal $\alpha$-Helix of IL8 (61-64). The C-terminal $\alpha$-Helix of IL8 is responsible for binding to glycosaminoglycans (GAGs) on endothelial cells for formation of a stable IL8 gradient and chemotaxis. Binding of IL8 to CXCR1 or CXCR2 occurs via its N-terminus. Unexpectedly, the reduction/alkylation of IL8 showed alkylation of lysine residues but this did not inhibit trypsin digestion.

*Speaker

# [P090] A Novel Data-Independent Acquisition Strategy for Non Targeted, Accurate Mass Contaminant Screening

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Companies and environmental regulatory authorities continue to investigate High Resolution Mass Spectrometry (HRMS), non-targeted, screening techniques to expand the scope of their screening methods. Improvements in mass spectrometer sensitivity and highly selective acquisition techniques, alongside advancements in the informatics used to process and review data, are facilitating the task.

Several fruit and vegetable samples, previously characterized by a collaborator, were screened against a pesticide library using several Data-Independent-Acquisitions (DIA). DIA strategies for complex mixture analysis, particularly within a contaminant screening environment offer significant efficiency advantages in that they enable a generic, non-biased strategy for data acquisition. The work will discuss the use of a novel DIA method – SONAR, in which precursor and product ion data are acquired with a sliding quadrupole window - for use in accurate mass screening applications and its use alongside more traditional DIA strategies such as full scan low and high collision energy acquisition (MSE), and its ion-mobility enhanced variant (HDMSE).

A comparison of the outcomes from MSE, HDMSE and SONAR screening sets will be presented.

All techniques were able to detect contaminants present. However, the comparison of each technique with increasing matrix complexity shows that the ability of non-selective full scan experiments (MSE) to generate clean product ion spectra is reduced when compared to HDMSE and SONAR datasets.

*Speaker

# [P091] Structural analysis of protein complex involving box C/D snoRNP assembly factor Bcd1p and histone chaperone Rtt106p

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Small nucleolar ribonucleoprotein particles (snoRNPs) are involved in ribosome biogenesis. Among them, C/D box snoRNPs catalyze site-specific 2'-O-methylation of RNA riboses1, one of the most prevalent post-transcriptional modification found in ribosomal RNAs. Eukaryotic C/D box snoRNPs biogenesis involves conserved assembly mechanisms2, which lead to the recruitment of four core proteins to one guide snoRNA. Bcd1 protein, an identified factor specifically involved in C/D box snoRNP biogenesis3,4 is able to interact with histone chaperon Rtt106 protein5 This protein is known to be involved in nucleosome formation associated with replication and transcription. This interaction could highlight a functional relation between snoRNPs biogenesis and chromatin remodeling.
Here we report the structural characterization of the interaction between Bcd1p and Rtt106p and particularly a combination of structural mass spectrometry (MS) approaches (native MS, ion mobility MS, H/D exchange MS) and more classical biophysical techniques. Native MS experiments revealed a 1:1 direct interaction between Bcd1p and Rtt106p complex. Ion mobility MS was then used to characterize conformational changes induced upon complex formation. Complementary information on interacting regions were obtained from H/D exchange MS (HDX-MS) and crosslinking MS (XL-MS) analyses. Altogether combination of native MS, IM-MS, HDX-MS and XL-MS experiments shed light on the structural characterization of Bcd1p/Rtt106p dimer, in agreement with results from other biophysical techniques.

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# [P092] Study of DNA i-motif folding in gas phase by ion mobility spectrometry coupled to native mass spectrometry

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Structural analysis of biomolecules is essential for understanding their function(s), interaction with other molecules, etc... Studying non-canonical DNA structures and the biophysics of their formation is important, given their role in gene regulation. Specifically, cytosine-rich DNA sequences can adopt in particular conditions (*e.g.*, acidic pH) the so-called i-motif structure, in which two anti-parallel duplexes are connected by intercalated hemiprotonated C-H+-C base pairs.

Here we explored how to use ion mobility spectrometry (IMS-MS) to discriminate whether intramolecular i-motif are folded or unfolded in solution. The experiments were performed as a function of the solution pH, and with mutant control sequences unable to form i-motifs. The IMS results were in agreement with classical spectroscopic approaches, such as circular dichroism and UV-melting. We will discuss also the parameters (electrospray conditions, charge state selection, and instrument tuning) that are critical to keep a memory of the solution conformational ensembles. In particular, our study revealed the importance of salt concentration for i-motif structural analysis via ESI. Furthermore, the analysis of i-motif in the presence of different ligands allowed to obtain a complementary insights on i-motif – ligand interaction compare to other techniques. For example, in contrast to previous observations (Waller Z., 2016) our results showed that mitoxantrone ligand is binding to unfolded form of telomeric i-motif sequence.

In conclusion, IMS-MS can be used to probe intramolecular folding of nucleic acids in solution and the effect of ligands on the folding equilibria, and the charge state selection and adjustment of salt concentration were critical for the success.

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# [P093] The synaptic vesicle cycle is governed by heterogeneous and macromolecular protein microdomains

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Synaptic vesicles are small storage organelles for neurotransmitters. They are densely packed with proteins and pass through a trafficking cycle in the nerve terminal. This includes neurotransmitter import, docking, priming and fusion with the presynaptic membrane, neurotransmitter release into the synaptic cleft as well as vesicles recycling. These processes are governed by non-covalent protein interactions that assemble on demand and dissociate when the task is completed. Although the major protein components of synaptic vesicles are identified we have only little knowledge about their interactions. Available models assume random distribution of the proteins in the membrane, however, there is strong evidence that they form functionally active assemblies. We set out to unravel these assemblies by employing chemical cross-linking combined with mass spectrometry of intact protein complexes. Synaptic vesicles were purified from rat brain and major proteins were identified by LC-MS/MS and database searching. Proteins within intact vesicles were then cross-linked using BS3 cross-linker. After digestion with Trypsin, cross-linked di-peptides were enriched by size exclusion chromatography and analysed by LC-MS/MS. Applied in this way, chemical cross-linking revealed first interaction networks in synaptic vesicles. These networks reveal many protein interactions with Synaptobrevin which plays a central role in complex formation. Local networks, for instance between Synaptophysin, Synapsin and the V-type ATPase, were also observed. Specific protein complexes were then targeted by native mass spectrometry to identify their stoichiometries. These results pave the way to unravel the networks in synaptic vesicles which are key to our understanding of signal transduction in the neuronal synapse.

[*]Speaker

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# [P094] Thermal desorption/GC-MS to explore VOCs profiling with XAD-2 at different sampling conditions

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Thermal desorption GC-MS (TD/GC-MS) was used to assess adsorption kinetics of an adsorbent for volatile compounds, XAD-2. XAD-2 is a macroreticular polymer characterized by a large surface area, good physical durability and stability at temperatures $<$ 200 ∘C. Though originally used to extract organic compounds from water, an initial study using liquid elution of compounds collected from the body odors of common marmosets adsorbed to XAD-2 showed additional detection of many (semi)polar compounds. Therefore, in a first work searching for efficient alternatives for VOCs profiling with air as the mobile phase and subsequent TD/GC-MS analysis, the conditions to provide a particularly low background and to achieve maximal recovery after subsequent release were evaluated (Marcillo, et al. 2017). As a result, XAD-2 was recommended as an alternative for analysis of VOCs TD/GC-MS profiling under standard sampling conditions. In fast active sampling of volatiles from body odor of mammals in common marmosets *(Callithrix jacchus)* (Ḱucklich, et al., 2017) and meerkats (*Suricata suricatta*) (Weiß, et al., 2017), the method resulted in an efficient and non-invasive volatiles analysis. Therefore, we tested XAD-2 in TD/GC-MS analysis using complex standard mixtures with a broad range of volatility, different chemical classes and different polarities loaded under standard and critical conditions of sampling to determine the adsorption kinetics of this sorbent material that may influence the accuracy of volatile profiling and, finally, to determine the methodological aspects for proper applicability, advantages and drawbacks for fast active sampling in comparison with the performance of a common adsorbent, Tenax TA.

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# [P095] Cardiolipin identification by means of LC-HRMS and Kendrick mass plots

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Cardiolipins (CL) are anionic phospholipids, which are exclusively located in mitochondrial membranes. This lipid class plays a fundamental role in the energy metabolism, especially in the inner mitochondrial membrane. While CL are essential for stability of protein complexes of the respiratory chain, they are also involved in various pathologies, such as neurodegenerative diseases. Due to four partly different acyl chains with various levels of saturation, CL are very complex phospholipids, making their analysis a very challenging task. Therefore, hyphenation of effective chromatographic techniques and mass spectrometric (MS) detection along with software-assisted data processing are powerful tools for CL identification and characterization.
The CL species were separated by reversed-phase high-performance liquid chromatography. Using an Orbitrap MS with negative electrospray ionization, the CL species were identified by their exact masses. Additionally, homologue CL species were visualized using Kendrick mass plots. By plotting the Kendrick mass defect against the Kendrick mass, CL species have been arranged by their structural characteristics, for example number of double bonds or chain length. The Kendrick mass plot capturing additional chromatographic data is a novel tool for graphical lipid identification such as CL homologues.
The structural characterization was performed using a linear ion trap mass spectrometer by means of data-dependent sequential (MS3) fragmentation experiments. The identity of various CL species was successfully determined based on their acyl chain combination by analysis of specific CL fragments.

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# [P096] Structure elucidation of the siderophores of Pseudomonas taiwanensis VLB120 bacteria by LC-HR-MS/MS

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Bacteria of the genus *Pseudomonas* are Gram negative and ubiquitous, and like most aerobic bacteria, they require iron for metabolism and growth. To facilitate iron acquisition from their environment, fluorescent pseudomonads synthesize yellow-green, water-soluble siderophores, known as pyoverdines, which have a high affinity for iron and form very stable chelate complexes. Furthermore, other structural related siderophores are synthesized in a small amount in addition to pyoverdines under iron deficient conditions.
The structure of pyoverdines produced by all fluorescent pseudomonads is constituted of three parts: (i) the chromophoric unit derived from 2,3-diamino-6,7-dihydroxyquinoline, (ii) a peptide moiety, consisting of 6 to 14 proteinogenic and non-proteinogenic amino acids bound to the chromophore via the carboxylic group and (iii) the acyl side-chain, which is linked to the C3-position of the chromophore. Pyoverdines of one bacterial strain differ only in this acyl side-chain. Limited information on siderophores of pseudomonads is available. Therefore, a detailed structural characterization was carried out by LC-HR-MS/MS.

The separation of the siderophores of *Pseudomonas taiwanensis* VLB120 was achieved by hydrophilic interaction liquid chromatography (HILIC). The analytes were detected by high-resolution mass spectrometry after positive electrospray ionization (ESI). The protonated molecule ions, the iron- and aluminium complexes and the doubly charged ions of two pyoverdines and one azotobactin were identified. By means of data-dependent and data-independent fragmentations, their structures were elucidated.
Additionally, 15N-labelled *Pseudomonas taiwanensis* VLB120 bacteria were analysed for verification of the number of assigned nitrogen atoms and confirmed the proposed structures, which are not yet described in the literature.

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# [P097] Protein-RNA Cross-Linking by 2-Iminothiolane and Nitrogen Mustard Coupled with Mass Spectrometry

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Cross-linking of RNA and proteins coupled with mass spectrometry (MS) is a stand-alone technique to identify RNA-protein interaction sites. During the process of gene expression, transcripts are regulated via intermolecular interactions between RNAs and proteins. The interaction sites within the protein sequence can be estimated by cryo-electron microscopy but not exactly determined. Cross-linking of the molecules with subsequent MS-analysis provides information at amino acid resolution. The current cross-linking method of choice is based on UV irradiation of the protein-RNA complexes, introducing covalent bonds between amino acids and nucleobases in close vicinity. However, cross-linking with UV-light works grossly inefficient and could therefore be expanded by the usage of chemical cross-linkers.
Here, we introduce 2-iminothiolane and mechlorethamine as potent alternatives for UV-crosslinking coupled with state-of-the-art MS. In contrast to UV-light, both chemicals act as spacer-molecules between protein and RNA resulting in complementary information on protein-positioning relative to the RNA during interaction.
Errors in protein-RNA interaction events often result in severe genetic disorders such as neurological diseases and cancer. Cross-linking of the molecules with 2-iminothiolane and mechlorethamine coupled with MS can contribute to the understanding of these complex medically relevant processes by identifying the interacting amino acid.

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# [P098] Molecular interactions of plasticizers and proliferator-activated receptor $\gamma$ determined by HDX-MS

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The most frequently occurring phthalate di-(2-ethylhexyl)phthalate (DEHP) has been reported to cause adverse effects on glucose homeostasis and insulin sensitivity, thus contributing to the metabolic syndrome. One of the key regulators of the connected pathways is the peroxisome proliferator-activated receptor gamma (PPAR$\gamma$). Since the endogenous ligand of PPAR$\gamma$ 15-deoxy-delta-12,14-prostaglandin J2 (15$\Delta$-PGJ2) features structural similarity to DEHP and its main metabolites mono(2-ethylhexyl)phthalate (MEHP) and mono(2-ethyl-5-oxohexyl)phthalate (MEOHP), which are produced during human hepatic metabolism, we tested the hypothesis of direct interactions between human PPAR$\gamma$ and DEHP or its transformation products.
In previous studies using hydrogen/deuterium exchange mass spectrometry (HDX-MS) and surface plasmon resonance analysis, we demonstrated that the metabolites MEHP and MEOHP, but not DEHP itself bind to the ligand binding pocket of PPAR$\gamma$. This binding leads to typical activation-associated conformational changes, as observed with its endogenous ligand 15$\Delta$-PGJ2. Furthermore, a reporter gene assay confirmed productive interaction of MEHP and MEOHP with PPAR$\gamma$, while DEHP failed to activate PPAR$\gamma$.
To further characterize and classify the effects of plasticizers and their metabolites on PPAR$\gamma$ activation, we will screen additional 30 common plasticizers and their transformation products. The obesogenic role of selected plasticizers will be evaluated by determining the ligand-specific DNA-binding motifs of the PPAR$\gamma$ complex using chromatin immunoprecipitation sequencing (ChIP). Ligand-specific PPAR$\gamma$ interacting proteins will be analyzed by affinity purification mass spectrometry. The obtained data will provide molecular evidence of metabolic syndrome related health effects of plasticizers, mediated by PPAR$\gamma$ activation, and will help to facilitate future risk assessment of plasticizers.

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# [P099] Analyzing subunit assemblies of the F1F0- ATP synthase and conformational changes of the heterodimeric ABC transport complex TmrAB

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Adenosine triphosphate (ATP) is a universal energy source of living cells and is used for numerous energy-consuming reactions. The major focus is on the F1F0 ATP synthase of *Acetobacterium woodii* and the heterodimeric ABC transport complex TmrAB from *Thermus thermophilus* which are responsible for ATP synthesis/hydrolysis and ATP-dependent membrane transport mechanism, respectively. We are interested in the assembly and process of different subunits and investigate requirements for the different assembly steps. For TmrAB we apply ion mobility measurements to investigate conformational changes involved in the transport in dependency of different substrates.
In LILBID-MS a droplet generator generates droplets of an aqueous sample. These are transferred into vacuum and irradiated with an IR laser ($\lambda = 2.94$ $\mu$m) which leads to an explosive expansion of the droplets, ionization and finally a detection by a time-of-flight analyzer. Synapt G2-S HDMS offers mass spectrometry and ion mobility (IM) measurements.
Recently we are able to show an assembly of $\alpha$- and $\beta$-subunits of the F1F0 ATP synthase. With IM experiments we monitored conformational changes of the heterodimeric ABC transport complex TmrAB related to substance transport over the transmembrane by adding ATP/ADP at permissive conditions.

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# [P100] GFP-guided Single-Cell Microchemical Analysis of Drosophila melanogaster neurons from intact brains

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Messenger molecules such as biogenic amines and neuropeptides which are released from neurons play key roles in cell-cell communication in neuronal networks. The knowledge of their qualitative and quantitative chemical composition in individual cells is crucial for our understanding of behavioral pattern and physiological processes in organisms. A biochemical characterization of both from such minute volume sample units as a single *Drosophila* cell is a challenging task; particularly for detection of biogenic amines. Here, strategies and results using different *gfp*-guided transgenic *Drosophila* fly strains for cell identification combined with direct single cell MALDI-TOF MS-based technology (SCMS) are presented. First, using the *dimmed* (c929)-> GAL4 line which uncover neurosecretory neurons in the *Drosophila* brain, a neurochemical map of ten types of *dimmed*-neurons from the brain/gnathal ganglion was created. Resulting mass spectra not only provided comprehensive data regarding mature products from 13 neuropeptide precursors but also evidence for the cellular co-localization of neuropeptides from different neuropeptide genes. Second, an optimized strategy for detection and quantification of biogenic amines from single *Drosophila* cells is demonstrated which contains a combination of chemical derivatization for ion structure stabilization and enhancement of ion signal intensities as well as derivatized standard application for quantification. Results are presented on two diverse tyraminergic/octopaminergic (TA/OA) neuron population, identified by a Tdc2-GAL4 driver line visualized with a UAS-mCD8::GFP transgene. Furthermore, the influence of this approach was also tested for neuropeptide detection. Resulting data shows that analyses of both chemical treated-biogenic amines and neuropeptides from the same sample is possible.

*Speaker

# [P101] MS analysis of protein compositions by the biosynthesis of APE

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Polyketide synthases (PKS) can be classified as type I or type II enzymes. The PKS I type are megasynthases, in which the domains are covalently linked together, while the PKS II type contain separate protein domains. Aryl-polyene ester (APE) is biosynthesized by the PKS II like type. Ten unbound proteins are known to be involved in this system, but the exact process of the biosynthesis of APEs is mostly unknown. Our aim is to understand how the proteins interact with each other and in which composition they are in solution.
To reconstitute the APE biosynthesis, all proteins that are involved in this process were purified from *X. doucetiae*. Components were mixed in vitro, and samples were desalted with Micro Spin Columns (Thermo scientific) into ammonium acetate or Tris buffer directly before MS analysis. ESI mass spectra of the enzymes were obtained in positive ion mode using a Synapt G2S (Waters) mass spectrometer.
The acyl carrier protein (ACP) is a small protein in PKS systems, which is responsible for carrying substrates cargo between active sites. We see binding of ACP to different proteins showing protein-specific differences in binding strength. Surprisingly we find that in this system the ketosynthase and the chain length factor as well as the dehydratases (DH1/DH2) build heterodimeric complexes. While the ketoreductase and thioesterase (TE) form homotetramers whereas the TE is able to bind four ACPs. From these results we are on the way to reconstruct the process of APE biosynthesis.

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# [P102] Does the presence of D/L-proline in the isobaric hypertrehalosaemic neuropeptides of cicadas explain their isomerism?

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It is known for more than 20 years that the neurosecretory glands of the cicadas, the corpora cardiaca (CC), synthesize two isobaric peptides with hypertrehalosaemic activity. The decapeptides have the same amino acid sequence (pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-Gly-Asn-NH2) but differ in retention time in reversed-phase liquid chromatography. A synthetic peptide with the above sequence co-eluted with the second, more hydrophobic peptide peak of the natural cicada CC extract. The modification associated with the less-hydrophobic peptide was not clear. Therefore, ion mobility separation, which is sensitive to changes in conformation, in conjunction with high-resolution mass spectrometry (nanoUPLC, Synapt G2 Si, Waters Corp.), was used to investigate this phenomenon in *Platypleura capensis*. Different drift times in buffer gas for both the intact peptides and some of their fragment ions were detected. Based on the ion mobility and fragment ion intensity of the corresponding ions it was concluded that the region Pro6-Ser7-Trp8 contained a different structural feature to that of the L-amino acids present in the known peptide [1]. The chromatographic behavior of synthetic peptides excluded the presence of d-Ser and d-Trp in these positions, whereas the synthetic peptide containing d-Pro had the same retention time as the less hydrophobic natural peptide. Likely, not only racemization is an option, but also *cis-trans* isomerization. Conformers may behave differently in biochemical processes due to steric hindrance. It remains to be seen whether the isobaric peptides have different functions in the cicadas. [1] Ḱonig S, Marco H, Ǵade G. Anal Bioanal Chem. 2017;409: 6415-20.

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European Mass Spectrometry Conference

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## Advances in Ultrahigh Resolution Mass Spectrometry Enable Exploration of Complex Biological Systems

Ljiljana Paša-Tolić[1], Jared Shaw[1], Malak Tfaily[1], Rosalie Chu[1], Nikola Tolić[1], Sylwia Stopka[2], Laith Samarah[2], Beverly Agtuca[3], Chris Anderton[1], Dušan Veličković[1], Akos Vertes[2], Gary Stacey[3], Mikhail Gorshkov[4], Qinghao Wu[1], David Koppenaal[1]

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Dr. Ljiljana Paša-Tolić is a Laboratory Fellow at Pacific Northwest National Laboratory (PNNL) and Lead Scientist for mass spectrometry (MS) at the Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility operated by PNNL for the U.S. Department of Energy. Her research centers on developing sophisticated analytical methods with emphasis on Fourier transform (FT) MS and micro-separations, as well as the application of these techniques to accurately quantify spatiotemporal changes in protein (metabolite) abundance, identity, and activity. She published over 200 peer-reviewed journal articles building an international expertise in developing proteomic strategies for identification and quantitation of proteins in intact state, designing and building breakthrough instrumentation and novel strategies for proteomics and metabolomics. Most recently, she led the development of the EMSL's 21 Tesla Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, and established the MS imaging (MSI) capability at EMSL comprised of SIMS-, MALDI- and several ambient ionization (e.g., Laser Ablation ESI, LAESI) FTMS platforms for applications ranging from environmental ecology to medicine and molecular histology.

### Abstract

Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS) offers the highest mass resolving power and mass accuracy of any mass analyzer. Since all key measures of performance improve with increased magnetic field strength, a recently deployed 21T FTICR spectrometer offers unique capability to address the key knowledge gaps in functional understanding of complex microbiomes. For instance, global analyses of soil organic matter combined with omics workflows, provide information on identity and quantity of intact biomolecules (metabolites, proteins) derived from plants and microbes needed to uncover soil-microbe-plant-atmosphere relationships and interdependencies. Spatial heterogeneity and relationships can be tackled using ambient MS imaging techniques, which minimize perturbation to the tissue by limiting sample preparation and performing the analysis *in situ*. For instance, direct ionization by laser ablation electrospray ionization (LAESI) coupled with the 21T FTICR provided simultaneous spatial distributions for hundreds of biomolecules (metabolites, lipids, peptides, xenobiotics) in biological tissues, many of which could be annotated through resolved isotopic fine structure readout directly from a biological tissue. Similarly, ultrahigh resolving power is needed for characterization of intact proteoforms using top-down MS, an approach essential for physiologically relevant studies of microbes and higher eukaryotes. Advances currently in progress on the 21T FTICR platform have a potential to push the current limits of top-down MS and facilitate high-throughput comprehensive characterization of the functional proteomes. Specifically, we have demonstrated unprecedented acquisition rates and resolution for large intact proteins by coupling of the 21T FTICR, 4X frequency multiplication, ion trapping field harmonization, and spectral data processing methods.

# Localomics: Combining Mass Spectrometry Imaging & Microproteomics

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Liam McDonnell obtained an $M_{CHEM}$ in Chemistry from the University of Oxford, UK, and a PhD in Chemistry from the University of Warwick, UK. Following a post-doctoral researcher position at the FOM institute for Atomic and Molecular Physics in Amsterdam, he started his own research group at Leiden University Medical Center, where he is Associate Professor. He is director of Proteomics at the Fondazione Pisana per la Scienza, and Vice-President of the Mass Spectrometry Imaging Society.

**Abstract:**
Mass spectrometry imaging (MSI) is able to simultaneously record the distributions of hundreds of molecules directly from tissue. These spatially-resolved molecular information can be combined with multivariate/clustering analysis to reveal regions of tissue with distinct molecular signatures, a process that has been termed MSI-based molecular histology and has been used to reveal tumor subpopulations, metabolically distinct cell layers, and tumor interface zones.
Rapid direct tissue analysis is essential for MSI in order to maintain spatial localization and acceptable measurement times. The absence of an explicit analyte separation/purification step means MSI lacks the depth of coverage of LC-MS/MS. Here, we demonstrate how MSI can be combined with high sensitivity microproteomics, even of the same tissue section.

# Tandem mass spectrometry and data processing

# [Keynote] Data Independent Acquisition Coupled to Visible Laser-Induced Dissociation at 473 nm (DIA-LID) for Specific Targeting of Cysteine-Containing Peptide Subset

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Thanks to unbiased sampling of all precursor ions, the interest to move towards bottom-up proteomic with Data Independent Acquisition (DIA) is continuously growing. DIA offers precision and reproducibility comparable to targeted methods, but has the advantage of enabling retrospective data testing with new proteins of interest. However, the chimeric nature of DIA MS/MS spectra inherent to transmission of multiple precursor ions makes the confident detection of low abundant peptides highly challenging, even with spectral library-based extraction strategy. We propose the introduction of specificity at the fragmentation step by visible laser-induced dissociation (LID) at 473 nm to test for the presence of cysteine-containing peptides (Cys-peptides) in DIA. The specific absorption and photofragmentation is induced by grafting a chromophore to the thiol group of Cys-peptides. The LID spectral library was built for 354 derivatized synthetic Cys-peptides used as surrogates of human kinases (1 peptide per protein), spanning the largest dynamic range possible in order to best reflect real sample complexity. The library was validated for DIA-LID analysis of the 354 derivatized Cys-peptides spiked in *Escherichia coli* proteome lysate and 272 (76%) tagged Cys-peptides were detected and identified using the established LID spectral library. By extracting ion chromatograms of 3 derivatized Cys-peptides spiked at different concentration levels in *Escherichia coli* proteome lysate, DIA-LID demonstrates a dynamic range of detection of at least 3 decades and coefficients of precision better than 20 %. Finally, the spectral library was used to search for endogenous kinases in human cellular extract.

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# [O1] Programmed Inter-Byte Fragmentation to Facilitate MS/MS Reading of Digital Information Molecularly Encoded in Sequence-Controlled Synthetic Polymers

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As inspired by DNA storing genetic information using four different bases, information can be implemented in synthetic polymers by using two different comonomers, arbitrarily defined as the 0- and 1-bits of the ASCII alphabet.[1] Such information can be read by tandem mass spectrometry (MS/MS), but efficiency of this sequencing step depends on the backbone chemistry. Using a DNA synthesizer which implements phosphoramidite chemistry, poly(phosphodiester)s can be prepared with a perfectly controlled sequence to store digital information in chains with more than 100 repeating units.[2] However, MS/MS sequencing of these polymers is challenging for long chains because of complex dissociation patterns.[3] Here we report that the structure of poly(phosphodiester)s can be specifically designed to achieve simplified MS/MS pattern and ensure full sequence coverage. A weak alkoxyamine (C-ON) linkage was placed between all bytes (groups of 8 monomers) and each byte was labeled with a location tag. Soft collisional activation induced exclusive cleavage of C-ON bonds, leading to the release of byte fragments at different $m/z$ values thanks to their tag, which allows their individual sequencing in a second activation step. This approach has allowed successful reading of up to eight bytes of information molecularly encoded in so-designed poly(phosphodiester)s.[4]

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A. Al Ouahabi et al., *Nat. Comm.* 8 (2017), 967.

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# [O2] A new R package for deconvolution of DIA data

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Data independent acquisition (DIA) of MS2 data represents an interesting alternative to data dependent acquisition (DDA) used in metabolomics and lipidomics. However, the relationship between precursor and fragment masses is lost and spectra have to be deconvoluted to obtain interpretable data. Here a R package as add-on to the well established XCMS package is presented, which performs this task. The package was developed around the new version of XCMS3 and MSnbase for a more efficient raw data processing. Reconstruction of tandem MS data is based on correlation of EICs traces from precursor and fragment candidates. Developed functions can be applied in different modes of action, e.g. searching of multiple fragment candidates (product ions), neutral loss search or utilizing the data in a MRM-like mode. Finally, data from different DIA modes (e.g. Bruker bbCID/mbCID or Sciex SWATH) obtained from authentic standards and biological samples were used for validation purposes. First, initial results show good agreements between reconstructed and traditionally collected tandem MS data.

*Speaker

# [O3] Ion Mobility Spectrometry Enabled Fragmentation of Doubly Charged Peptide Ions in MALDI-TOF for Protein Identification

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State-of-the-art protein bottom-up identification in MALDI-MS employs singly charged peptide ions, mainly formed in the ionization process. However, fragmentation of these precursor ions requires higher collision energies and yields often fragment ion spectra of lower quality, hampering the identification of a protein by peptide fragment fingerprinting (PFF). Recently, doubly charged ions were also observed in MALDI. Using ion mobility spectrometry, these doubly charged peptide ions can be selected for protein identification, as they should yield fragment ion mass spectra of higher quality, enabling more confident PFF. Different matrix compounds were screened for the most abundant formation of doubly charged peptide ions. These matrix compounds included several halogenated a-cyano cinnamic acid derivates and 2,5-dihydroxy benzoic acid (DHB). A tryptic enolase digest (Waters, Manchester) was used as standard sample. The highest abundance of doubly charged peptide ions was observed using DHB as matrix compound, yielding even some peptide ions with a charge of 3+. Using ion mobility spectrometry (IMS) the higher charge states were visualized and a rule file for the removal of the +1 charged ions created. Optimization of the collision energy was performed to efficiently fragment +2 charged peptide ions. Finally, an automatic HDMSe acquisition method for the targeted detection and fragmentation of doubly charged peptide ions was developed using the standard protein enolase and applied to a digest of the ene reductase GOX-8. Data was acquired using a MALDI-Q-IMS-ToF mass spectrometer (Synapt G2Si, Waters, Manchester). Data analysis was performed using DriftscopeTMv2.9, BioLynxTM, and MSe DataViewer v.1.2(Waters Corp., Manchester).

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# Environment

# [Keynote] A New Single Particle Aerosol Laser Mass Spectrometer for Dual TOFMS Analysis of the Individual Airborne Aerosol Particles: Detection of Polycyclic Aromatic Hydrocarbons and Inorganic Compounds

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Single-particle laser ionization mass spectrometry (ATOFMS) based on laser desorption/ionization (LDI) is established for characterization of airborne particles. Current ATOFMS-technologies mainly detect inorganic species. Recently ATOFMS-approaches for on-line detection of polycyclic aromatic hydrocarbons (PAH) on individual airborne aerosol-particles were developed (Bente et al., Anal.Chem. 2008, 8991ff), using IR-laser desorption (LD) and a resonance-enhanced multiphoton post-ionization step (REMPI) for PAH detection. A drawback of the method is the loss of the LDI-information (inorganic composition). A new multi-step laser ionization ATOFMS-concept allows detection of organic species (LD-REMPI) and elemental signatures (LDI) from the same individual aerosol particles (Passig et al., Anal.Chem. 2017, 6341ff), based on sequential application of laser pulses and an extraction-field polarity-inversion in the dual-TOFMS between the LD/REMPI- and LDI-laser ionization processes. The particle firstly is in-ion-source IR-laser-desorbed and the PAH are REMPI-ionized. After the PAH-ions have left the ion source, the extraction-field in the ion source is reversed and the same particle core, still flying through the ion source at ¯˜ 300 m/s, is hit by a third laser pulse for LDI. The LDI-cations now are detected separately in the second TOF-tube. By this procedure particle size, REMPI-spectrum (PAH-molecules) and LDI-spectrum (inorganics) from the very same individual aerosol particle are detected. The technique has been further improved to also allow bipolar LDI-results. Measurements of e.g. wood-, lignite- or diesel-combustion particles show different organic and inorganic fingerprints. Ambient air measurements show that the approach is promising for aerosol particle anaylsis, including source apportionment and internal/external mixing state-analysis of aerosol compounds.

*Speaker

# [O1] Polarity extended chromatography coupled with HRMS for non-target screening in the aqueous environment

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Trace organic compounds are important in environmental analysis, because they impact water quality and introduce potential (eco)toxicological effects.
Current analytical methods mostly rely on gas chromatography (GC) or reversed-phase liquid chromatography (RPLC) coupled with (tandem) mass spectrometry. However, neither method can easily separate very polar molecules.

Two chromatographic separation strategies, a serial RPLC- hydrophilic interaction liquid chromatography (RPLC-HILIC) coupling and an analytical scale supercritical fluid chromatography (SFC) system will be presented, and their separation effectiveness as polarity-extended chromatographic methods for 274 environmentally relevant compounds were validated in a recent publication [1].

Compounds tested were grouped into three polarity classes, "very polar, "polar", and "non-polar". Nearly all compounds could be retained in both systems with relative standard deviations of retention times typically between 2 and 5%.

Both techniques have considerable benefits when combined with accurate mass spectrometric detection. Molecules RT and accurate mass were recorded in a database for each set up. This information was used for compound screening measurements like "hidden-target screening" in complex environmental matrices (such as wastewater treatment plant effluents).

Results of both techniques are complementary and useful for all types of molecules polarity. The orthogonality of both techniques provides additional benefits for the identification of known and unknown compounds by HRMS or tandem-HRMS [2].

S. Bieber, G. Greco, S. Grosse, T. Letzel. Analytical Chemistry 2017, 89 (15), 7907-7914
S. Bieber, T. Letzel. Journal of Chromatograhy B, submitted

*Speaker

# [O2] Non-targeted characterization of PAXHs contaminated soil using API FT Orbitrap MS

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Over 0.5 million contamination sites have been identified in Europe as a byproduct of ongoing industrialization, which leads to an annual clean-up cost ranging from 2 to 17 billion Euros. Among others PAHs produced during production processes in coking and manufactured gas plants are of great concern. The targeted analysis of 16 EPA PAHs has been applied over 40 years to monitor the contamination level of PAHs in soil and evaluate the thermal, chemical and/or biological remediation technologies. However these representatives don't really represent the whole PAH contaminants.

Here we present a comprehensive non-targeted analysis of a PAH contaminated soil by FT Orbitrap MS using various API methods, including ESI, APCI and APPI, in both polarities. Prior to the mass spectrometric analysis, 5.00 g of air dried, sieved and homogenized potting soil or contaminated soil were extracted by Soxhlet extraction using the following solvents: toluene, dichloromethane, acetone : *n*-hexane (1:1, *v/v*) or methanol.

With our method we are able to determine pure and alkyl derivatives of PAHs, moreover heterocyclic aromatic compounds containing N, S or O. Our results showed a 2 to 7-fold higher amount of solvent extractable organics in the contaminated than in the potting soil. Distinct class and DBE vs. carbon number distributions were found for the contaminated soil compared to the potting soil. Besides highly aromatized PAHs with a DBE up to 70, low to high molecular weight azaarenes were also detected. Finally our results offer a deeper insight into what are the contaminants in soil.

*Speaker

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# [O3] Metal speciation and mobility in clay - From ICP-MS batch and CE-ICP-MS Speciation to miniaturised clay column experiments (MCCE) using LC-ICP-MS

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Nowadays, there is a broad scientific consensus on the technical merits of the disposal of high-level nuclear waste (HLW) in deep and stable geological clay formations. A wide set of geochemical parameters can influence the mobility of radionuclides originated from a leakage in a waste disposal for example competing ions released from the clay by infiltration of percolating water, natural organic matter (NOM) as complex forming ligands, changes in temperature or pH-milieu of the aquifer. In this study trivalent europium(III) (homologue of americium(III)) and uranium(VI) were used and their retention in Opalinus clay in the presence or absence of NOM were studied. As methods, capillary electrophoresis hyphenated with inductively coupled plasma mass spectrometry (CE-ICP-MS) was used to study the complexation behaviour of Eu(III) and U(VI) with HA. The influence of metal concentration, the presence of competing cations from clay dissolution as well as cations from clay porewater on the complexation behaviour was analysed. For the sorption/desorption behaviour common batch experiments with clay mineral suspensions are performed, and in comparison a new developed setup of miniaturised clay column experiments (MCCE) with compacted clay was used to study the influence of NOM on the metal mobility in clay. Online coupling of the MCCE with ICP-MS leads to quantitative information on the elemental composition of the eluent directly after determination of the UV/Vis-active compounds in the diode array detector of the LC.

*Speaker

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# Metabolomics and fluxomics

# [Keynote] Single-cell mass spectrometry of metabolites extracted from live cells by Fluidic Force Microscopy

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There is a growing awareness that cell-to-cell heterogeneity is present in any cell population, which has fostered the development of specific methods and approaches to enable studies at the single cell level. Whereas analysis of subcellular amounts of DNA and RNA has become feasible, to date, single-cell metabolomics is still in its infancy. However, there is high interest to overcome current limitations since changes in metabolite levels can be regarded as the ultimate response to genetic and/or environmental triggers. Besides required sensitivity of analytical instruments, appropriate sampling methods have to be developed. Here, we coupled metabolite extraction by fluid force microscopy (FluidFM) with MALDI-TOF-MS for metabolite detection. FluidFM represents a modified atomic force microscope provided with a microchanneled cantilever, which is mounted on top of an optical microscope. The instrument enables minimal cell perturbation and quantitative extraction of intracellular molecules without affecting viability [1]. Defined amounts of cell extracts were disposed on special microarrays for mass spectrometry (MAMS) and samples were prepared following a protocol that provides high sensitivity for metabolite analysis with MALDI-TOF [2]. With the developed method, we could detect 20 cytoplasmic metabolites of individual HeLa cells without removing them from its environment or compromising their viability. Moreover, labeling incorporation into different metabolites was shown upon C-13 glucose feeding experiments. The approach not only offers the opportunity to perform time series but also to combine different omics approaches at single-cell level.

*Guillaume-Gentil et al. 2016 Cell*

*Guillaume-Gentil et al. 2017 Anal Chem*

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# [O1] High-resolution mass spectrometry to decipher microbial origin of sulfonolipids inside mammalian gut

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Sulfonolipids (SLs) are classified into the group of sphingolipids. They have a sulfonic acid group in the sphingoid base, and are structurally close to the class of ceramides. In previous studies, they were found in diatoms and some bacteria such as *Capnocytophaga*, *Cytophaga*, *Flexibacter*, *Sporocytophaga* or *Algoriphagus*. So far, SLs were not identified in mammalian gut. Therefore, we aim to investigate whether SLs are present in mouse gut and which microbes are capable of SLs production. We screened the luminal content of cecal extracts by FT-ICR-MS, followed by identification and characterization applying LC-MS/MS and NMR. We narrowed down potential mass signals of SLs based on the previously reported molecular formula of sulfobacin A, B and flavocristamide A, supported by the isotopic fine structure analysis, derived from FT-ICR-MS analysis. Extensive LC-MS/MS analysis of fragmentation patterns allowed us to identify eighteen SLs, varying in their capnoid and fatty acid composition. Two LC-peaks were isolated and as an example one LC-peak was confirmed as sulfobacin B by NMR. Semi-targeted LC-MS/MS of SLs revealed that high-fat diet is significantly increasing SLs profile in the gut of mice. The microbial original of SLs was elaborated by metagenome analysis by searching sphingolipid biosynthesis genes and homologues and their taxonomic assignment. Several bacterial candidates were proposed and two genera including *Alistipes* and *Odoribacter* were able to produce SLs in pure cultures, confirmed by LC-MS/MS analysis

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# [O2] Determination of stable isotope labeled glucose tracers by high-resolution mass spectrometry in dual- and triple-tracer studies

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Determination of glucose tracer enrichments in human sample blood derived from multiple-tracer studies usually relies on a mix of stable isotope labeled and radioactive tracers, because with nominal mass resolution (traditionally GC-MS) mass spectral interferences rapidly increase with the number of stable tracers used. Due to this fact the number of stable isotope tracers is usually restricted to two at maximum by the statistical uncertainties arising from the isotope corrections of mass spectral interferences. Here we show that these limitations can be overcome by applying high-resolution mass spectrometry (HRMS). Our method relies on Orbitrap mass spectrometry of deprotonated glucose in negative ESI mode at a resolution of almost 200.000. This lets us monitor the exact masses of various triple tracer combinations (e.g., [1-2H1]-, [6,6-2H2]-, [1,6-13C2]glucose). With this instrumental setup we are able to completely separate 2H and 13C tracers, thereby allowing the enrichment of a tracer to be simply calculated from the observed ion intensities using a standard curve with curve parameters unaffected by the presence of other tracers. Generally, this HRMS method delivers low limits of detection and good repeatability in the tested 0.1-15% enrichment range. Furthermore, short sample preparation and analysis times render this method highly suitable for high throughput determination of multiple glucose tracer enrichments in plasma samples. In summary this new mass spectrometry based method for determination of multiple stable tracer enrichments should in the long run be able to replace any radioactive tracers still used in human glucose metabolism studies.

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# [O3] Planetary Scale Metabolomics - Molecular Imaging of a Phytoplankton Bloom in the California Current Ecosystem

Daniel Petras *† [1], Brandon Stephens [2], Sara Rivera [2], Lihini Aluwihare [2], Pieter Dorrestein [1]

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**Dissolved organic matter (DOM) is one of the most complex community metabolomes on earth and primarily the product of interactions between the upper ocean's microbial food web and downstream abiotic chemical modifications. Analyzing the chemical composition of DOM is important for determining the rate and extent of element exchange between inorganic reservoirs and the marine biosphere and of central importance for illuminating microbe-microbe interactions within these communities. To study the community metabolome during a phytoplankton bloom in the California Current Ecosystem, we applied large scale water sampling, covering different stages of the bloom. After solid phase extraction and subsequent non-targeted high-resolution liquid-chromatography tandem mass spectrometry (LC-MS/MS) analysis, we aligned exact masses from extracted ion chromatograms with MS/MS information in spectral networks. Besides the annotation of a multitude of primary metabolites, we could also identify species-specific secondary metabolites such as algal toxins. Our results showed that different stages of the bloom have clearly distinguishable chemotypes, depending on microbial community composition, nutrient concentrations, and physical properties of the water mass. Through correlation analysis and multivariable statistics, we could determine molecular drivers of the different stages, enabling a connection of molecules to community members and environmental factors. Besides important biological implications, this study is the first large scale application of LC-MS/MS for the analysis of DOM, showing the enormous potential of this analytical approach. We anticipate, that these developments, together with next generation sequencing and meta-proteomics, will pave the way to a more comprehensive understanding of the role of DOM in marine ecosystems.**

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# Waters Workshop
# Wednesday 12:00 – 13:00
# Room 0.06

# Sciex Workshop
# Wednesday 13:00 – 14:00
# Room 0.23

# Pharmaceutical applications and nutrition

# [Keynote] Multiplexed proteome dynamics profiling reveals mechanisms controlling protein homeostasis

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Protein homeostasis integrates the balanced control of protein synthesis and degradation with the regulation of mRNA metabolism. Maintaining proteome homeostasis requires the concerted action of a multitude of proteins. The regulation of e.g. protein degradation is extremely complex involving hundreds of proteins such as E3 ligases, deubiquitinases and proteases to orchestrate proteasomal, lysosomal and other degradation pathways. External stimuli and disease can modulate any of these processes and thus alter the proteome. However, sensitive proteome-wide profiling approaches to distinguish dynamic changes in protein synthesis- and degradation across a range of conditions at high throughput are lacking. We developed 'multiplexed proteome dynamics profiling', mPDP. This quantitative mass spectrometry-based proteome profiling method combines dynamic light-to-heavy and heavy-to-light SILAC labelling with isobaric mass tagging to enable the sensitive and comprehensive simultaneous analysis of changes in protein degradation and synthesis in a single mass spectrometric experiment of biological replicates for up to five different treatment conditions. We applied this strategy to investigate how manipulating the HSP90 chaperone system affects the proteome. mPDP enabled classification of HSP90 dependent proteins either requiring HSP90 for stabilization constitutively versus during synthesis only. Constitutive HSP90 clients have lower thermal stability and higher affinity for the chaperone compared to synthesis clients. Further, HSP90 dependency can vary between cell types and can be conditional to external stimuli such as T-cell receptor activation.

*Speaker
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# [O1] Analysis of brain tumors after fluorescence-guided resection by the complementary use of HILIC-ESI-MS/MS and MALDI-MS/MS

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Fluorescence-guided surgery (FGS) has evolved as an important technique for brain tumor resection. After oral application and transport of the proagent 5-aminolevulinic acid (5-ALA) across the disrupted blood brain barrier, protoporphyrin IX (PpIX) is formed as an intermediate product of the heme biosynthesis cascade and accumulates within the tumor. By intraoperative fluorescence microscopy, the specific PpIX fluorescence can be used to differentiate tumor from healthy brain tissue. While FGS is an established technique for the resection of glioblastomas being the most aggressive malignant brain tumor, the application for low grade gliomas and other types of brain tumors is still under investigation as the observation of fluorescence is heterogeneous. To further examine the biochemical processes related to FGS, the complementary analysis by liquid chromatography mass spectrometry (LC-MS) and MS imaging (MSI) is presented. For spatially resolved analysis, cryosections of different human tumor samples were investigated by matrix-assisted laser desorption ionization (MALDI)-MS/MS. The drug distribution maps were correlated to the evaluation by histological staining. Furthermore, an ultrasonication-based method for drug extraction from the tissue was developed. Direct analysis of the polar analytes without the need of time-consuming derivatization was realized by the use of hydrophilic interaction liquid chromatography-electrospray ionization (HILIC-ESI)-MS/MS providing good limits of detection and quantification. By means of ultra-high performance LC (UHPLC), a fast separation of 5-ALA from potentially interfering isobaric compounds of endogenous origin was accomplished. Consequently, this study proves the value of the complementary use of spatially resolved and LC-based MS methods to address complex biomedical challenges.

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# [O2] Volatile Compound Fingerprinting by Headspace Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS) for the Authenticity Assessment of Honey as Benchtop Alternative to 1H NMR Profiling

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[2]

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This work describes a simple approach of untargeted profiling of volatile compounds for the authentication of the botanical origin of honey based on resolution-optimized HS-GC-IMS, combined with optimized chemometric techniques, namely PCA, LDA and kNN. A direct comparison of the PCA-LDA models between HS-GC-IMS and 1H NMR data demonstrated that HS-GC-IMS profiling could be used as a complementary tool to the NMR-based profiling of honey samples. While NMR profiling still requires comparatively precise sample preparation, in particular, pH adjustment, HS-GC-IMS fingerprinting may be considered as an alternative approach for a truly fully automatable, cost-efficient and in particular, highly sensitive method. It was demonstrated that all tested honey samples could be distinguished based on their botanical origin. The loadings plots revealed the volatile compounds responsible for the differences among different monofloral honeys. The HS-GC-IMS-based PCA-LDA model was composed of two linear functions of discrimination and 10 selected PCs that discriminated canola, acacia and honeydew honeys with a predictive accuracy of 98.6 %. Application of the LDA model on an external test set of ten authentic honeys clearly proved the high predictive ability of the model by correctly classifying them into three variety groups with a correct classification of 100 %. The constructed model presents a simple and efficient method of analysis and may serve as a basis for the authentication of other food types.

[*]Speaker

# [O3] Advanced glycation end-products in the bovine milk proteome

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Thermal milk processing ensures its safety and longer storage, but also induces Maillard reactions modifying proteins yielding the heterogeneous advanced glycation end-products (AGEs) that can likewise be formed by dicarbonyls. AGEs may reduce the nutritional value of milk, may alter protein functions, trigger allergies, and elevate the "*in-vivo* AGE-pool" raising concerns about harmful effects. Therefore, 14 AGEs formed in milk proteins during processing/storage were analyzed in raw milk and different brands of pasteurized, ultra-high temperature treated (UHT), and lactose-free pasteurized and UHT (ULF) milk, and infant formulas (IFs). Additionally, UHT and ULF were stored for three months (_˜23∘C) and IF for one year (4∘C). AGE-modified peptides were identified by nRP-UPLC-ESI-MS/MS using DDA in CID-mode (unmodified peptides identified by DIA were excluded) and confirmed in a targeted DDA approach (ETD mode). Overall, 132 AGE-modified peptides (118 modification sites, 62 proteins) were identified in CID- and ETD-modes. Most AGEs were detected at lysine residues (mainly formyl- and carboxymethyllysine). The number of AGE-modification sites increased with harsher processing similarly for regular and lactose-free products, but remained stable during storage. This was supported by the peptide quantities. Beta-lactoglobulin as main target (eleven modified residues) was modified at K77, K135, and K138 by five AGE-types indicating their high susceptibility. Most AGE types/sites were identified for the first time. Similar modifications can be formed from lipid peroxidation products (LPPs), which are typically referred to as advanced lipoxidation end-products (ALEs). Currently we are profiling LPPs as potential precursors of ALEs to identify and quantify ALE-modified milk proteins.

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# Instrumentation 2

# [Keynote] Extending the gas phase structural biology toolbox: Circular Dichroism mass spectrometry of G-quadruplexes.

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Native mass spectrometry is now widely accepted as a useful tool for structural biology studies, particular for the study of non-covalent oligomers of biomolecules. Despite this, there are currently many unknowns within native mass spectrometry which make it unclear to what extent it can be used as a structural tool. Hence, the development of probes of the gas phase structure of biomolecules is of high interest. Spectroscopy based techniques are particularly attractive as they can provide the possibility for comparison of structure in both solution and gas phase, therefore providing a bridge between the two media and allowing to study more deeply the link between solution and gas phase biomolecular structure. To this end, we report here the first results of gas phase circular dichroism on several G quadruplex structures measured by monitoring of electron photodetachment. G quadruplexes with parallel and antiparallel strand topologies are considered, and circular dichroism in gas phase and solution phase are compared. The results show for the first time that gas phase circular dichroism of large gaseous biomolecules is feasible, and can be an important and versatile new tool for native mass spectrometry studies.

*Speaker

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# [O1] Comparison of TLC-LTP-MS and TLC-FAPA-MS: Fundamental Studies and Selected Applications

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Ambient desorption/ionization mass spectrometry (ADI-MS) has emerged as an important analytical field for direct sample analysis since 2004, when desorption electrospray ionization (DESI) was introduced. The main goal of ADI-MS is to minimize sample preparation procedures (*e.g.*, solvent extraction) and to overcome the long analysis times, often encountered with chromatographic separations, by directly desorbing the target analytes from the sample surface. Usually high-resolution mass spectrometry is used to distinguish large amounts of different analyte molecules inside a sample mixture. Nevertheless, ADI-MS techniques are susceptible to matrix effects and as a result the analyte response may vary from sample to sample. Because of these effects, fast screening techniques, such as thin-layer chromatography (TLC), might be used to quickly separate matrix components from that target analytes before direct MS sampling via ADI techniques to prevent matrix interferences. In the presented study, the low-temperature plasma (LTP) probe and the Flowing Atmospheric-Pressure Afterglow (FAPA) will be compared for direct surface sampling of model analytes (*e.g.*, caffeine, ibuprofen, and acetaminophen) from TLC plates. Fundamental features of desorption properties for the plasma-based ADI sources will be discussed. First, different stationary phases for TLC, *e.g.*, normal-phase, reversed-phase, and cyano-phase, will be compared for their capability to be coupled with these ADI sources. Second, the influence of the TLC phase thickness will be discussed in regard to the desorption process. Third, the effect of transient microenvironments in relation to the desorption/ionization process will be discussed.

*Speaker

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# [O2] Development of Micro-Time-Of-Flight mass spectrometer for Chemical Threat in situ detection

Frédéric Progent *† [1], Alexandre Sonnette [1], Jérôme Tupinier [1], Pierre-Etienne Buthier [1], Jean-Christophe Lictevout [1], Sebastien Vigne [1], Thomas Alava [2]

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**M**ass **S**pectrometry (MS) has become a reference analytical tool and is now implemented in many laboratories. Despite the widespread implementation of this analytical technique, classical MS instrumentation remains heavy and expensive laboratory equipments.
In this context, the development of a portable gas chromatography-MS is of great interest, especially for *in situ* gas analysis. To be efficient on the field, the device should be small, light and low power consuming. In order to address the size constraints, we used the **M**icro **E**lectro **M**echanical **S**ystem technology (MEMS). This technology has been developed by the microelectronic industry and has tremendous applications in the sensors area. MEMS developed in this work have a size of only 1.5 cm × 3 cm for micro-mass spectrometer.
Last developments and optimization of our new generation of microfabricated time-of-flight mass spectrometer ($\mu$-TOF) are focused on ionization, ions extraction and their focusing. Using electron impact ionization (EI 70 eV), ions are generated in an ionization chamber, then extracted and focalized by electrostatic lenses constituted by 6 electrodes. Ion beam is collected in linear mode in a Micro Channel Plate (MCP) detector and mass spectra are recorded. Using decreasingly concentrated gas mixtures of six first alkanes in helium, we show the ability of microfabricated time-of-flight mass spectrometer to detect up to 100 ppm of alkanes in helium. Resolution will be improved using orthogonal injection. This work shows encouraging results and pulls the $\mu$-TOF one step closer towards a fully integrated portable analytical system.

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# [O3] Laser-spark ionization mass spectrometry

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A versatile ionization scheme for atmospheric pressure MS is presented. It is based on a quasi-continuous laser-induced plasma (LIP), generated by a 26 kHz pulsed DPSS-laser, which is ignited in front of the MS inlet. Analytes are determined with different sampling regimes, comprising either an ambient desorption/ionization mechanism, a liquid-phase or gas-phase sample introduction.
The MS signal closely resembles the ionization behavior of APCI-like plasma-based sources, such as DBD or DART. Though LIPs are known to efficiently atomize/ionize any sample material, mass spectra of intact molecular ions are recorded, exhibiting low fragment-ion content. To understand this contradictory behavior, the plasma properties are investigated that lead to the formation of molecular ions. Comprehensive studies include optical emission spectroscopy, shadowgraph imaging and mass spectrometry diagnostics.
The results show that the ionization of analyte does not occur in the plasma itself, but in the cold adjacent gas layer. The pulsed character of LIPs induces an expanding shockwave, which concentrically expands around the plasma core and sweeps the molecules toward the plasma edges, where they are ionized either directly by the self-emission of the hot core or via interaction with secondary reactants. However, this unidirectional transport causes a rarefaction inside the plasma center, which leads to a decrease in plasma intensity and number density. Thus, a restoration of the former gaseous medium by other dynamically equilibrated diffusion processes would be favorable. Besides gas replenishing, we demonstrate the beneficial use of an acoustical standing wave inside an ultrasonic resonator on the performance of the LIP.

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# Ion chemistry

# [Keynote] Photo-induced linkage isomerization in the gas phase probed by tandem ion mobility and laser spectroscopy

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Ruthenium complexes involving sulfoxide ligands display unique photochromic properties with potential applications to the design of smart materials. Namely, a modification of the linkage mode of the sulfoxide ligand (from S to O or O to S) can be triggered by light absorption, accompanied by dramatic changes in the optical spectrum of the complex. Numerous experimental and theoretical studies have been undertaken to understand this photo-induced linkage isomerization mechanism. We used a combination of mass spectrometry, ion mobility spectrometry (IMS), and laser spectroscopy to probe in details the ground and excited state isomerization pathways of a prototypal example of such ruthenium complex. Namely, a Ru(bipyridine)2 complex binding two dimethyl sulfoxyde (DMSO) molecules (Ru-bpy2-DMSO2) has been investigated. Three different isomers of the complex were possible to distinguish from ion mobility measurements, which were assigned to the three possible binding schemes for the two DMSO ligands (SS, SO, and OO). Tandem-IMS experiments were then carried out to follow the isomerization pathways from each isomer. This could be done in the ground state by subjecting selected ions to collisional activation, as well as through electronic excitation by laser irradiation. In particular, we show that photo-induced linkage isomerization is possible for isolated [Ru-bpy2-DMSO2]2+ ions, thus not implying ligand exchange with solvent molecules. Finally, based on IMS-resolved action spectroscopy measurements, we were able to characterize separately the optical properties of the different linkage isomers identified. These results are in line with recent theoretical predictions.

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# [O1] Mechanistic Insight in the Copper Catalyzed Oxidative Derivatization of Tetrahydroisoquinolines

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The development and optimization of new organic reactions often is a highly challenging task. Undirected screening of a multitude of different reaction parameters typically blocks a lot of resources without guarantee for satisfying improvements. In this context, mechanistic insight in kinetics, relevant intermediates, as well as side products or unproductive resting states can be of great help. Modern mass spectrometry offers powerful tools for mechanistic studies. Even complicated reaction mixtures can be studied in detail by sophisticated combinations of electrospray ionization with online microreactor techniques.[1,2] We herein present elucidating mechanistic results gained for the copper-catalyzed oxidative derivatization of tetrahydroisoquinolines developed in the Menche lab.[3]
We were able to successfully detect and characterize several postulated reaction intermediates by high-resolution tandem mass spectrometry and also monitor their temporal evolution in reacting solutions.[4] In particular, substituent effects on the reactivity were probed with an electrochemical cell directly coupled to the electrospray ionization source. Based on the experimental results, a consistent catalytic cycle was proposed and an improved reaction protocol was developed.

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# [O2] Structure and Reactivity of Gas-Phase Phenylhydroxycarbenes: Hydrogen Tunneling at Room Temperature

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We report initial spectroscopic evidence for H-tunneling at room temperature, observed in hydroxy-

-(trimethylammonio)phenyl
carbene in the gas phase. This carbene is generated by decarboxylation of oxo[4-(trimethylammonio)phenyl]acet
acid upon collision induced dissociation (CID) tandem mass spectrometry, and structurally characterized by isomer-selective two-color infrared-infrared-spectroscopy and high-level calculations. The charge-tagged phenylhydroxycarbene undergoes clean 1,2-H-shift to the corresponding aldehyde, evidenced by isomer-selective IRMPD- depletion experiments. The deuterated (OD) carbene analogue showed negligible 1,2-D-shift reactivity, providing clear evidence for hydrogen atom tunneling in the H-isotopomer. This is the first experimental confirmation that hydrogen atom tunneling can govern 1,2-H-shift reactions at room temperature; a result of broad significance for a wide range of (bio)chemical processes, including enzymatic transformations and organocatalysis. **Mathias Sch'afer**, Katrin Peckelsen, Mathias Paul, Jonathan Martens, **Jos Oomens**,
Giel Berden, **Albrecht Berkessel**, **Anthony J. H. M. Meijer**, Hydrogen Tunneling Above Room Temperature: A Gas-Phase Study of Phenylhydroxycarbene, Journal of the American Chemical Society **139** (2017) 5779–5786.

*Speaker

# [O3] Mass Spectrometry and Ion Mobility Spectrometry for Investigating the Interlocked Nature of Catenanes

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We report an elaborate characterization of imine-based macrocycles, formed with the efficient condensation of dialdehyde and aliphatic diamine in pure water. The identity of the formed macrocyles are characterised based on the mass-spectrometry and ion mobility spectrometry. The latter is also combined with the collision induced experiments to further demonstrate the interlocked nature of the cycle; therefore, the macrocycle is unambiguously assigned as a [2]catenane. The interlocked structure is well supported by the results from NMR measurements.
Within the [2]catenane libraries, we identified a family of homologous amphiphilic [2]catenanes, whose self-assembly is primarily driven by the hydrophobic effect. The length and odd-even character of the diamine alkyl linker dictate both the yield and the conformation of the [2]catenanes, whose particular thermodynamic stability further shifts the overall equilibrium in favour of imine condensation.
These findings highlight that mass spectrometry combined with ion mobility spectrometry can be effectively used to characterize the structure of interlocked supramolecular structures. Additionally, our results demonstrate the role played by solvophobic effects in the self-assembly of complex architectures.

*Speaker

# Wednesday Posters

# [P001] Evaluation of electronic effects of ligands with VUV spectroscopy

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An important feature of metal catalysts lies in the crucial role of ancillary ligands on the electronic structure of the metal center [1]. Determining how those ligands affect the reactivity of the metal center and quantifying this effect is thus of great importance. Various methods have been proposed to evaluate the electronic influence of ligands such as the Tolman Electronic Parameter [2]. All these approaches suffer however from some limitations. In particular they do not give access to the relative contributions of the ligand in terms of $\sigma$-donation and $\pi$-back donation effects as described by the Dewar-Chatt-Ducanson model [3]. In this context, a multi-fields collaborative network has been developped with the objective of exploring new approaches to describe and rationalize electronic properties of ligands bound to a metal. We present here the results obtained with VUV-spectroscopy on Molybdenum complexes, Mo(CO)5L, with L = various phosphine ligands. Mass selected photoelectron spectroscopy was used to probe the electronic configuration of the complexes by measuring their ionization energies which are related to discrete energy levels of molecular orbitals [4].

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# [P002] Comparison of atmospheric pressure photo ionisation with 8.6/9.4 and 10/10.6 eV to atmospheric pressure chemical ionisation for the analysis of complex mixtures

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For the last decades, atmospheric pressure ionisation techniques raise in importance for state-of-the-art mass spectrometric analyses, and non-targeted analyses often need to cover a broad chemical space. In this study, evolved gas analysis coupled to a 7 T Fourier transform ion cyclotron resonance mass spectrometry is applied to investigate the gas-phase ionisation behaviour of complex mixtures with different API techniques.
APPI utilising a Xenon lamp (8.4/9.6 eV) was applied and compared to APPI utilising a Krypton lamp (10/10.6 eV) and APCI. Various standard substances and complex samples were introduced into the ionisation source by hyphenation to a thermobalance.
Based on the pyrolysis of polystyrene it turned out that also with Xe-APPI a broad oligomeric pattern can be detected. The pattern changes drastically from APCI to APPI. Despite the fact that all decomposition products are pure hydrocarbons other species are ionised more efficiently in Kr- and Xe-APPI. This effect was also observed for petroleum distillation cuts. The analysis of the elemental composition assignments reveal a clear shift of the distribution between protonated and radical cations. APCI exposed mostly protonated ions, whereas APPI showed mostly radical ions. The proportion of odd-species was even higher for Xe-APPI.
In summary, Xe-APPI will result in a lower ionisation efficiency and higher limit-of-detection. Nonetheless, for investigations where sensitivity is not the primary aspect Xe-APPI can be applied, and the absence of protonated compounds leads to a further simplification of the spectra. This aspect is of high interest for ultra-complex samples, such as shown for Petroleomics.

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# [P003] Homogenous Langmuir-Blodgett films of gold nanoparticles for quantification in surface-assisted laser desorption/ionization mass spectrometry

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The inhomogeneity of the surface in surface-assisted laser desorption/ionization (SALDI) mass spectrometry (MS) often causes significant shot-to-shot variations of ion signals and spot-and-spot reproducibility issues, which limit quantitative analyses in SALDI-MS. Therefore, preparing a reproducible, homogenous and reliable layer of surface material is a fundamental requirement for quantitative SALDI MS.
Langmuir-Blodgett (LB) technology can be readily performed at low cost and high throughput levels, to achieve highly-ordered films of nanoparticels. Gold nanoparticle (AuNP) LB films with different densities were synthesized here, initially characterized by their UV/Vis spectra, followed by SEM analysis to show homogeneity and the highly ordered structures of the films.
AuNP LB films were expected to be an effective surface for quantification in SALDI-MS because the highly ordered layer of AuNPs dissipates the laser energy homogenously over a large area and sweet spots are avoided. Due to the homogeneity and repeatability of these films, the reproducibility of the generated ion signals from these films should be highly improved. Initially, the AuNP LB film has been applied to measure linoleic acid. The results illustrated that signal intensity, background and signal-to-noise ratio were greatly improved as compared to MALDI using CHCA as matrix. The density of the AuNP LB films was expected to affect desorption and ionization of analytes. The signal intensity of linoleic acid increased as the density of the film increased. Other analytes such as amino acids, small peptides and synthetic drugs could also be readily measured with AuNP LB films by SALDI-MS.

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# [P004] Automated Presentation of Solid Phase Extraction Fibers to Facilitate High-Throughput Ambient Ionization-MS Analysis

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Unattended thermal desorption analysis of chemicals isolated from biological fluids by using solid phase extraction fibers has been facilitated by using a liquid handling robot. The integration of the robot with a Direct Analysis in Real Time (DART®) ionization source mass spectrometry system permitted rapid, reliable determination of drugs in biological samples including urine and oral fluids, as well as pesticides in plants and fruits. We demonstrate the utility of SPME-based isolation for moderate throughput analysis of the extracted drugs using multiple passes at different desorption gas temperatures to investigate the potential for reducing the matrix effects normally encountered in the DART analysis of these complex mixtures. Utilizing a combination of DART®-HR/MS, a liquid handling robot (Andrew 1000, Andrew Alliance) programmed for precise movement and positioning of the SPME fibers we demonstrate moderately high-throughput analysis of samples with good precision and accuracy.

*Speaker

# [P005] Cluster Ion Formation of Saccharose in Positive-Ion Direct Analysis in Real Time Mass Spectrometry

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The analysis of mono-, di, and trisaccharides by positive-ion direct analysis in real time mass spectrometry (DART-MS) [1] revealed that all of these sugars yielded [M+NH4]+ ions while some additionally formed abundant [Mn+NH4]+ cluster ions [2].
Cluster ion formation of highly polar or ionic analytes is common in DART-MS, can extend to high mass [3], and may be exploited for mass calibration [4].

Saccharose, the most common sugar, is among the [Mn+NH4]+ ion forming species, and thus, may potentially be used as mass calibrant.

However, the factors influencing the extent of saccharose cluster ion formation such as temperature of the DART gas and sample load as well as instrumental factors like trapping conditions of ions prior to mass analysis have to be well defined. Therefore, this study deals with the optimization of experimental conditions and identifies critical parameters.
A Bruker Apex-Qe Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer was used. Ions were collected for 0.3–1.0 s prior to ICR mass analysis in RF-only accumulation hexapole. Ions of the range $m/z$ 200–2500 were excited and detected using standard setting from previous DART work [4]. The DART-SVP ionization source was mounted on the ESI interface of the ion source via the Vapur Interface. Transmission mode DART was employed by mounting the Open Source. External mass calibration in positive-ion DART mode was established on an ionic liquid (IL)[4]. For DART-MS, sample solutions were applied to the Open Source cards. The temperature of the DART source was set to 200–450∘C.

*Speaker

# [P006] Characterization of LILBID-MS using DNA structures

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LILBID-MS is a sensitive mass spectrometric ionization method in which specific noncovalent complexes are transferred into gas phase via micro-droplets of a native-like aqueous buffer, which are irradiated by an IR-Laser. This leads to an explosive expansion of the sample droplets. As a result of the desorption process, the ions receive an energy input from the laser pulse, which can be controlled via the intensity of the desorption laser.
This MS technique is able to detect the intact single- and double-stranded oligonucleotide complexes in the gas phase. Under soft desorption conditions, we observe nearly no dissociation of the duplex for 15-20 bp, while harsh laser conditions lead to complete dissociation of the *ds*DNA. So *ds*DNAs present a suitable model for the investigation of the softness of LILBID. We tested LILBID's tolerance towards salt concentration in buffer using the DNA samples, which are annealed under different buffer conditions. This allows us now to optimize the salt concentration to ensure the stability of the double helix without compromising the spectral resolution.

*ds*DNA melting can be observed with a heatable droplet generator. The relative amount of duplex was defined as the peak of all duplex signals, divided by the sum of all peak areas. By plotting this ratio of duplex to total signal against the temperature of a solution it is possible to create a melting curve. Comparison of apparent *Tm* from these curves with measured *Tm* with UV/*vis* spectroscopy allows us to assess the energy transfer during desorption under different laser conditions.

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# [P007] Ultra-high resolution mass spectrometric characterization of a pyrolysis biofuel by using different ionization techniques and spectra-stitching method

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Biofuel derived from biomass feedstocks invokes the interests of scientific community because of the cheap price, large quantity availability and sustainability of feedstocks which allows it's production on a larger industrial scale. Biofuels present as a good model of highly complex mixture. In order to achieve an in-depth compositional analysis of a bio-oil, it is of tremendous importance to utilize complementary techniques. Previously we have demonstrated 3s transient data (mass resolution of 960k at m/z= 400) for petroleomic study by using a research-type Orbitrap Elite and revealed the significant coverage improvements. However, such high resolution mass spectrometry has never been used for bio-oil studies. In this study, a combination of techniques, such as different resolving power (120k; 240k; 480k and 960k), ionization methods (positive APCI and APPI; ESI with both positive and negative mode) and scan mode (full and spectra stitching mode), were investigated for bio-oil characterization. Finally, a total number of 34472 compositions were detected, which is a significant improvement in comparison with previous study. Also the better data depth allows in detailed studies of ionization discrimination effects by analysis of classes distributions, specific class DBE versus C distribution, unique compositions DBE/C normalization, C/O values, etc.

*Speaker

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# [P008] SICRIT Mass Spectrometry: Simple, Smart and Sensitive

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*Soft **I**onization by **C**hemical **R**eaction **I**n **T**ransfer*" (SICRIT) is new a ionization technology introducing mass spectrometry in new fields of application. SICRIT directly merges the atmospheric pressure inlet of the MS with a small and rigid dielectric barrier discharge based plasma ionization (DBDI) source. This significantly enhances ion transmission into the MS and thereby the sensitivity. The ionization process is very soft usually yielding solely MH+ analyte species. As a further advantage, the source does not need any additional gas or liquid supplies and can be attached to virtually any atmospheric pressure inlet MS. Due to its flow-through design, it can be used for direct analysis without any sample pretreatment or in combination with e.g. LC, GC, SPME or laser ablation.
Several studies were conducted on various instruments and directly coupled to chromatographic analysis systems, e.g. a nano-LC, a GC, and a laser ablation system. The source has proven to be capable of efficiently ionizing a wide range of substance classes including chemical warfare agents (CWA), PAH, pesticides, and illicit drugs with general sensitivities found to be in the ppq/low ppt range.
Our results show that the SICRIT ionization source is very sensitive and versatile for screening or in-field-applications as well as for interfacing to any chromatography or sample delivery system.

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# [P009] Ambient Ionization of Peptide and Protein Ions: Insights from Mass Spectrometry and Molecular Dynamics

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Ambient ionization techniques have received considerable attention in recent years because of their simplicity, high sensitivity, ease of sample preparations, and wide range of applications. Surface acoustic wave nebulization (SAWN) is one of the new techniques that imparts less internal energy to the nebulized ions than electrospray. However, how SAWN-generated analytes become ionized, and what factors govern their ionization mechanism is still not well understood. Herein, we report a comparative investigation of solvent assisted inlet ionization (SAII) and SAWN with substance P, ubiquitin and carbonic anhydrase using varying interface conditions. The charge state patterns of peptide and protein ions were similar for SAII and SAWN although overall ion intensity in SAWN was typically higher. For both techniques, increasing the capillary inlet temperature up to 450∘C resulted in increased ion intensity. For peptide, the charge state distributions were affected by the temperature, and a global decrease in high charge state was observed with increasing temperature. A similar pattern was observed for ubiquitin and carbonic anhydrase ions. Molecular Dynamics simulations revealed that, at high temperature, the N-terminal of Substance P folded inward, yielding a globular shape, which may explain the decrease in abundance of multiply charged species. Our results suggest that, in spite of the use of very soft ionization techniques, and depending on the inlet temperature, ions produced by SAII and SAWN may not always retain their original solution state conformation upon introduction into the MS interface.

*Speaker

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# [P010] How does low temperature plasma ionization (LTPI) performs compared to the established methods

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Ambient ionization, i.e. the direct ionization from samples at atmospheric pressure with minimal or no sample preparation is a rapidly expanding field in analytical research and developed dramatically in the recent past. These techniques offer an enormous potential saving time and resources. In particular, plasma-based ambient ionisation techniques are intriguing due to their simple setup, solvent-free operation and, consequently, less waste production. Even though there have been many developments regarding plasma ionization, it is still difficult to quantify a sample due to many factors such as ionization efficiency, matrix, etc. We have faced up to this question and compared the relative ionization efficiency of aromatic amino compounds in low temperature plasma ionization (LTPI) with the established methods of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). For the evaluation of the influence of the analyte on the ionization efficiency, a set of 30 anilines was prepared in 50% ACN for each aniline and the signal intensity was determined with each of the three methods. Compared to ESI and APCI, plasma ionization shows a higher selectivity to the analytes. While the signal intensities in the ESI follow the solution basicity of the analyte, in the LTPI the evaporation enthalpy and the non-polar surface area are key players of ionization efficiency.

*Speaker

# [P011] Native Mass Spectrometry of Biomolecules: A Comparison of nESI-MS and LILBID-MS

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Native mass spectrometry has emerged as an important tool in structural biology. Advantages of MS in contrast to the other tools like X-ray crystallography, nuclear magnetic resonance or cryo-EM are for instance its lower limits of detection, its speed and its applicability towards heterogeneous samples.
However the investigation of membrane proteins and their complexes remain challenging, not only for MS. Until the beginning of 2017, about 30 membrane proteins have been investigated so far using mainly conventional or modified commercially available native MS instruments. This number underlines the need of better MS sample preparation protocols and/or the need of MS instruments which are more suited towards the analysis of membrane protein complexes.

In this work we compare the performance of the complimentary methods nESI and LILBID in the analysis of the water soluble protein Avidin (tetramer) and the dimeric membrane protein EmrE. While with nESI, in most cases, it wasn't possible to avoid a certain amount of dissociation, we were able to demonstrate a gentle analyte release via IR-laser desorption in LILBID-MS, preserving almost the entire intact protein complex.
In addition, we performed a comparative screening of different buffers, concentrations and additives to explore the current limitations of both MS techniques. LILBID-MS proved to be more tolerant to additives like salt and non-volatile buffers like TRIS and HEPES due to a different ion release process. The advantages of nESI are, as of now, its better resolution, more widespread use and the commercial availability of native MS-IMS instruments.

*Speaker

# [P012] Combination of 2,5-Dihydroxybenzoic acid and 2,5-Dihydroxyacetophenone Matrices for unequivocal assignment of Phosphatidylethanolamine Species in complex Mixtures

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Unequivocal assignment of phospholipid peaks in complex mixtures is difficult if only the $m/z$ values but no tandem mass spectrometry (MS) data are available. This is usually the case for matrix-assisted laser/desorption ionization time-of-flight (MALDI-TOF) MS imaging experiments. The situation is even more complex since many matrices induce the loss of a methyl group from phosphatidylcholine (PC) and thus render it detectable as negative ion.
Selected lipid mixtures of known compositions were investigated by negative ion MALDI-TOF MS. In addition to established matrices such as 2,5-dihydroxybenzoic acid (DHB) and 9-aminoacridine (9-AA), different binary matrices, including 2,5-dihydroxyacetophenone (2,5-DHAP) as matrix additive to DHB, were tested to probe their performance in both ionization modes. Beside artificial PC and phosphatidylethanolamine (PE) mixtures of known compositions, egg yolk and liver extracts were selected as biologically relevant systems.
The majority of the herein used binary MALDI matrices lead to the loss of a methyl group from PC in the negative ion mode, which makes the clear identification of PE species ambiguous. However, this problem does not apply if a mixture of DHB and 2,5-DHAP is used. Therefore, the application of DHB / 2,5-DHAP as matrix is a simple method to unequivocally identify PEs even in complex mixtures as negative ions and without the necessity to separate the individual lipid classes prior to MS detection.

*Speaker

# [P013] Laser desorption low temperature plasma (LTP) postionization mass spectrometry: first results for nonvolatile and apolar compounds

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Ambient mass spectrometry (AMS) allows to investigate the molecular makeup of complex samples under ambient conditions. Despite widespread use, analysis of lowly volatile molecules with plasma-based AMS techniques have been limited due to a low analyte desorption efficiency. Laser desorption ionization (LDI) techniques, on the other hand, enable efficient ablation of samples, but analyte ionization efficiencies of apolar compounds are typically rather low. After combining the benefits of these orthogonal techniques and surpass their drawbacks, first results using a newly developed low temperature plasma (LTP) postionization/LDI (337 nm) ion source are presented. Nonvolatile and low-polarity compounds such as steroids and UV filters were analyzed in order to compare LTP, LDI and LDI + LTP performances. Compared to LTP and LDI, the developed ion source resulted in a signal enhancement for avobenzone and octocrylene of more than 10-fold. LDI+LTP was less effective for progesterone (P4) and testosterone (T) detection due to the lack of a suitable 337 nm chromophore. Embedding T and P4 in 2,5-dihydroxybenzoic acid crystals improved the desorption but not the ionization efficiency of these steroid compounds. Only LTP+LDI resulted in a 71-fold (P4) and 44-fold (T) increase in abundance of protonated P4 and T compared to LDI or LTP mass spectra, respectively. As a next step to extend the applicability of the developed postionization source, natural products containing UV chromophores will be analyzed with optimized laser wavelength and energy.

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# [P014] Visualization of the MALDI plume expansion in an elevated pressure ion source by use of a shadowgraphy technique with high spatio-temporal resolution

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MALDI-MS imaging (MSI) is increasingly performed with elevated pressure ion sources. A buffer gas pressure in the mbar range results in both collisional cooling, which stabilizes thermally labile ions, and a plume confinement effect. By irradiating the plume with a second UV-LASER the latter can be exploited for inducing secondary MALDI-like ionization processes (MALDI-2) and boosting the ion signals. To further optimize the postionization approach, precise knowledge about the spatial and temporal development (expansion) of the particle plume is required. Both plume composition and expansion dynamics will generally depend on several relevant parameters, in particular buffer gas pressure, focal spot size of the MALDI LASER, LASER fluence, and type of MALDI matrix.

Here we describe a custom-built chamber that enables visualization of the plume expansion by use of a fast shadowgraphy technique. To achieve the required high spatio-temporal resolution in the ns- and $\mu$m-range, respectively, the homogenized beam of an $N^2$-LASER of 337 nm wavelength and 3 ns pulse duration is used to illuminate the MALDI plume. Upon propagation through the plume, photons are both absorbed and scattered. The so modified beam is picked up with a high-NA objective and enlarged onto a sensitive CCD sensor (Spiricon SP620U), providing a region of interest (1 $mm^2$) on 1.44 megapixel. Differential images are obtained by illumination with and without use of the MALDI LASER.

In the poster, design considerations, a first prototype of the shawdography chamber, and – presumably – first results of this "in-progress" study will be presented.

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# [P015] Investigating LTP ionization products of terpene species via FT-ICR-MS and Twin-Trap-MS

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Introduction

Previous LTP ion mobility spectrometry studies [1;2] have shown signs of terpenes forming large clusters inside of a LTP ion source. In this work we present further studies of terpene systems using a Bruker ICR mass spectrometer and Twin-Trap [3] mass spectrometer.

Experimental

Mass analysis were performed on a 7.05 T FT-ICR system as well as on a Twin-Trap mass spectrometer, provided by *Bruker Daltonik*, were used. A LTP torch built from scratch according to the design of Nørgaard et al. [4] with slight variations was used as an ion source. Plasma gas and sample gas were Argon (99.999 %) by *Air Liquid Deutschland GmbH*. As a Test system (S)-(-)-Limonene and $\beta$-Caryophyllene, purchased from *Sigma Aldrich*, were chosen.

Results

Mass spectra of the investigated systems show a great variety of formed species. The two systems were chosen due to the setup of the two mass spectrometers. The ICR has got a mass cut off at lower m/z than the Twin-Trap but the Twin-Trap provides us with much faster measuring times. For these reasons we compared spectra of both setups for more detailed information. Both molecules show a great variety of oxidized species. Clusters were formed mostly with Hydrogen, Oxygen and Nitrogen. Cluster size for both molecules goes up to four molecules.

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# [P016] REMPI and MATI spectroscopy of pyridine derivatives

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Pyridine derivatives are important building-blocks in biochemical relevant molecules. To understand their reactivity it is helpful to get an insight the geometrical and electronic structure in different states. This information is obtained by the *Resonance-Enhanced-Multi-Photon-Ionization* (REMPI)- and *Mass-Analyzed-Threshold-Ionization* (MATI)-spectroscopy.
The picolines, 3-Fluoro- and 3-Chloropyridine have been investigated by means of REMPI- and MATI-spectroscopy. The measurements were conducted with two dye lasers from *Laser Analytical Systems* and *Lambda Physics* and a home-built time-of-flight-mass-spectrometer.

The excitation energy of the picolines, 3-Fluoro- and 3-Chloropyridine have been determined with a very high accuracy. The influence of the position of the methyl-group and the halogen atoms has been identified. All derivatives experience an in-plane geometry distortion along the eigenvector of the vibration 6a upon S1 excitation. Furthermore, an out-of-plane geometry distortion along the eigenvector of low frequency vibrations, which have a varying intensity among the derivatives, has been observed.

The adiabatic ionization energy of the picolines, 3-Fluoro- and 3-Chloropyridine has been determined by means of MATI-spectroscopy. The pyridine derivatives experience a geometry distortion along the eigenvector of the vibration 8b upon ionization from the S1-state. In addition, the measurement of MATI spectra via the active vibrations in the S1-state offered the possibility to verify the assignment in the REMPI spectrum of the S1-state. This was especially true for the low frequency vibrations, which are symmetry-forbidden and were not taken into account for the simulation of the vibrational spectra.
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# [P017] Investigation of polycyclic aromatic hydrocarbons as matrices for MALDI mass spectrometry of small molecules and lipids

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MALDI-MS is widely used for the analysis of a wide range of chemical compounds, including metabolites, lipids, and peptides/proteins. However, a major limitation for the analysis of small molecules (e.g., primary and secondary metabolites, small lipids) is given by the (often high) matrix-derived chemical background. Typically, this consists of a manifold of distinct matrix ions (monomers, dimers, etc.), photochemical products, matrix fragments, and further unspecific clusters. Moreover, also non-covalent matrix-analyte complexes are frequently observed. The problem is particular apparent in MALDI MS imaging applications where up to hundreds of compounds need to be analyzed without prior separation.
One possible solution is be the use of matrices that provide sufficient desorption/ionization properties but yet produce only few distinct matrix ion signals. Here we investigated a set of polycyclic aromatic hydrocarbons (PAH) with increasing molecular weight and chemical complexity as potential MALDI matrices for the analysis of small molecules (in particular less polar metabolites and small lipids). PAHs were characterized with regard to their solubility, characteristics for achieving uniform layers on tissue sections by spray- or sublimation coating, their fragmentation and clustering behavior, and last but not least their ionization efficiencies.
First MALDI- and MALDI-2-MS results of our "in-progress" study, performed with a MALDI QStar pulsar i and a MALDI(-2) Synapt G2-2 mass spectrometer, will be presented.

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# [P018] The selected matrix influences the MALDI-TOF mass spectral patterns of partially deuterated glycosaminoglycan disaccharides

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**Rationale**

If carbohydrates are investigated by NMR spectroscopy, they are normally dissolved in deuterated solvents, such as D2O. The incorporation of deuterium leads to a high complexity of subsequently recorded mass spectra and reduced sensitivity because different deuterated ions become detectable. Here, we demonstrate that the applied MALDI matrix has a considerable impact on the observed isotopic distribution.

**Methods**

Unsaturated disaccharides of chondroitin (CS) and dermatan sulfate (DS) were prepared by enzymatic digestion of the polysaccharides in D2O and analyzed by MALDI-TOF MS using 2,5-dihydroxybenzoic acid (DHB) and 9-aminoacridine (9-AA) according to previously published protocols.

**Results**

The extent of deuteration of a given compound can be easily determined by using the mass shift between the non-deuterated and deuterated ions. However, such a determination is more difficult when considering sugars due to their high content of exchangeable groups. Therefore, both, the solvent and the matrix, have a considerable impact on the MS pattern. Additionally, there are significant differences if the spectra are recorded at different laser fluences.

**Conclusions**

Great caution should be taken when the deuterium content of disaccharides is determined by MALDI MS. Aside from the use of non-protic solvents, DHB is the matrix of choice, whereas 9-AA shows a considerable influence on the observed isotope pattern in dependence on the applied laser fluence.

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**Keywords**
Disaccharides; Chondroitin/Dermatan Sulfate; MALDI-TOF MS; Deuteration; DHB; 9-AA.

# [P019] REMPI-Spectroscopy of Non-Covalent Anisole-Clusters

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Non-covalent intermolecular interactions play an important part in understanding as well as explaining supramolecular organization and recognition processes, e.g. DNA base pairs.
First investigations on benzene- and phenol-dimers yielded different electronic structures to those of the monomers.

REMPI experiments showed two electronic ground states (red shifted for the electron donor, blue shifted for the electron acceptor). Hydrogen bonds were assumed to form between the molecules and thus develop clusters

More current investigations on anisole-clusters showed different results compared to benzene and phenol. Further experiments with varying partner were conducted to further investigate if the observed behaviour is universally valid.

Experiments were carried out on a modified custom-build ReToF-MS using Argon as a carrier gas. The molecules were ionized using a tunable dye-laser pumped by a XeCl Excimer laser.

The anisole-clusters showed only a single electronic ground state. The ground state display a red shift compared to the anisole-dimer depending on the clustering partner which was measured simultaneously the account for instrumental errors.

To be able to properly assign the observed signals quantum chemical calculations need to be performed.

The clusters are assumed to stabilize via $\pi$-$\pi$-interactions. Van der Waals forces are another possibility though that would more likely lead to two sperate 0-0 transitions. A stabilization via dipol-dipol-interactions can practically be ruled out.
To further solidify the obtained results and expand the range of possible clustering partners current investigations focus on using clustering partners that display a greater similarity to DNA base pairs, e.g. anilines and toluidines.

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# [P020] Molecular integrity of dendrimers designed around triphenylamine or porphyrin based heart with up to 10 kDa molecular weight : High resolution electrospray ionization experiments as key methodology.

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The synthesis of dendrimers needs ultra-skilled chemists. With the size growth, solubility and chemical reactivity are definitively modified, the purification becomes a bottleneck step. Triphenylamine based dendrimers are developed by the "Molecular Imaging and Photonic" research group in Bordeaux. In order to reach a convenient mass accuracy, several experiments and multiple parameter optimizations are needed. At the end, electrospray ionization in positive ion mode with high resolution mass spectrometer provides multiply charged ions like small intact proteins.
We described spectra presenting measurements of multiply charged ions on a Thermo-Fisher Q-Exactive and a Bruker MaXis 4G.

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# [P021] Characterization by high resolution mass spectrometry of organometallics with hexahelicene, merocyanine or porphyrin unit.

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The engineering of new molecules with specific magnetic or optical properties is a real big deal to design nanoelectronic devices for the future. Organometallics containing hexahelicene unit and merocyanine ligand are developed in the "Organometallics : Materials and Catalysis" team. The ionization of these compounds should be helped by the metal, but the story is not that easy, the analyst really needs hours of measurement and optimization to take the best advantage of instrumental parameters and to achieve a valuable and calibrated spectrum within a few PPM exact mass accuracy. We described spectra presenting measurements in chlorinated solvents of cationated molecule, cation and cluster ion on a Thermo-Fisher Q-Exactive and a Bruker MaXis 4G.

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# [P022] Fragmentation of Oligosaccharides and Suppression of Metal-Salt Induced Adducts Using Electrospray Ionization

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**Introduction**

Oligosaccharides, especially as part of glycoproteins, are important for many biological processes. Therefore, oligosaccharides have been already part of several studies using mass spectrometry for characterization through fragmentation templates. It is known that alkaline adduct-ions have an influence on CID and PD induced fragmentation mechanisms of oligosaccharides [1] [2]. For proteins the use of ammonium-acetate as buffer loading and to counteract cation induced signal suppression in electrospray ionization is published [2]. To increase the intensity of the non-adducted molecular ion the application of ammonium-acetate to oligosaccharide containing samples is of interest.

**Experimental Section**

The experiments were performed on an APEX Qe FT-ICR mass spectrometer, equipped with a 9.4 T superconducting magnet (Bruker Daltonik, Bremen, Germany). The samples were dissolved in MeOH/H2O and ionized using an Apollo III ESI-source. To analyse the suppression of alkali-adducts NH4Ac was added in different concentrations to the samples. Fragmentations of the molecular cations were achieved by SORI-CID with argon as collision gas.

**Preliminary Data**

Several spectra of maltopentaose with different concentrations of NH4Ac could be recorded. The intensity of the non-adducted molecular ion could be highly increased. The effect on chromophore labelled maltopentaose and subsequent CID/PD fragmentation is part of current research.

**Literature**

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# [P023] Charging and Charge Switching of unsaturated lipids by in-source Paternò-B'uchi Functionalization

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Investigations of lipid structure and abundance can aid in the understanding of lipid biochemistry and reveal possible lipid-based disease biomarkers. Electrospray ionization (ESI) is often utilized to study complex biological lipid mixtures, thereby forming intact lipid ions that are sensitively detected by high performance mass spectrometers. However, functional groups such as acids or bases are required in order to achieve high ESI ionization yields and dictate the ionization polarity. The absence of functional groups in aliphatic lipids such as cholesterol esters or hydrocarbon pheromones hinders their detection by ESI mass spectrometry (MS), and functional groups in, e.g., fatty acids predefine the ionization polarity and in turn can complicate combination of ESI MS with chromatographic methods.

To overcome these limitations, we present an in-source method that allows to functionalize unsaturated lipid compounds via the Paternò-B'uchi reaction (PB reaction) in nanoESI emitter tips in less than 30 s. Addition of 3-acetylpyridine to lipid double bonds via PB reaction introduces a basic group that is readily protonated under acidic conditions. Following PB reaction, protonated positive lipid ions are generated irrespective of other functional groups, thereby increasing the sensitivity for detection of unsaturated lipids in positive ion mode by up to 630 times. Additionally, discrimination between double bond isomers is possible by fragmentation of photoproduct ions yielding double bond specific fragment ions that enable relative double bond isomer quantification.

We applied this method to several unsaturated lipid compounds including mono- and polyunsaturated fatty acids, triglycerides, hexene isomers, cholesteryl esters, lipid extracts and olefin mixtures.

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# [P024] Metabolic phenotyping and strain characterisation of Pseudomonas aeruginosa using rapid evaporative ionisation mass spectrometry (REIMS)

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Rapid evaporative ionisation mass spectrometry (REIMS) is a novel technique for the real-time analysis of biological material. It works by applying heating on a sample, resulting in the evaporation of analyte-containing vapour, further channelled to a mass spectrometer. A platform customised for automated and high-throughput laser-REIMS analysis was used to characterise the metabolome of 700 *P. aeruginosa* clinical isolates, collected from acute non-respiratory infection sites, bronchiectasis and cystic fibrosis (CF) patients with acute or chronic infection. A highly-diversified metabolic profile was detected, comprising phospholipids which form the structure of the bacterial membrane, and virulence-related metabolites. MS/MS confirmed the identity of 17 rhamnolipids with surfactant and antimicrobial properties, and 18 quorum sensing molecules, known to regulate virulence and persistence mechanisms in *P. aeruginosa*. Respiratory isolates showed a higher diversity and a distinct phospholipid profile, which was attributed to the chronic nature of most respiratory infections and adaptation to the specific lung environment. The detected metabolic profile allowed to classify individual *P. aeruginosa* isolates after repeated culturing, and achieved 83% accuracy in accordance to MLST types. The expressed metabolome of *P. aeruginosa* showed significant changes during the establishment of pulmonary infection in CF. We observed a diversification of the metabolic profiles alongside that of the phenotypes, with a general decrease of the production of rhamnolipids which appeared to be key in the early stage of infection, and important modifications in the secreted quorum sensing profiles, playing a crucial role in the long-term adaptation to the host.

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# [P025] A Novel Solution for EtG/EtS Analysis in Human Urine by LC-MS/MS

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Introduction

Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) are unique biomarkers of alcohol use. EtG and EtS analysis offers many advantages for abstinence monitoring including the detection window, stability in stored specimens, and specificity. EtG and EtS are both polar, making them difficult to retain via reversed-phase chromatography. Both compounds are also very sensitive to matrix interferences which can result in being unable to achieve low limits of detection and can also make quantitation impossible. In this study, a simple dilute and shoot method was developed and validated for the analysis of EtG and EtS in human urine by LC-MS/MS.

Results

EtG and EtS were successfully resolved from matrix interference. The selectivity lots did not show additional interferences that would impact quantitation. The calibration linearity was acceptable for both analytes with R2 values $\geq 0.999$ and % deviation of less than 10.0%. Three levels of QC samples were analyzed for accuracy and precision across multiple days, instrument platforms, and column lots. Mean accuracy values ranged from 90%-101% of the nominal concentration for QC low, mid, and high samples and 89-105% for the QC LLOQ for both analytes. The %RSD did not exceed 10% for any set of QC samples throughout the study.

Conclusion/Discussion

An easy dilute and shoot method was developed and validated for the quantitative measurement of EtG and EtS in human urine. The analytical method was demonstrated to be fast, reproducible, and rugged.

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# [P026] Endogenous reporter neuropeptides for the measurement of protease dysregulation in Complex Regional Pain Syndrome

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CRPS is a severe and often disabling syndrome, which develops after trauma in ¸˜2-3% of all cases; most often after distal radius fractures. Neuropeptides such as bradykinin (BK) and substance P (SP) as well as metalloproteases like angiotensin-converting enzyme (ACE) participate in inflammatory processes and contribute to the pathophysiological mechanisms in CRPS. [1]
Modulation of BK signaling is largely determined by ACE which metabolizes the BK nonapeptide into BK1-7 and BK1-5, but degradation by aminopeptidase P (APP, cleaves position 1-2) and carboxypeptidase N (CPN, cleaves position 8-9) is also known.

Using TLC-based separation of BK products following serum incubation we have demonstrated that ACE activity is reduced in CRPS patient sera compared to healthy controls. [2] Dabsylation of reporter peptides enables differentiation from peptides naturally occuring in serum as well as adding a chromophore label to eliminate the need for a staining procedure after a TLC-run.

Isolated ACE cleaves dabsyl-BK more rapidly than BK and cleavage was only slowed down after adding ACE-inhibitor teprotide whereas BK degradation was completely inhibited as shown by mass spectrometry (Q-TOF Premier, Waters).

Following incubation of DBK in serum enzymatic cleavage products of CPN and ACE could be shown by mass spectrometry as well as TLC.

For further elucidation of the pathophysiology of CRPS we labeled SP (sequence RPKPQQFFGLM). After serum incubation for 120 min, fragment KPQQFFGLM is the most abundant.

Monitoring of dabsylated neuropeptides holds high promises not only for the investigation of CRPS.

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*Speaker

# [P027] Investigating the role of vitamin E in Alzheimer's disease using a new high-throughput extraction and LC-MS/MS method

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Hallmarks of Alzheimer's disease (AD) are an increased amyloid burden and enhanced levels of reactive oxidative species (ROS). Some studies suggested that vitamin E is associated with a reduced risk of AD due to its antioxidative properties, however, epidemiological studies and nutritional approaches of vitamin E treatment are controversial. Here we investigate the effect of tocopherol and tocotrienol on AD-relevant processes. In line with literature, Vitamin E reduces ROS level and additionally decreases cholesterol, a known risk factor in AD. Besides the positive effects, amyloid-b (Ab) levels were elevated by Vitamin E treatment accompanied by an increase in the activity of the involved amyloidogenic enzymes. In addition to the enhanced Ab production, degradation was inhibited further emphasizing that Vitamin E has heterogeneous effects linked to AD. To clarify the impact of Vitamin E in AD we analysed the $\alpha$-tocopherol level and the expression of the Tocopherol binding protein (TBP) of human post mortem brain samples. A new extraction method for $\alpha$-tocopherol from brain tissue, requiring only 1.5 % or less sample volume compared to previous published methods was established. The method comprises sample preparation by saponification, liquid/liquid extraction and measuring $\alpha$-tocopherol by LC-ESI-MS/MS. This method revealed significant difference of $\alpha$-tocopherol brain levels between AD patients and control group. In line with these findings TBP was altered in AD patients. Our results might help to understand the controversial findings of previous studies demonstrating

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that besides positive neuroprotective properties, Vitamin E also have negative characteristics in respect to Alzheimer's disease.

# [P028] Link between altered ceramide homeostasis and amyloidogenic processing in Alzheimer's disease

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Alzheimer's disease (AD) is an irreversible neurodegenerative disorder and characterized by extracellular plaques in the brain mainly consisting of amyloid-$\beta$ (A$\beta$) peptides. A$\beta$ is derived from sequential cleavage of the transmembrane amyloid precursor protein (APP) by $\beta$- and $\gamma$-secretase. The active centers of the $\gamma$-secretase complex are formed by presenilin1 or 2 (PS1, PS2).
Ceramides are core molecules of sphingolipids which are a major class of membrane lipids. Early processes in AD are linked with alterations in individual ceramide species level, for example a significant increase in Cer18 level.
Here we investigate the impact of APP processing on regulating ceramide homeostasis by applying a method for qualitative analysis of individual ceramide species in biological samples based on electrospray ionization mass spectrometry. Our results indicate that the $\gamma$-secretase influences ceramide metabolism. In both mouse embryonic fibroblasts devoid of presenilin (MEF PS1/2-/-) and those carrying the familial AD mutation (FAD) in PS1 that show a decreased $\gamma$-secretase activity (MEF-T354I) Cer18 level were elevated. Furthermore, we observed a significant increase in total ceramide level, in particular Cer18, in the human neuroblastoma cell line SH-SY5Y with a CRISPR-KO of PS1 (SH-SY5Y PS1-/-). In line with these findings human *post mortem* brain samples of FAD patients, showing a decreased $\gamma$-secretase activity, revealed elevated ceramide levels. Our results emphasize an abundant role of presenilin and the $\gamma$-secretase mediated processing of APP in regulating the ceramide homeostasis and might help to explain alterations of ceramide levels found in brains of AD patients.

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# [P029] DIA based analysis of CSF before and after spinal cord injury to identify therapeutic target proteins

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Spinal cord injury (SCI) is a debilitating traumatic injury, which has a severe impact on the patients quality of life. The exact underlying mechanisms and pathways that inhibit neuronal recovery have not been fully elucidated. Due to this, there are no therapeutic options available, only physiotherapy to improve what mobility remains. Additionally, a small percentage of patients show full recovery where other patients with similar SCI do not recover to the same degree for unknown reasons. Due to this, there is a high need for predictive biomarkers for determining this clinical outcome, and therapeutic targets. The identification of proteins which impact inhibition of repair and outgrowth are essential for the discovery of potential biomarkers. Due to the need for having samples before SCI occurs, in order to identify proteins differentially expressed due to SCI, an animal model was essential. Therefore, in this study rat CSF samples taken 7 days before surgically induced SCI as well as 7 and 28 days after were taken. These samples were analyzed via DIA based quantitative proteomic approach. Before mass spectrometric analysis protein concentration was determined with amino acid analysis, and changes in protein concentration across all time points could already be seen. Using a spectral library we could quantify 419 proteins, and of these 14 proteins showed direct regulation across all samples due to SCI and maintenance pathways.

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# [P030] An LC-MS/MS-based candidate reference method for the clinical assessment of the emerging iron biomarker hepcidin-25 in human serum

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Hepcidin-25 has attracted much attention ever since its discovery in 2001. It is widely recognized that this peptide hormone plays a major role in the regulation of iron levels in mammals and can reveal important clinical information about several iron-related disorders [1]. However, the development of a reliable assay to quantify hepcidin proved to be problematic and serum hepcidin-25 concentrations determined by various assays differ substantially. Challenges arise in the MS analysis of hepcidin due to the "sticky" character of the peptide and the lack of suitable standards [2].
With the aim to tackle the current difficulties in hepcidin quantification and improve the status of this promising biomarker in the clinical field, we developed a rapid and robust analytical strategy for the quantification of hepcidin-25 in human samples based on HPLC-MS/MS (QqQ) as a reference method candidate to be implemented in routine laboratories. The novelty of the method is the use of amino- and fluoro-silanized autosampler vials to reduce hepcidin interaction to laboratory glassware surfaces. Furthermore, we have investigated two sample preparation strategies and two chromatographic separation conditions where the use of acidic mobile phases was compared with a novel approach involving solvents at high pH containing 0.1% of ammonia. Both methods were carefully validated and applied to clinical samples in an intra-laboratory comparison of two LC-MS/MS methods using the same hepcidin-25 calibrators with a very good correlation of the results.

Girelli D et al, *Blood,* ***2016****, 127(23):2809-13*
van der Vorm LN et al, *Clin Chem*, **2016**, 62(7):993-1001

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# [P031] Proteomic analysis of CD34 and CD123 co-expressing stem cells from patients with acute myeloid leukemia (AML) and from healthy stem cell donors

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Although being under investigation, epitopes for a targeted immunotherapy of acute myeloid leukemia (AML) are rare. The proteomic characterization of primary hematopoietic stem cells from patients with AML with respect to a control group of healthy donors has the potential to identify differentially abundant proteins that can either be used as therapeutic target on the cellular surface or as proxies for affected molecular pathways. Previous studies on AML proteomes lacked in using primary patient material, suitable control groups, proteomic coverage or reliability of the used quantitative methods. Here, we aimed to establish a protocol to comprehensively and reproducibly quantify proteins from 2 x 106 FACS-sorted CD34+CD123+ stem cells originating from patients with AML and healthy donors. By applying a TMT 10-plex based-labelling approach, 5 samples of each group were directly comparable in one LC-MS run. By further using a cellular fractionation, 1751 and 995 proteins were quantified in at least 4 out of 5 replicates in both donor groups in the cytosolic and membrane fraction, respectively. In total, 221 proteins were in significantly altered abundance. Functional analysis of those proteins revealed their contribution in regulation of the cell cycle, cytokine production and isoprenoid metabolism, all known to be dysregulated in AML. Moreover, SPI1 and IL 13 were predicted to be upregulated contributing to the myeloid development and autocrine cytokine production in AML. Thus, the here described method allows a comprehensive insight into primary stem cells from AML patients serving as a basis for future biomarker discoveries and individualized proteogenomic characterizations.

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# [P032] Native MS demonstrates the supportive impact of carbohydrates on norovirus infection

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The important role of natural carbohydrates as attachment factors in viral infection pathways is well known. Surfaces of human target cells are covered with various glycans. However, the related entry processes are not understood. Specific carbohydrates appear to increase the susceptibility to virus infections.

Our research focuses on the norovirus (NoV) glycan binding process, including stoichiometry, binding affinity and glycan preference, which are deciphered by native mass spectrometry (MS). The technique is based on electrospray ionization (ESI) and allows soft ionization of target proteins and preserves non-covalent interactions.

NoVs cause acute viral gastroenteritis in humans and leads to 200.000 deaths annually. The main structure of interest for glycan attachment is major capsid protein protruding (P) domain. We analyzed the binding of various glycans to P dimers from human NoV strains. Crystal structures show the availability of two glycan binding sites at low glycan concentration. When increased the concentration to millimolar levels. The results in conjunction with other techniques strongly suggest that cooperative multi step binding plays an vital role in viral cell entry. MS results clearly revealedl four glycan binding events on the P dimer instead of two.
Furthermore,Recorded MS data indicates charge state dependency of glycan binding. That could be a hint for glycan mediated structural changes on P during glycan binding. In addition, it showed glycan preference depends on the assembly of carbohydrate residues.

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# [P033] MRM-MS analysis of the mothers' serum proteins enables IUGR risk assessment of preterm babies prior to giving birth

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A multiplexing serological assay based on LC-MRM/MS was applied for distinguishing maternal peripheral blood serum samples of pregnant women that either belonged to the IUGR group (n=15) or to the control group (n=15). Two different work-up procedures (MS1 and MS2) were tested and resulted in comparable protein concentration data, showing that both, peptide mixtures (MS1) and protein mixtures (MS2) from serum can be shipped unharmed at room temperature as lyophilized powders prior to quantitative analysis. Assessment of fifteen concentrations of apolipoproteins and of proteins that belong to the lipid transport system was performed with all 30 maternal serum samples, consuming only 10 $\mu$l of serum per assay from each patient. Of all 15 investigated proteins the serum concentrations of apolipoprotein B100 showed the greatest power for discriminating IUGR from CTRL samples. Separation confidence of the apolipoprotein B100-based discrimination reached sensitivities of 0.73 and 0.67, specificities of 0.93 and 0.93, positive predictive values of 0.92 and 0.91, as well as negative predictive values of 0.78 and 0.74 for MS1 and MS2, respectively. With the reached separation efficiency the minimally required total sample size with all the apolipoprotein B100 protein concentrations was 16 for the first (MS1) and 14 for the second measurement series (MS2). Our data indicate the potential of LC-MRM/MS to become of clinical importance in the future for IUGR risk assessment based on maternal apolipoprotein B100 serum levels.

*Speaker

# [P034] Multiplexed LC-MS/MS Analysis for the Quantification of Serum Adipokines

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Since 1980, the percentage of people being overweight or obese has more than doubled. The excessive accumulation of adipose tissue represents a massive health risk. Adipose tissue acts as an endocrine organ, secreting several hormones and cytokine (adipokines), thus modulating a variety of biological and physiological processes, like food intake, insulin action and glucose metabolism. Commonly, adipokine level are measured by ELISA, a very time consuming and expensive method, especially if serum levels of several adipokines need to be determined. To overcome these limitations we aim to establish a LC-MS/MS-based assay for the simultaneous analysis of 15 adipokines in a single MS run.
In a first step, we developed an efficient digestion protocol for the reproducible generation of proteolytic peptides in order to ensure competent quantification. Since adipokines belong to the lowest abundant serum proteins, analysing untreated serum hampered reliable identification of several adipokines. This problem was solved by the depletion of high abundant serum proteins using antibodies columns. Furthermore, isotopic-labelled peptides for each adipokine were used for quantification by generating an external calibration curve. In a preliminary study, we show that even with a small sample set, significantly altered adipokine levels between lean and obese individuals can be detected.
In conclusion, we successfully established a multiplex LC-MS-based assay for the identification and quantification of human serum adipokines. This method offers a valuable tool for the detection and characterization of biomarkers associated to obesity and obesity-related comorbidities.

*Speaker

# [P035] Investigating assembly of Ebola virus nucleoprotein and accessory proteins with native mass spectrometry

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Ebola virus is classified as high-risk pathogen. Recent clinical trials showed the effectivity of an Ebola vaccine, but there are still no therapeutics available. EBOV is a negative single stranded RNA virus with genome size of 19 kb. Its particles are heterogeneous, filamentous and enveloped with helical symmetry. Being its main component, the 83 kDa nucleoprotein (NP) is essential for formation of the nucleocapsid. NP's first 450 amino acids contain domains for self-assembly and also for binding of RNA. Within this part of the protein a peptide motif is present, which was found to be highly conserved among *Filoviridae*.
This study addresses biophysical and structural investigations on EBOV proteins using primarily native mass spectrometry. We aim to identify the motif's function, its role in self-assembly, besides the effect of RNA and other EBOV proteins on this process. NP mutants will establish whether the motif is crucial for assembly and hence for infectivity.
Recombinant NP is expressed in *E. coli*. During size-exclusion chromatography, retention time of NP-containing fractions was lower than expected, suggesting existence of oligomers with larger hydrodynamic radius than monomers. Native MS is used to identify NP monomers and assemblies, furthermore to run interaction studies with binding partners. Monomeric and dimeric nucleoprotein was detected in buffers with low-salt concentration. At higher ionic strength, closer to physiological salt concentration, peak distributions representing an oligomeric formation, were gathered. These were mathematically assigned as circular oligomer of 24 to 25 NP units and confirmed by electron microscopy showing ring-like particles (Ø30-40 nm).

*Speaker

# [P036] Revisiting Ehrlichia ruminantium life cycle using proteomics: the host and the bacterium perspectives.

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*Ehrlichia ruminantium* (*ER*) is the etiologic agent of Heartwater, an infectious disease of ruminants, characterised by a disseminated vascular endothelial cell infection. *ER* is transmitted by *Amblyomma* ticks in nearly all sub-Saharan countries of Africa and neighbouring islands, also in the Caribbean. *ER* is an obligate intracellular bacterium with a biphasic developmental cycle in endothelial cells, alternating the elementary bodies (EBs, small extracellular infectious forms) and the reticulate bodies (RBs, larger intracellular non-infectious replicative forms). As little is known regarding the interactions *ER*-host endothelial cells and particularly the RBs, we focused on the identification and characterisation of both the host and *ER* proteins modified during *ER* life cycle, from the cell invasion until lysis. We performed DIGE and nanoLC-MS/MS analyses (SameSpots software, Orbitrap Elite mass spectrometer, data treatment using MaxQuant software with Perseus for statistics) of proteins purified every 24h from bovine endothelial cells infected by *ER*. The overall results from total protein extracts indicated that *ER* induced important changes in key endothelial cell metabolic networks including cell death, nutrients trafficking/metabolism of amino acids and glucose, cell osmosis and cytoskeletal modifications, and signalling related to immune response. Moreover, we identified for the first time *ER* proteins and key effectors differentially expressed by either EBs or RBs within host cells. This comprehensive analysis allowed us to reconstruct for the first time the interactions of *ER* with its host, and provide a basis for further qualification of key proteins associated with *ER* replication that could be targeted to disrupt *ER* transmission.

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# [P037] Miniaturized dispersive liquid-liquid microextraction and MALDI MS using ionic liquid matrices for the detection of bacterial communication molecules and virulence factors

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The identification and quantification of molecules involved in bacterial communication are major prerequisites for the understanding of interspecies interactions at the molecular level. We developed a procedure allowing to determine 2-Heptyl-4-quinolone (HHQ) and 2-Heptyl-3-hydroxy-4(1*H*)-quinolone (PQS) and the virulence factor pyocyanin (PYO) formed by the Gram-negative bacterium *Pseudomonas aeruginosa.* The method is based on dispersive liquid-liquid microextraction from small supernatant volumes (below 10 $\mu$L) followed by quantitative matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS). The use of ionic liquid matrix led to a lowered limit of detection for pyocyanin and, due to suppression of matrix background signals, easy to interpret mass spectra compared to crystalline matrices. Using an isotope-labeled pyocyanin standard synthesized in small-scale synthesis, quantitative analysis spanning approximately one order of magnitude (0.5 fmol to 250 fmol) was feasible. The method was successfully applied to the detection of the signaling molecules PQS and HHQ in cultures of *P. aeruginosa* strains isolated from sputum of cystic fibrosis patients and allowed a highly sensitive quantification of PYO from these cultures. Hence, the developed method bears the potential to be used for screening purposes in clinical settings and will help to decipher the molecular basis of bacterial communication.

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# [P038] Evaluation of successive phosphorylation steps of nucleosides/tides by Frontal Affinity Chromatography-HRMS

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In chemotherapies of viral diseases, nucleosides had led a considerable progress in the fight against viral infections such as herpes, hepatitis or AIDS. Thymidyne, thymidylate and nucleoside diphosphate kinases are key enzymes in the activation of nucleoside analogues. This activation process requires three successive phosphorylation steps to produce the active triphosphorylated molecule that target the viral DNA polymerase or reverse transcriptase. The main objective of our study is to have a better understanding of the three phosphorylation steps to screen new nucleoside analogues with higher activities and safety.
A specific and sensitive analytical methodology based on an "on-line enzymatic assay" by UHPLC-HRMS for the monitoring of the three phosphorylation steps was performed. This work was dedicated to get quickly reliable data concerning the conversion of a nucleoside into in monophosphate, diphopshate and triphosphate nucleotide. An immobilization of the Thymidine (TK), Thymidylate (TMPK) and Nucleoside Diphosphate (NDPK) kinases on silica beads was optimized first of all. The last ones have been packed into necessary bioreactors to perform a Frontal Affinity Chromatography (FAC) which enables the rapid and reliable screening. This "on-line" enzymatic cascade was first optimized by the monitoring of the endogenous conversion and new synthesized nucleoside analogues. This methodology development allows identifying the capacity of new potential candidates to be mono-, di- or tri-phosphorylated forms.

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# [P039] Analyse de tensioactifs non-ioniques par chromatographie liquide à haute performance couplée à un détecteur à décharge Corona et à spectromètre de masse APPI- LTQ- Orbitrap

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L'étude a pour but de déterminer des différences qualitatives et/ou quantitatives entre les Macrogolglycérides Linoléiques et Oléiques, il s'agit d'excipients à usage pharmaceutique utilisés dans des formulations administrées par voie orale, topique, rectale et vaginale. La composition chimique de ces produits n'est pas détaillée dans la pharmacopée européenne, mais il est primordial de la connaitre afin de comprendre leurs rôles dans les formulations pharmaceutiques/cosmétiques, pour cela on a cherché un outil puissant et sensible pour élucider leur composition.
L'outil analytique employé est la chromatographie liquide à haute performance (HPLC), en phase inverse et une phase mobile polaire non aqueuse. La phase stationnaire est une colonne C18 et la phase mobile est un mélange Acétonitrile/Acétone. En premier, couplé à un détecteur à décharge Corona, pour optimiser la composition et le mode d'élution de la phase mobile, en second, couplé à un spectromètre de masse pour permettre l'identification des différents com-

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posés. La source d'ionisation en masse est la Photo-ionisation à pression atmosphérique (APPI) et un analyseur hybride LTQ-Orbitrap® (trappe ionique linéaire couplé à une trappe orbitale à transformé de Fourrier).
**Mots clés** : Tensioactifs, HPLC, spectrométrie de masse, APPI, trappe ionique, trappe orbitale.

# [P040] Derivatization methods for the measurement of nitric oxide species with liquid chromatography- HCD tandem mass spectrometry

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Nitric oxide (NO) plays a pivotal role in vascular tone homeostasis and inflammation. During aging and in case of cardiovascular diseases, endogenous NO production decreases. Administration of *S*-nitrosothiols (RSNOs) such as *S*-nitrosoglutathione (GSNO), which mimic physiological reservoirs of NO, appears promising to restore the NO pool. NO species are distributed in the blood and tissues mainly as nitrate, nitrite ions, and RSNOs. The present work aimed at the development of a sensitive liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method based on the use of the stable nitrogen isotope 15N (exhibiting a very low natural abundance), and able to differentiate and quantify NO released from 15N labelled GSNO and other sources (endogenous synthesis and diet). Because of its low molecular mass, NO (30 u) has to be derivatized to allow its measurement. Two derivatization methods have been compared: *(i)* 2,3-diaminonaphthalene (DAN) giving rise to a naphthotriazole (NAT) adduct, and *(ii)* Griess reagents (*i.e.* naphthylethylenediamine and sulfanilamide) forming an azo adduct. Fragmentation of both adducts shows: *(i)* a loss of 15N for triazole, while the azo adduct fragments keep labeled nitrogen; *(ii)* consistent blank values in both 15N labeled adducts due to the 13C interference from unlabeled (14N) adducts; *(iii)* a reaction between the precursor ion [15NAT+H]+ and acetonitrile, under Higher energy Collision Dissociation (HCD) mode producing an original transition (m/z 171 → 156) compared to traditional CID. The DAN assay based on this latter provides a lower limit of quantification (*ca.* 5 nM) than with the Griess derivative.

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# [P041] Morphological Analysis of Mass Spectrometry Imaging Data to Discover Heterogeneity Markers in Oncology

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The detection of biomarkers within tissue are based on molecular intensities. A biomarker is a molecule to describe tissue, disease and histology. It may change either intensity or morphology. We developed a tool that quantify the morphological information of such as surface, coverage of each molecules. This has been used to identify characteristics of multiple samples, discover markers defined by their tissue features&heterogeneity. As tumors are heterogeneous, we perform morphometry analysis of tissue & identify heterogenous markers.10$\mu$m thick tissue sections of P815 tumor xenografts were thaw mounted onto ITOcoated slides, & 1,5DAN matrix was coated using TM-Sprayer. MSI were performed using 7T MALDI FTI-ICR. MSI data were recorded in - ion mode at 30$\mu$mpx. Data acquisition, visualization were performed using the Flex&Multimaging.A workflow based on custom python scripts was applied, &plots made using matplotlib, objects having a surface - than 4px were deleted. We developed a tool based on morphologics that allows the detection & selection of onco-metabolites&other markers.Segmentation was obtained with Otsu.We selected the 1,000most intense molecules to detect heterogeneously distributed ions.We validate a workflow it permits the identification of ions with a distribution within the necrosis&proliferative regions. We apply the flow to a mIDO tumor model which has an overexpression of IDO which converts Tryp into Kyn. We investigated their parameters&found those markers are up&down regulated depending on the level of IDO expression, metabolites&lipids which have heterogeneous distributions.The soft has the capacity to investigate the changes&to create of statistical models.

*Speaker

# [P042] 2D-LC-MS/MS in elucidation of drug metabolites

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The knowledge about the metabolic degradation of new drug entity is crucial for its optimization in research and drug approval. The objectives of optimization are increasing metabolic stability, with the results of lower hepatic clearance and higher drug exposure, Secondary objective is the identification of pharmacological active products or potential toxicological relevant metabolites.For metabolism studies of in vitro or in vivo samples the LC/MS is the technology of choice.

Modern mass spectrometers have a high sensitivity and in combination with an UPLC- system, this combination allows the identification of metabolites in low dosed samples from pharmacokinetic studies.

Due to the high complexity of the endogenous matrix, especially in vivo samples such as plasma, bile, excreta or organ tissue homogenates, the identification of the metabolites formed in vivo is the main challenge.

The matrix has a negative effect on the analysis result and can lead to incorrect final conclusions. Therefore various offline sample preparation procedures, e.g. plasma precipitation in combination with offline SPE for the plasma samples are applied.

With the use of an online 2D-LC-MS/MS System, an automated sample preparation with subsequent analysis can be carried out. Interfering matrix components are separated in the first dimension followed by a second dimension and MS analysis.
The poster describes the configurations of different 2D-LC systems, their control and application possibilities with examples from metabolism studies.

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# [P043] Development of metabolite based MALDI MS assays

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Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has become a promising tool in drug discovery. Mass spectrometers of the latest generation enable high throughput, label free analysis of assays in 1536-well format [1]. Munteanu *et al.* demonstrated feasibility of whole cell MALDI MS-based assays, i.e. the delineation of concentration response curves for histone deacetylase inhibitors incl. calculation of EC50 values from histone acetylation-associated mass shifts [2]. While whole cell assays are well established for abundant, pharmacodynamic protein markers, analogous assays for metabolites are lacking. A central reason is the higher complexity of spectra in the low molecular mass range [3]. In this study, we employed a score-based method development approach to establish a small molecule fingerprinting workflow applicable for whole cells. Our IT-supported data acquisition and processing pipeline enabled the identification of lipid marker molecules that show reproducible concentration responses to tyrosine kinase inhibitors. Re-measurements of spots that exposed highest feature intensity using ultra high-resolution FT-ICR MS enabled structural elucidation of the respective marker molecules.

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# [P044] Application of GC-MS to assess the practical relevance of marine red algae

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Red algae are broadly used in food industry, cosmetics, medicine etc. In spite of growing interest to different algal products, there are still few species of macrophytic red algae that are thoroughly investigated from the biochemical perspective and extensively exploited. Here we used a GC-MS based metabolomics approach to reveal the red algal species accumulating compounds of medical and nutritional value.
Samples of 11 species of macrophytic red algae were ground and extracted with methanol. GC-MS analysis of the extracts was carried out on an Agilent HP-MSD 6980/5973N with electron impact ionization. Peak deconvolution was accomplished using AMDIS; GMD and NIST14 libraries were used for peak identification; quantitation of metabolites was done with Xcalibur.

All the studied species contained relatively high amounts of soluble carbohydrates, free amino acids and polyunsaturated fatty acids. *Furcellaria fastigiata* and *Phyllophora brodiaei* had the highest concentration of specific algal metabolite floridoside (2-O-$\alpha$-d-galactopyranosylglycerol), which is now explored as an immune response modulator, a drug for cancer therapy, and a non-toxic antifouling agent. *Corallina officinalis* showed specific accumulation of trehalose – a compound having a broad application both in the food and medical industries. *Ph. brodiaei* and *Phycodrys sinuosa* contained a considerable amount of arachidonic acid. High concentration of beta-alanine, which is widely used to enhance athletic performance, was found in *Palmaria palmata*. Several species accumulated considerable amounts of essential amino acids. As a whole, our data provide comprehensive information about the new potential sources of valuable algal products.
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# [P045] (Re-)Discovery of compounds targeting DnaN

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After the first age of intense search for antibiotics active against *Mycobacterium tuberculosis* in the 1960s a lot of compounds found with good activity were neglected because Rifampicin became available. Griselimycin is one of these abandoned molecules. It is a cyclic peptide isolated from *Streptomyces* spp. and showed a low MIC of 1 ug/ml in broth culture as well as a good activity in macrophages. First studies in human were promising, but revealed poor pharmacokinetics of Griselimycin such as a rapid metabolism and therefore a short half-time. Due to the increasing prevalence of MDR-Tuberculosis Griselimycin was rediscovered and structurally modified by Sanofi to overcome these problems. In 2015 the target of Griselimycin was identified guided by the occurrence of a resistance gene in the biosynthetic gene cluster encoding for a second homolog of a protein taking part in the DNA-replication system as the sliding clamp (DnaN).As DnaN represents a new antibiotic target, there will be no cross-resistances with the already commercially available drugs. First experiments also indicate that there is no binding to the human sliding clamp and the frequency of resistance is low. Mycoplanecins are another group of natural products, showing high structural similarity with the Griselimycins. They were isolated from *Actinoplanes awajinensis* in 1983, but contrary to the producer of Griselimycin, this strain is able to produce a variety of natural derivatives. Characterization of these derivatives will help to get a better understanding of the structure-activity-relationship and the influence of structural variations to pharmacokinetic properties of the molecule.

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# [P046] Automated electrochemistry/mass spectrometry as a powerful tool for the rapid simulation of phase-I metabolism

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Phase-I reactions are catalysed mainly by CYP450 enzymes. Electrochemistry (EC)/ESI-MS has proven to successfully mimic many of these oxidation reactions based on a purely instrumental approach. EC/ESI-MS allows identification of unknown oxidation products and short-lived intermediates. The generation of oxidation products at different potentials takes place in an electrochemical cell coupled online to high-resolution (HR)-ESI-MS. The results are shown in three-dimensional mass voltammograms and the oxidation products are identified via their accurate masses. For drug development, it is necessary to analyse a large number of pro-drugs, which is expensive and time-consuming. Combining an autosampler with the EC/ESI-MS setup and developing a software tool enables high-throughput analysis. EC/ESI-MS automation has been successfully applied to the investigation of phenothiazines and sun screen agents. An optimization of different system parameters has been performed. The injection volumes and the flow rates were matched to determine the time periods during which the signals of the analytes were sufficiently constant. Rinsing steps were introduced in order to clean the system and to prevent carry-over effects. The necessary time interval between the application of two potential ramps was determined. Thus, the oxidation products are washed out of the electrochemical cell. This allowed to perform several EC/MS measurements with a single injection by the autosampler. It has been shown that the automated EC/MS is a powerful alternative to established EC/MS approaches. This system is less time consuming and may thus allow to carry out significantly more analyses in a given period of time at lower costs.

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# [P048] Targeted quantification of sub-stoichiometric protein malonylation in the low attomole range

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With the advent of mass spectrometry, numerous post-translational protein modifications (PTMs) have been discovered, but their exact biological role is often unknown. Lysine malonylation of mTOR (a major regulator of cellular signaling) is still poorly understood, because its sub-stoichiometric nature renders functional analysis quite challenging. Here we present a 1D-nano-LC-MS/MS assay for highly sensitive targeted quantification of mTOR K1218 malonylation in primary human endothelial cells.
We synthesized the isotope-labeled malonylated mTOR peptide IVK*GYTLADEEEDPLIYQHR (Fmoc-Lys(mono-tert-butyl malonate)-OH was synthesized in-house; Peng et al., Mol Cell Proteomics, 2011) and three additional non-modified peptides of known concentration as internal standard for quantification. Peptides were spiked into digests of anti-mTOR IP eluates and analyzed by nanoLC-MS using a Q Exactive HF in PRM mode. The maximum ion injection time was set to 1500 msec to enable a higher sensitivity.
Our targeted assay allowed for the reproducible detection of all mTOR peptides in 16 out of 18 biological replicates with a throughput of _˜15 samples per day. The longer ion injection time enabled the specific detection of the malonylated peptide down to _˜2.5 amol. Upon knock-down of fatty acid synthase (4 vs 4 samples) we observed a _˜10-fold increase of mTOR malonylation (0.5 ± 0.3 % vs 4.6 ± 3.3 %) emphasizing a role of mTOR K1218 in metabolic signaling.

*Speaker

# [P049] Replacing Immunoassays with MS-based Technology: Quantitative Proteomics Assays Enabling Deep Molecular Phenotyping of the Mouse

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Mice are the most commonly used laboratory animals worldwide. With the recent introduction of CRISPR/Cas9 and haploid embryonic stem cell approach for the generation of genetic modification, the number of genetically modified mice is rapidly growing. However, detailed characterization of the new mouse models is still challenging as most phenotypic information is still derived from time-consuming antibody-based analyses that provided limited data (e.g. histology & outdated biochemical analyses). Here, the whole proteome quantitation in mice will be done by developing a new MS-based targeted approach for the rapid quantitative analysis of 3000 mouse proteins in a single assay i.e. one capable of accessing a significant portion (_˜20%) of the mouse proteome. The project includes the creation of tissue- and cell-specific spectral libraries (i), selection and synthesis of surrogate stable isotope labeled standard (SIS) peptide (ii), development of MRM and PRM assays for 20 mouse tissues (iii) and determination of reference values for proteins in tissue (iv). Finally, the Mouse Proteome Atlas: Publically available interactive quantitative map of mouse proteome will be created.

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# [P050] Evaluation of different phosphopeptide enrichment strategies with regard to different biological sample types

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In the human proteome approximately 30% of the proteins are temporary phosphorylated. These phosphorylation sites have various influences in the cell like activate or inhibit the function of proteins and whole pathways, influence on proliferation or apoptosis, changes in the localization or structure of a protein. Abnormal phosphorylation pattern can therefore often be linked to severe diseases like cancer or diabetes. Mass spectrometric analysis of phosphorylations became more important because it can not only provide information about the phosphorylation status and site of a given protein but also do this for many thousands of phosphorylations in parallel.

Even though phosphorylations are of high interest, its analysis is hindered due to their substoichiometric occurrence which strongly depends on the starting material (cell line, tissue). For this reasons we tested different enrichment strategies with a view to the applicability with diverse biological samples.

For primary cell lines it turned out that a fractionation via hydrophilic interaction liquid chromatography (HILIC) prior the phosphopeptide enrichment by magnetic titanium dioxide beads showed the best results in terms of overall phosphopeptide quantity. This way of processing is enabled due to the huge amount of sample which is approachable by cell culture. In the case of tissue, where the amount of sample is often very limited, excessive fractionation is not always possible. Therefore we established here a workflow that still allows a high number of phosphopeptide identification out of scarce tissue samples by using a combination of immobilized metal affinity chromatography (IMAC) and nonmagnetic titanium dioxide beads.

*Speaker

# [P051] Structural Dynamics and Flexibility of the Neuronal Calcium-Sensor Synaptotagmin-1

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Synaptic vesicles are small storage organelles for neurotransmitters. They fuse with the presynaptic membrane in response to intracellular calcium signals. Binding of $Ca^2$ ions to Synaptotagmin-1 (Syt-1) is regulating this process. To gain insights into the structure and dynamics of Syt-1 we integrated chemical cross-linking and mass spectrometry with molecular dynamics simulations of Syt-1 in different conformations and thus described its flexible movements in varying environment.
Purified synaptic vesicles were cross-linked with the lysine-reactive cross-linker BS3. Proteins were digested with trypsin and the resulting peptides were analyzed by LC/MS-MS. Cross-links were identified by database searching and manually validated by their spectral quality. For Syt-1 mainly intra-protein cross-links were identified. These were located in the structured calcium binding domains but also in the flexible linker regions suggesting a high flexibility with multiple conformations in the absence of calcium.
To address the flexibility of Syt-1, we generated molecular dynamics simulations starting from two high-resolution structures of an open and a closed conformation. To combine these structures with experimental data obtained from cross-linking experiments, we used DynamXL - a new software tool that incorporates side-chain flexibility into the calculation of inter-amino acid distances. For all structures determined *in silico* more than one conformation is needed to satisfy all restraints imposed by chemical cross-linking suggesting that Syt-1 is flexible in the absence of Ca2+ ions. Currently, we investigate conformational changes in the presence of various lipids or interaction partners to get insights how the flexibility of Syt-1 is affected by its function.

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# [P052] Influencing the Selectivity of Small Proteins and Peptides on the Raptor$^{TM}$ ARC-18

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**Introduction**

With the influx of biotherapeutics in medical research and healthcare, the analysis of small proteins and peptides by liquid chromatography (LC) continues to grow. Many of these analyses utilize acid-modified mobile phases to improve peak shape; however their effects on selectivity and retention are often not well understood. In this presentation we will explore the effects of acid type and concentration, temperature, and gradient slope on the selectivity and retention of several peptide probes using the sterically protected superficially porous Raptor$^{TM}$ARC-18 LC column (stable to pH 1).

**Results**

Several peptides and small proteins were used as probes to aid in the understanding of selectivity differences which occur when method conditions are manipulated. Through these experiments it is apparent that the retention of peptides containing basic amino acids (e.g. arginine) exhibit considerably large changes in retention when switching between formic acid and TFA. Variations in acid concentration had a comparable effect. Similarly, changes in elution order were observed in the resulting peptide map following the analysis of a bovine serum albumin (BSA) trypsin digest standard (Protea®). Column oven temperature and gradient steepness were shown to impact the band spacing of closely eluting compounds with temperatures $\geq 60\circ$C and shallower gradients showing the greatest improvement in resolution. For this reason sterically protected columns that are stable at low pH and high temperatures are often preferred for peptide applications.

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# [P053] Probing plant glycated proteome by mass spectrometry: at the interface of proteomics and metabolomics

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Glycation is a post-translational modification of free protein amine and guanidine groups with carbonyl compounds. In the first step, reducing sugars, aldoses and ketoses, interact with lysyl $\epsilon$-amino groups yielding Amadori and Heyns compounds. These early glycation products are involved in further transformation and formation of advanced glycation end-products (AGEs). In mammals, these compounds have an impact in diabetes mellitus and serve as reliable markers of ageing. Accordingly, for several proteins, a clear relation between AGE formation, accompanying structural changes and loss of function was demonstrated. During the last decade, AGEs were identified in exhaustive hydrolyzates of plant proteins. Recently, we comprehensively characterized the constitutive glycated plant proteome, which proved to be essentially different from the mammalian one. Thereby, we relied on a combination of metabolomic and proteomic approaches, as well as employing model peptide-based glycation systems. Thus, we demonstrated that ageing of plant organs is accompanied by accumulation of glycation products in leaves, seeds and such specialized structures, as legume root nodules. Thereby, specific sites of AGE formation were identified. These glycation hotspots indicate an essential degree of site specificity of the protein Maillard reaction. Finally, experiments with light, drought, heavy metal and gravitational stresses revealed pronounced effects on the patterns of protein glycation both qualitatively and quantitatively. Generally, the patterns could be interpreted in the context of corresponding characteristic plant metabolites with a high glycation potential. However, despite relatively high levels of protein glycation, the biological role of this phenomenon in plants still needs further evaluation.

*Speaker

# [P054] Towards MS/MS-free high throughput proteome characterization

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Methods of "shotgun" proteomics are based on the separation of proteolytic peptides followed by tandem mass spectrometry (MS/MS). The latter is the crucial step in the workflow, in which the eluting peptides are sequentially isolated and fragmented to produce MS/MS spectra. These spectra are sequence specific and compared with the theoretical ones generated from the applicable database. However, the MS/MS-based proteome analysis suffers from a number of limitations: it complicates identification of low abundant proteins and only a small fraction of all peptide-like features from peptide mass spectra (MS1) are selected for the MS/MS and subsequent identification. Typically, these are the most abundant features and the rest ones are left behind the analysis. There are alternatives to MS/MS-based analysis: peptide mass fingerprinting (PMF) and accurate mass and time tags (AMT) methods, both giving the needed throughput to proteome characterization. In these methods, protein identification is based on comparison of measured and theoretically calculated masses of peptides generated from enzymatic digestion of protein sample. However, they work for the relatively simple mixtures reduced typically to a few proteins and the relatively small proteomes for PMF and AMT, respectively. In this work we present the MS/MS-free method for protein identification which uses the features of both PMF and AMT approaches, as well as parallel digestion of the analyzed sample using proteases with complementary sequence specificities. Further, we discuss the efficiency, attainable proteome-wide protein sequence coverage, ultimate capabilities, and practical implementation of the proposed MS1-only method.

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# [P055] Quantification of individual glycation sites in human plasma proteins by stable isotope dilution with bi-labeled standard peptides

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Type 2 diabetes mellitus (T2DM) is a widely spread disease associated with a complex group of chronic disorders, characterized by hyperglycemia, insulin resistance and insulin deficiency. In human blood, hyperglycemia ultimately results in enhancement of protein glycation – a posttranslational modification formed by reaction of glucose with free protein amino groups. The resulting fructosamines (Amadori compounds) readily undergo further degradation yielding highly heterogeneous group of advanced glycation end products (AGEs), known to be pro-inflammatory in humans. Amadori compounds are used in clinical diagnosis as the markers of early stages of diabetes mellitus (e.g. HbA1C, glycated albumin). Recently, individual plasma protein glycation sites were proposed as promising T2DM biomarkers sensitive to short-term and long-term fluctuations of plasma glucose. Label-free relative quantification of glycation sites in plasma proteins of T2DM patients and nondiabetic individuals showed a disease-related increase of glycation level. However, corresponding strategies for absolute quantification, applicable in a regular clinical practice, are still not established. Therefore, here we propose a new analytical approach based on reproducible and precise quantification of multiple glycated peptides in human plasma tryptic digests. The standard glycated peptides comprised a 13C,15N-labeled lysyl residue, a dabsyl moiety for determination of standard amounts, and a cleavable linker. Specified amounts of these peptides were spiked into plasma samples prior to tryptic digestion and quantification relying on stable isotope dilution. The method was demonstrated to be applicable for quantification of individual glycated sites in T2DM patients and non-diabetic controls and was used for developing a multi-biomarker diagnostic approach.

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# [P056] Age-related changes in root nodules of common beans (Phaseolus vulgaris)

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Glycation is referred to as a reaction of protein lysyl and arginyl residues with carbonyl compounds. In the later steps of this process, advanced glycation end products (AGEs) are formed. In mammals, these modifications underlie aging and directly affect cellular structure and functions. Several years ago, formation of AGEs was demonstrated also in plant leaf proteins. Recently, we have shown that the proteins of legume root nodules are glycated as well. This fact raised the question, if ageing of root nodules is accompanied by glycation, and if yes – how it affects plant physiology. Therefore, we addressed these questions in common bean (*P. vulgaris*) plants, considering young, mature and senescent developmental steps (21, 33 and 47 days after inoculation, respectively). Here we characterize the changes in nodule glycated proteome accompanying aging of *Phaseolus vulgaris* root nodules by a bottom-up proteomics approach. Peptide identification relied on database search against a combined legume database comprising *Phaseolus vulgaris*, *Medicago truncatula* and *Lotus japonicus* sequences and a symbiotic bacteria database (*Rhizobium leguminosarum bv. Phaseoli*). Label-free quantification relied on Progenesis QI and statistical data interpretation was done with the Perseus software. The age-related changes were addressed by targeted quantification of AGE sites using LCquan, with normalization on protein expression data. Totally, 101 glycation sites were identified in nodule proteins. Thereby, for mature developmental steps an age-dependent increase in numbers of modification sites was observed. These patterns dominated by N$\epsilon$-carboxymethyllysine (CML) and glyoxal-derived hydroimidazolone (Glarg). The glycated proteins were mostly involved in stress and protein metabolism.

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# [P057] In-Depth Characterization Of Intact Protein Standards Using HRAM Top Down Mass Spectrometry With Multiple MSMS Strategies

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Complete characterization of intact proteins by mass spectrometry is both possible and increasingly popular today thanks to the latest technological developments made for LC and MS hardware, instrument control software, and data processing software. Having witnessed the explosive growth of bottom-up proteomics and the subsequent evolution of high-quality standards to normalize platform performance and assist with method development for new applications, we recognize a similar need for the Top-down proteomics field. We have developed such a standard mixture for LC and MS quality control, as well as application and method development, and here we present the top-down analysis of this mixture using various fragmentation techniques and MSn capabilities available on a modified Orbitrap Fusion Lumos Tribrid.

We performed direct infusion experiments and LC-MS experiments using a Pierce intact protein standard mix ranging from ~9 kDa to ~70kDa MW on an Orbitrap Fusion Lumos Tribrid MS modified with a 213 nm UVPD source and coupled with a Vanquish UHPLC system. Intact proteins were separated using 2.1 mm by 50 mm Acclaim columns with a 4 $\mu$m particle size. We performed CID, HCD, ETD, EThcD and UVPD fragmentation MSn experiments for structural investigation. Intact Protein spectra were deconvoluted with ReSpect or Xtract in Biopharma Finder 2.0. MS/MS spectra were analyzed with ProSightPC 4.1 and the ProSightPD node in Proteome Discoverer 2.1.

We will present here the range of optimized values in each fragmentation mode for proteins across the MW range, and discuss theoretical reasoning behind these experimentally determined settings.

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# [P058] Proteomic analysis of the angiogenic endothelial extracellular matrix and of the Lysyl-Oxidase-like 2 (LOXL2) substrates

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Interactions between endothelial cells and their microenvironment are key regulators of angiogenesis and vascular function. These interactions consist in both the response of endothelial cells to signals from the microenvironment and generation of the vascular wall microenvironment by these cells, as capillaries are formed. In this context, the composition of the extracellular matrix is a key factor, through the expression of structural and signaling proteins, as well as of enzymes involved in post-translational modifications of the components of the matrix. Indeed, such remodeling of the ECM content impacts its structure and stiffness, which directly affects the mechanical constraints exerted on cells and thus modulates angiogenic potential and vascular permeability.
Specifically, enzymes of the lysyl oxidase families (LOXL2) are responsible for the cross-linking of elastin and collagens. In addition to their function in the development of the cardiovascular system, they are involved in the generation and maintenance of a pathological microenvironment in fibrotic and tumoral contexts and in cardiovascular diseases. The expression of LOXL2 is tightly regulated by hypoxia.
These studies face major analytical challenges related to the specific extraction of ECM proteins. ECM proteins such as collagens are highly modified (around 70 potentially hydroxylated and glycosylated lysines and 250 potentially hydroxylated prolines) and present numerous cross-linking sites, hindering tryptic digestion and subsequent bioninformatic identification. The different analytical steps were optimized to better characterize collagens and their modifications. The role of LOXL2 in cross-linking was investigated as well as the proteomic composition of the ECM in normoxia vs. hypoxia.

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# [P059] Enrichment strategies for improvement of mass spec analysis of chemical cross-linked peptides

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**Background**

Chemical cross-linking in combination with mass spectrometry is a powerful method to determine protein-protein interactions. However, this method suffers from low identification rates without enrichment/fractionation, as the typical yield of cross-linked peptides is less than 1 % of total identified peptides. In this study, we evaluated multiple, widely used enrichment/fractionation techniques and benchmarked newly developed SCX spin columns for cross-linked peptide analysis using an Orbitrap Fusion Lumos mass spectrometer.

**Methods**

Different crosslinkers including DSS, DSSO and DSBU were used to crosslink protein and protein complex standards. Cross-linked samples were reduced, alkylated and digested with trypsin. Cross-linked peptides were pre-fractionated on SCX stage tips, SCX spin columns and SEC Superdex Peptide PC column (GE Health). Enriched samples were separated using a 50cm Thermo Scientific$^{TM}$ EASY-Spray$^{TM}$ column and an EASY-nLC$^{TM}$ 1200 UPLC system in 60min gradient, followed by detection on the Thermo Scientific$^{TM}$ Orbitrap$^{TM}$ Fusion Lumos$^{TM}$ mass spectrometer. Data were analyzed using Thermo Scientific$^{TM}$ Proteome Discoverer$^{TM}$2.2 software and XlinkX node.

**Results**

Our method was developed by optimizing enrichment efficiency with respect to pH and ionic strengths of the buffers/solutions used and benchmarked against well- established methods. For newly developed SCX spin columns, we identified a similar number of crosslinked peptides as by other traditional enrichment methods in only 2 fractions.

**Conclusions**

Overall, the combination of MS-cleavable crosslinking with MS3 acquisition methods and SCX pre-fractionation enables sensitive and selective analysis of protein-protein interactions in com-

*Speaker

plex samples.

# [P060] Revolutionary Proteome Profiling And Quantitation Without Compromising Speed, Sensitivity, And Selectivity

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For large cohort studies in translational proteomics research, data independent acquisition (DIA) methods are used to provide a global view of protein abundance changes among the samples. Here, a novel high resolution MS1 based quantitation DIA (HRMS1) method was developed on a Thermo Scientific$^{TM}$ Q Exactive$^{TM}$ HF-X mass spectrometer to optimize usage of scan speed, ion injection time (sensitivity), and high resolution (selectivity). Conventional DIA quantitation is based on the intensities of fragments in MS2 scans. Instead of quantifying the peptides on MS2 intensities, MS1 scans offer potentially higher sensitivity since the peptide precursor is not fragmented into multiple fragments. High resolution detection removes interferences from analytes of interest, achieving accurate and precise quantitation. The Q Exactive HF-X system enables collection of a higher number of ions per time. The combination of the HRMS1 method and Q Exactive HF-X MS enables collection of a high number of ions within several milliseconds for confident identification and sensitive quantitation on the MS1 level. Separation of the tryptic peptides in complex proteomes was performed with 1-hour total run time on the same capillary LC coupled to both versions of the Q Exactive HF system. $>$ 4400 proteins and _˜35,000 peptide precursors are identified and quantified on the modified system, which produces _˜15% more than the standard system.

*Speaker

# [P061] Performance Evaluation of the Q Exactive HF-X Mass Spectrometer

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Orbitrap-based mass spectrometers are increasingly being used for many different applications. Each application imposes special requirements on the mass spectrometer. Modern mass spectrometers have improved sensitivity, accuracy, high resolution, and/or increased scanning speed. These directly result in significant benefits for applications such as proteomics, environmental/food safety, metabolomics, lipidomics and many more. To further address existing and new requirements from a broad field of applications, new technological developments and performance improvements on the existing Thermo Scientific$^{TM}$ Q Exactive$^{TM}$ HF instrument were undertaken.

We made both hardware and software changes to enhance the performance of the Q Exactive HF instrument.

The Q Exactive HF-X consists of a brighter ion source to improve ion transmission. Using our standard positive calibration mixture at a flow rate of 5uL/min, we observed up to fourfold improvement in ion transmission; injection times were approximately 2.5x shorter and electrometer currents correspondingly higher relative to the unmodified Q Exactive HF instrument. Through optimization of the electronics switching times and the measurement strategy, combined with a resolution setting of 7,500 @ m/z 200, a maximum scan speed of over 40Hz can be obtained. The productivity of this resolution setting was verified using a complex HeLa digest sample. On average, 25,000 unique peptides were identified using 30min gradient. The same number of peptides could only be identified on the Q Exactive HF using a 60min gradient. A direct comparison with a 60min gradient on the Q Exactive HF-X showed approximately 39% more identified peptides.

*Speaker

# [P062] Implementation of Real-Time Update for Time Scheduled Targeted Peptide Quantification (PRM) on a new Quadrupole Orbitrap Benchtop Mass Spectrometer

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In targeted peptide quantification, it is necessary to cover hundreds of targets at high absolute sensitivity at the early stages of the biomarker development pipeline. Nano-LC is commonly utilized with time-scheduled retention time lists to achieve this. The aim is to keep retention time windows as narrow as possible to reduce the number of overlapping RT windows at a given timepoint. This is rendered difficult by the relatively high retention time variations of nano-LC systems. On-the-fly adjustment of RT-windows by means of 'landmark' peptides is a sophisticated method to address these challenges. Here we present the implementation of this concept in the method editor on a new benchtop Orbitrap instrument, resulting in a significantly higher number of reliably quantifiable peptides. The use of dynamic RT, which updates RT information during acquisition, significantly improved the detection of unique peptides, even when small (

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# [P063] Improving high-throughput top-down proteomics using a modified hybrid quadrupole-ultra-high-field-Orbitrap mass spectrometer

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Over the last few years, top-down mass spectrometry has benefited from enormous improvements in FTMS instrumentation in terms of resolution, speed, sensitivity and fragmentation techniques. In spite of all these improvements, there is still a need for higher sensitivity and more intelligent data-dependent acquisition methods in order to successfully conduct high-throughput large scale top-down proteomics studies. Furthermore, improvement to the current generation of charge state assignment and deconvolution algorithms to handle complex top-down data will lead to more efficient, complete and accurate protein identification.
Several hardware and software improvements have been implemented into an existing Q Exactive HF instrument: (a) a brighter ion source interface using a high-capacity transfer tube and an ion funnel for improved ion transmission, (b) on-the-fly charge state assignment and deconvolution for both, medium and high resolution Orbitrap spectra using advanced algorithms, and (c) improved data-dependent decisions for 'High-High' and 'Medium-High' methods by selecting only one charge per protein and applying optimum collision energy for HCD fragmentation.
Top-down LC-MS/MS analyses of several intact protein mixtures were carried out. We started with an E.Coli protein extract, which was analyzed using 15min LC gradient and TopN 'High-High' method. With the modified instrument we were able to identify 35% more proteins than with the non-modified one. Furthermore, purified E.Coli and Human ribosome protein complexes were analyzed using a combination of 'High-High' and 'Medium-High' methods. For E.Coli ribosome sample, all 67 expected ribosomal proteins as well as other ribosome-associated proteins with MWs ranging from 4.4 to 61kDa, were unambiguosly identified.

*Speaker

# [P064] 2D-precursor selection for trapped ion mobility with parallel accumulation serial fragmentation (TIMS-PASEF)

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With the previously introduced "Parallel Accumulation Serial Fragmentation" method (PASEF, Meier et al., JPR 2015, PMID: 26538118) for ion mobility (IMS) quadrupole time of flight (QTOF) instruments, 5-10x faster data dependent acquisition of fragment ion spectra became possible. This approach requires a fast two-dimensional precursor selection algorithm using mass as well as ion mobility information.
In TIMS-PASEF mode, peptide ions elute from the IMS device as a condensed packages. For most efficient MSMS acquisition the quadrupole isolation window needs to switch its isolation position exactly synchronized to these elution times in the fastest possible order. The corresponding algorithm was developed and evaluated using tryptic digests of human HeLa cell lysates, separated by 90min nanoLC gradients. Data were analyzed using DataAnalysis(Bruker), Mascot (Matrix Science) and MaxQuant (MPI of Biochemistry).

The precursor selection algorithm detects m/z and mobility positions of all precursors in the MS1-IMS scan. Then they are scheduled for measurement across 10-20 consecutive IMS experiments aiming for most efficient utilization of measurement time. Low intensity precursors are measured multiple times to achieve sufficient spectra quality. Elution length of an individual precursor ion is dependent on IMS resolution which is a function of mobility scan time. For tryptic peptides, mobility scan times of 25-200 ms resulted in average mobility resolutions up to 80 and elution lengths of 1.8-10.6 ms. With a 100ms IMS separation up to 900.000 MS/MS spectra can be acquired during a 90 min Hela nanoLC-run, resulting in about 200.000 unique MSMS spectra.

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# [P065] Electron-transfer/higher-energy collision dissociation (EThcD) of doubly charged labile phosphorylated peptides

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Covalent post-translational modifications (PTMs) influence the structure and function of proteins. Protein phosphorylation is one of the most studied PTMs and regulates nearly all areas of cell biology, including cell signaling, enzyme activities, apoptosis and protein-protein interactions. In recent years labile protein phosphorylations of arginine, histidine, cysteine and lysine; as well as pyrophosphorylations of serine and threonine have gained more attention in phosphoproteomic studies. However, the reliable localization of those posttranslational modifications via tandem mass spectrometry remains a challenge. Common fragmentation techniques such as collision induced dissociation (CID) and higher-energy collision dissociation (HCD) are limited due to extensive neutral loss of the modification. Electron transfer dissociation (ETD) has shown to preserve labile modifications, but is restricted to higher charge states. Here, we report the ability of electron-transfer/higher-energy collision dissociation (EThcD) to fragment doubly charged phosphorylated peptides, the most prevalent ion species in phosphoproteomic studies, without the loss of labile phosphorylations. To study the EThcD fragmentation behavior of those delicate modifications, we synthesized peptides containing phosphorylations of arginine, histidine, cysteine and lysine as well as pyrophosphorylations on serine residues. This allowed us to evaluate the optimal fragmentation conditions, demonstrating that EThcD is the method of choice for unambiguous assignment of tryptic, labile phosphorylated peptides.

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# [P066] Mass Spectrometry-Based Interaction Studies of Escherichia coli Formate Channel A

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The formate channel A (FocA) of Escherichia coli translocates formate across the membrane in a bidirectional fashion and forms a homopentamer within the bacterial inner membrane [1]. However, there is no clear mechanistic understanding how formate passage through the FocA channel is achieved and controlled. Previous studies had indicated a specific interaction between FocA and the enzyme pyruvate formate-lyase (PflB) [2] suggesting that the N-terminus of FocA is involved in the regulation of formate transport [3]. To validate this hypothesis, we studied the FocA/PflB interaction by chemical cross-linking/mass spectrometry (MS) using full-length FocA, an N-terminally truncated FocA variant, and an N-terminal FocA peptide. We also screened for additional FocA interaction partners with an affinity enrichment approach combined with chemical cross-linking/MS. Chemical cross-linking of full-length FocA and PflB yielded four intermolecular cross-links, preferentially within the N-terminal region of FocA. This result was confirmed by cross-linking experiments of PflB with the N-terminal FocA peptide, for which seven intermolecular cross-links were identified. The interaction network of FocA variants with anaerobically grown E. coli cell lysates revealed 136 protein interaction partners for full-length FocA, 32 proteins for the N-terminally truncated FocA variant, and 38 proteins for the N-terminal FocA peptide. These results underline the importance of the N-terminal region of FocA and show a complex protein interaction network, including enzymes and proteins involved in formate metabolism. [1] Wang et al. (2009), Nature. 462:467-72. [2] Doberenz et al. (2014), J Mol Biol. 426:2827–2839. [3] Hunger et al. (2014), Biol Chem. 395:813-25.

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# [P067] Spectral library based approach to protein arginine phosphorylation in Staphylococcus aureus

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*Staphylococcus aureus* emerged as an important human pathogen and is the causative agent of a high number of nosocomial infections. Therefore, investigation of the phosphoproteome will help to decipher molecular and cellular mechanisms that underlie pathogenesis and virulence. In addition to the well-known phosphorylations at ser/thr/tyr/cys/his/asp residues, the phosphorylation at arginine residues plays an essential, but mostly still unknown role in bacteria. Therefore, we decided to study arginine phosphorylations in greater detail.
*S. aureus* COL possesses the protein PtpB which is assumed to be an arginine phosphatase. Hence, we applied a gel-free method to analyze the changes in the phosphoproteome of the deletion mutant *ptpB* and the wild type, thereby focusing on arginine phosphorylations. This way, we could identify eight arginine phosphorylations in wild type samples (B´asell *et al.,* 2014) and 207 arginine phosphosites exclusively within the mutant (Junker *et al.*, 2017). This identification of putative targets of PtpB allows further investigation of the physiological relevance of arginine phosphorylations.
In order to enhance the reliability of identified phosphorylation sites at arginine residues, a subset of arginine phosphorylated peptides was chemically synthesised. Combined spectral libraries based on phosphoenriched samples, synthetic arginine phosphorylated peptides and classical proteome samples contain 960 phosphopeptides (396 at arginine) and provide a sophisticated tool for the analysis of phosphorylations. Furthermore, the combination of proteome and phosphoproteome quantifications of wild type and mutant under control and stress conditions indicates drawbacks to amino acid metabolism and therefore reveals further insights into the physiological role of this important human pathogen.

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# [P068] Towards comprehensive signaling pathway monitoring using advanced PRM methods

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Introduction

Targeted mass spectrometry quantification based on high-resolution and accurate-mass (HRAM) parallel reaction monitoring (PRM) measurements is increasingly adopted by the proteomics community. The PRM technique offers unmatched degrees of selectivity and analytical sensitivity, typically required to analyze peptides in complex samples. Here the performance of PRM has been revisited to evaluate the advantages enabled by the latest technology developments offered by a Q Exactive HF and the most advanced acquisition methods.

Methods

The analyses were performed on a Q Exactive HF (Thermo Fisher Scientific) operated with a broad range of PRM methods, including several variants of IS-PRM (some of which using instrument programming interface). Under its main implementation, the IS-PRM technique alternated between i) a "watch mode", in which internal standards (IS) were continuously measured in their (dynamically corrected) elution time monitoring windows at fast scanning rates, and ii) a "quantitative mode" (triggered by the real-time detection of the IS) which measured the corresponding pair of IS and endogenous peptides serially over their elution profile.

Preliminary Data

The new features and methods were evaluated and applied to the monitoring of signaling pathways in various human cancer cell lines, including the measurement of peptide dilution series (40 peptides) and very large screening experiments (600-700 peptides). The brighter ion source of the Q Exactive HF yielded an overall improvement in the sensitivity of measurements. The improved scanning rate in the watch mode of IS-PRM and the new variant of IS-PRM were

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decisive to the broad coverage of signaling pathway achieved.

# [P069] Parallel accumulation – serial fragmentation (PASEF) on a novel trapped ion mobility spectrometry (TIMS) – QTOF instrument for highly improved shotgun proteomics

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In data dependent acquisition experiments only around 20% of the eluting peptide features are targeted for MS/MS due to limitations in speed, sensitivity and resolution. We recently described that the novel parallel accumulation – serial fragmentation (PASEF) method is able to increase the sequencing speed and sensitivity of MS/MS scans on a TIMS-QTOF significantly (Meier et al., JPR 2015, PMID: 26538118). Here we show further data acquired with PASEF-TIMS-MS/MS in shotgun proteomics experiments.
In a TIMS-QTOF instrument ions are accumulated for a user-defined time and released from the TIMS device depending on their mobility cross section. By applying the PASEF method multiple precursors per TIMS scan were selected by sub-millisecond switching of the quadrupole isolation window. Raw data were analyzed using DataAnalysis (Bruker Daltonics) and MaxQuant (MPI of Biochemistry).

Different accumulation and release times (25-100 ms) corresponding to median ion mobility resolutions of up 78 were tested. The sensitivity was improved by targeting low abundant precursor ions several times. As an ultimate test for the benefit of the high speed and sensitivity of PASEF, just 200 ng of a human cancer cell line (HeLa) protein digest were analyzed in a 90 min nanoLC gradient. From this run 200.000 peptide features were detected. With only 12 ng of digest still more than 50000 peptide features were observed. Application of PASEF allowed the selection of 13 precursors within 100 ms release time: this corresponds to ca. 130 MS/MS spectra per second and demonstrates the fast targeting of peptide features.

*Speaker

# [P070] Evaluation of a Deuterated (D12) Version of the MS/MS-Cleavable Cross-Linker Disuccinimidyl Dibutyric Urea (DSBU)

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Previous studies have demonstrated the benefits of the amine reactive, MS/MS-cleavable cross-linker DSBU (disuccinimidyl dibutyric urea; formerly BuUrBu or urea-linker) for structural proteomics studies based on cross-linking/mass spectrometry (MS) [1, 2]. Especially in combination with the customized software tool MeroX [3], DSBU allows an automated, robust, and reliable analysis of cross-linked products [1].
To further advance the automation of the cross-linking/MS approach, we now introduce a deuterated (D12) version of the DSBU-linker, combining the advantages of MS-cleavable linkers and isotope labeling. One major benefit of using mixtures composed of labeled and non-labeled (deuterated) cross-linkers is the possibility to exploit the characteristic mass differences of MS signals for targeted data-dependent MS/MS experiments. Here, the basis for a reliable assignment of cross-linked species is a mass difference between the two isotopic species that is large enough to create non overlapping isotope patterns of the single species. Also, the elution profiles of the differentially labeled cross-linked species have to overlap during LC/MS analysis. We show that the D12-labeled DSBU linker meets both requirements and as such is beneficial for structural proteomics studies based on cross-linking/MS.

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*Speaker

# [P071] Protein Interactions of the p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass-Spectrometry

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The tumor suppressor p53, also referred to as the "guardian of the genome", is a transcription factor that plays a key role in preventing the development of cancer. It binds to specific response element DNA as a tetramer of initial dimers and enhances the transcription rate of selected genes that are involved in cell cycle arrest, apoptosis or senescence. The p53 monomer encompasses about 40 % of intrinsically disordered regions (IDRs) responsible for the specific binding of a variety of different proteins [1]. Despite the broad spectrum of known p53 interaction partners, the identification of p53 binding proteins has never been conducted with recombinant expressed full-length p53. We will perform p53 interaction partner analysis with LoVo colon adenocarcinoma cell lysates [2] using a combined affinity enrichment cross-linking/MS approach with the MS-cleavable cross-linker disuccinimidyl dibutyric urea (DSBU). The experiments will be performed using cytosolic as well as nuclear fractions to localize specific compartment-related protein interaction networks. Candidate p53 binding partners will be subjected to detailed structural analyses employing a multi-disciplinary approach combining chemical cross-linking/MS and native MS. These studies will offer further insights into the protein interaction network of p53 in colon carcinoma cell lines. Also, information will be obtained on the conformational versatility of the so far understudied IDRs in p53 upon binding to different protein interaction partners.

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Drewinko *et al.*, J. Natl. Cancer Inst., 1978, **61**, 75-83

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# [P072] Future directions for data-independent acquisition modes utilising a scanning quadrupole on Q-ToF instruments

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Data independent acquisition (DIA) methods utilising a resolving quadrupole have recently gained in popularity. The reduction in sensitivity caused by the filter is offset by the corresponding gain in selectivity. However the cycle time in experiments where the quadrupole is stepped over a wide m/z range (typically > 1s) is incompatible with fast chromatography. The recently introduced SONAR method utilises a scanning quadrupole and ultra-fast acquisition system, giving an order of magnitude reduction in cycle time and a similar increase in selectivity x sensitivity. In the original method, the instrument alternates between low and elevated energy in successive scans of equal duration. We consider modifications to the basic SONAR method that further improve DIA performance.

Qualitative and quantitative SONAR experiments were carried out on Xevo G2XS Q-ToF instruments with modified instrument control software. Various loadings of a tryptic digest of a K562 human cell line spiked with a four protein tryptic digest standard were injected onto a nano-LC system equipped with a reversed-phase C18 column. Different gradient lengths (45, 60 and 90 minute) were employed to elute peptides from the column into the mass spectrometer. Data was visualised in Waters DriftScope software and processed using a modified version of Waters ProteinLynx Global Server, Progenesis QIP and Skyline.
Three different modifications to the basic SONAR method are considered and discussed.

*Speaker

# [P073] The Caenorhabditis elegans proteome response to naturally associated microbiome members of the genus Ochrobactrum

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The nematode *Caenorhabditis elegans* interacts with a variety of bacteria as it feeds on microbes, and a number of these both associate and persist within the worm's intestine. Host-microbe interactions in *C. elegans* have been analysed primarily at the transcriptome level with the host response often been monitored after challenge with pathogens. We assessed the proteome of *C. elegans* after growth on bacteria capable of colonising its gut, via a comparative analysis of the nematode exposed to two naturally associated *Ochrobactrum* spp. (MYb71, MYb237) versus *C. elegans* grown on *E. coli* OP50. A total of 4,677 *C. elegans* proteins were identified, 3,941 quantified. Significant alterations in protein abundances were observed for 122 proteins, 48 higher and 74 lower in abundance. We observed an increase in abundance of proteins potentially regulated via host signalling pathways, in addition to proteins involved in processing of foreign entities (e.g. lipase, proteases, glutathione metabolism). Decreased in abundance were proteins involved in both degradation and biosynthesis of amino acids, and enzymes associated with the degradation of peptidoglycan (lysozymes). The protein level differences between *C. elegans* grown on native microbiome members compared to the laboratory food bacterium may help to identify molecular processes involved in host-microbe interactions.

*Speaker

# [P074] Aptamer-Protein interaction. Epitope and affinity study using biosensor and mass spectrometry

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C-Met is a glycosylated receptor tyrosine kinase (RTK) of the hepatocyte growth factor (HGF) [1]. Upon ligand binding and autophosphorylation, C-Met transmits intercellular signals by its unique multi-substrate docking site, which are essential for tissue repair, embryonic development, wound healing and liver regeneration [2]. However, pathophysiological activation of the C-Met pathway leads to tumorigenesis, schizophrenia and cardiomyocytes death [3,4].
The tyrosine kinase protein has been found as a biomarker for cancer diagnosis. However there is no major clinical impact to date [5], from the study of this important biomarker no inhibitors and monoclonal antibodies have been proven to be very effective.
Here we report a study of the C-Met interaction with two DNA aptamers that bind the target protein (C-Met) with high affinity and specificity. Nucleic acid-based aptamers have very similar properties with monoclonal antibodies. In comparison with antibodies, they are chemically synthesized [6].To determine the affinity strength of the two aptamers to the protein, surface plasmon resonance(SPR) biosensor technology was employed. Furthermore, by using epitope excision with MALDI mass spectrometry we could determine the epitope regions on the protein.By combining MALDI MS and SPR biosensor we showed that the interaction of both aptamers has very strong affinity, and that the aptamers undergo conformational changes after the first binding event. More interesting is that the second binding event is 100 times stronger. Further analysis revealed the binding epitopes for both aptamers and confirmed that the protein has 2 different binding sites for each aptamer.

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# [P075] Elucidating the mechanism of peptide modification by chemical modifiers with MALDI-MS

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Post-translational modifications and artificial modifications of peptides and proteins are of principal interest in proteomics and pharmaceutical sciences. Modification of proteins and peptides at several reactive amino acid residues can cause a change in activity and lead to pharmacological effects. Chemical modifiers can occur as natural products in secondary metabolic pathways or can artificially be applied as drugs. Among reactive amino acids, cysteine plays a special role due its unique chemical properties. We assessed MALDI-MS analysis of synthetic peptides bearing only one reactive amino acid residue among unreactive alanine-residues was applied to investigate the reaction with different chemical modifiers. The specificity towards different reactive amino acids was inspected, especially towards cysteines. Using high-resolution mass spectrometry, we investigated the mechanism of binding of the chemical modifiers. We grouped these modifiers into directly interacting Michael acceptors, which show the typical electron-withdrawing chemical-moiety and a double-bound, and untypical covalent modifiers, which either need to be activated to a Michael-acceptors or react via a radical-driven mechanism. To support our investigations and elucidate hidden substance specific redox properties, we additionally applied an UV-VIS-based radical scavenging and reduction assay. We proved the already published mechanisms for several substances, such as U73122, Afatinib, Nitrooleic acid and Quinones. We newly discovered the exclusive reaction of hydroquinones with cysteines after activation by oxidation or via a radical mechanism. UV-VIS data supported this mechanism by showing high radical scavenging and reductive potential of hydroquinones. Additionally, we found several MALDI matrices to modify cysteines covalently.

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# [P076] Improvement of mitochondria extract from Saccharomyces cerevisiae characterization in shotgun proteomics using sheathless capillary electrophoresis coupled to tandem mass spectrometry

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In this work, we describe the characterization of a quantity-limited sample (100ng) of yeast mitochondria by shotgun bottom-up proteomics. Sample characterization was carried out by sheathless capillary electrophoresis, equipped with a high sensitivity porous tip and coupled to tandem mass spectrometry (CESI-MS/MS) and concomitantly with a state-of-art nano flow liquid chromatography coupled to a similar mass spectrometry (MS) system (nanoLC-MS/MS). With single injections, both nanoLC-MS/MS and CESI-MS/MS 60 min-long separation experiments allowed to identify 271 proteins (976 unique peptides) and 300 proteins (1765 unique peptides) respectively, demonstrating a significant specificity and complementarity in identification depending on the physicochemical separation employed. Such complementary, maximizing the number of analytes detected, presents a powerful tool to deepen a biological sample's proteomic characterization. A comprehensive study of the specificity provided by each separating technique was also performed using the different properties of the identified peptides: molecular weight (MW), mass-to-charge ratio (m/z), isoelectric point (pI), sequence coverage or MS/MS spectral quality enabled to determine the contribution of each separation. For example, CESI-MS/MS enables to identify larger peptides and eases the detection of those having extreme pI without impairing spectral quality. The addition of peptides, and therefore proteins identified by both techniques allowed to increase significantly the sequence coverages and then the confidence of characterization. In this study, we also demonstrated that the 2 yeast enolase isoenzymes were

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both characterized in the CESI-MS/MS dataset. The observation of discriminant proteotypic peptides is facilitated when a high number of precursors with high-quality MS/MS spectra are generated.

# [P077] Structural Insights into Retinal Guanylyl Cyclase/GCAP-2 Interaction by Cross-linking/Mass Spectrometry

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Retinal guanylyl cyclases (ROS-GC) are transmembrane proteins that are regulated by guanylyl cyclase-activating proteins (GCAPs) in dependency of intracellular Ca2+ concentration [1]. However, the exact mechanisms of how GCAP-1 and -2 activate their target proteins are still not fully elucidated. Previously conducted cross-linking/MS studies using GCAP-2 and a short peptide derived from catalytic domain of ROS-GC1 had pointed to a well-defined structure of the GCAP-2/ROS-GC peptide complex in its Ca2+-bound state [2].
To clarify whether GCAP-1 and -2 possess overlapping or separate binding sites in the intracellular region of ROS-GC, we extended these initial cross-linking studies [3]. For this, three ROS-GC peptides were employed: The first peptide comprises the binding motif of GCAP-2, the second one contains an *N*-terminal extension, and the third one represents the potential binding motif of GCAP-1. For our studies, we used the external cross-linkers disuccinimidyl dibutyric urea (DSBU) and 1,1-carbonylimidazole (CDI) as well as incorporated photo-methionine into GCAP-2.

The majority of cross-links were identified between GCAP-2 and second ROS-GC peptide from the catalytic domain. This finding confirms the interaction site of GCAP-2 with the catalytic domain of ROS-GC. However, several cross-links point to additional interaction sites of the ROS-GC peptides with GCAP-2. Further cross-linking experiments will be conducted between GCAP-2 and the complete intracellular region of ROS-GC to delineate the interactions between both proteins.

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# [P078] Proteomics of single starch granules

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Starch granules display a broad structural and morphological heterogeneity. Moreover, the abundance of starch associated proteins is likely under the control of regulatory mechanisms related to the initiation and biosynthesis of the granules. To decipher these mechanisms and avoid biases due to intra-sample heterogeneity, the work reported here aimed at identifying starch-bound proteins and determining their stoichiometry in miniaturized samples. Granules from potato are the largest among common starch sources, with a diameter reaching up to 100 $\mu$m. Theoretical calculations show that the amount of the various proteins in a single 100 $\mu$m starch granule from potato lies between 178 fmol and 75 amol, which is within the detection limit of nanoLC-nanoESI-MS/MS shotgun mass spectrometry. In our experimental workflow, starch granules are surface stripped with SDS, prior to glucan denaturation by heat. Then, the proteins are reduced, alkylated and digested by trypsin using eFASP method. Finally, peptides are analyzed on a Q-Exactive Orbitrap mass spectrometer and raw data are faced to several potato databanks. Protein quantification is realized using AQUA Peptides. Analyses have been realized with 300 mg (around 10ˆ6 granules) of size fractionated starch. Then we miniaturized the protocol to analyze the proteome of 30 granules and of a single starch granule. First data allow us to identify new proteins associated to potato tuber starch, and to establish protein stoichiometry inside the starch granule. We are also able to detect and quantify proteins with 30 granules, and the granule bound starch synthase (GBSS) with only one granule.

*Speaker

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# [P080] Proteom analysis of human neuromelanin granules in the background of dementia with Lewy bodies

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Dementia with Lewy bodies is known to be the second most frequent dementia following Alzheimer's disease. It is an atypical parkinsonism and together with Parkinson's disease and Multisystem Atrophy it is classified as a synucleinopathy due to the presence of Lewy bodies. The prevalence of dementia with Lewy bodies is 0.7% among the population older than 65 years. Besides other characteristics a loss of dopaminergic neurons containing neuromelanin in the *substantia nigra pars compacta* during the course of dementia with Lewy bodies is found. Neuromelanin, a dark, insoluble pigment, forms so called neuromelanin granules together with lipids and proteins. It is widely debated if those granules are neuroprotective or neurodegenerative since there is evidence for both sides. A protein biochemical characterization of neuromelanin granules could allow deeper insight in the function of these granules in the pathogenesis of dementia with Lewy bodies. In a pilot study neuromelanin granules from *post mortem substantia nigra pars compacta* tissue of four dementia with Lewy bodies patients and five control patients without any indications for a neurodegenerative disease were collected. The donor-specifically samples were isolated via laser microdissection and analyzed by mass spectrometry. This study revealed 536 proteins to be considered as a protein biochemical essential composition of the neuromelanin granules while 116 proteins were identified as significantly differential. To affirm the results and increase the statistical significance the study will be extended by 18 dementia with Lewy bodies cases.

*Speaker

# [P081] Making Glycoproteomics via Mass Spectrometry More Accessible to the greater Scientific Community

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We have recently developed a method that, for the first time, is capable of facilitating both, glycan structure and attachment site analysis for both N- and O-glycans alike. This method termed '*Isotope Targeted Glycoproteomics*' (IsoTaG) is based on the introduction of an isotopic label that also functions as a (cleavable) enrichment handle, allowing for enrichment of metabolically labelled glycoproteins. We found an unprecedented enrichment and detection of low abundant glycoproteins without either the need to truncate glycan structures or complex sample fractionation.
We aim to make this method a widespread tool for both, glycoproteomics experts and non-experts and have begun the transfer of the method to interested laboratories by supplying them with a novel approach to generate meaningful glycoproteomic datasets. We also plan an evaluation of the IsoTag workflow *via* interlaboratory comparison of identical samples. This small round-robin will be the basis for standardized procedures for an IsoTag 'kit'. These will then be tested for general applicability by dissemination - along with a set of standards - to a larger group of laboratories (mainly MS core facilities). After evaluation these results will be available in an online repository (www.IsoStamp.org).
We also demonstrate the versatility of the IsoTag method through use in additional systems and are currently in the process of developing new IsoTag probe systems for new or specialized applications. We are highly interested in starting collaborations to test and improve our IsoTag technology and are able to provide the IsoTag probes, standards and protocols to interested laboratories.

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# [P082] TimsTOF Pro and PASEF for High Sensitivity Proteomics and Phosphoproteomics

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**Background:** We evaluate the performance level of the first serial trapped ion mobility spectrometry quadrupole time of flight (TIMS-QTOF) instrument enabling a "Parallel Accumulation - Serial Fragmentation" method (PASEF, Meier et al., JPR 2015, PMID: 26538118) on extremely low ample amounts.
**Material & Methods:** Low peptide sample amounts (12ng - 100 ng) of a HeLa protein digest and IMAC phosphopeptide enrichments from 200 $\mu$g proteolytic digests were HPLC separated (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) and analyzed on a high-resolution timsTOF Pro instrument (Bruker Daltonics) on different LC gradients from 30 - 90 min. Post-processing analysis was performed in Mascot 2.5.1 (MatrixScience). Peptides and phosphopeptides were filtered to $< 1\%$ PSM FDR.

**Results:** High-throughput DDA measurements acquired from 100 ng of an Hela Cell tryptic digest and separated using 30-90 min gradient length resulting in identifications of 25,000-45,000 unique peptide and 4000 to 5300 protein groups. 12 ng of the same digest separated with a 60 min gradient resulted in 13000 unique peptide identified, corresponding to 3000 protein groups. Phosphopeptides enriched from 200 $\mu$g starting material resulted in 14,000 unique peptide identified after a 90 min LC-TIMS-PASEF-MS experiment

**Conclusion**: The sensitivity of TIMS-PASEF experiments is preserved @ MS/MS acquisition speeds exceeding 100 Hz. Furthermore, the ion mobility dimension separation facilitates the detection and fragmentation of phosphopeptides. These combined features will enables to make a significant step towards comprehensive proteome analysis.

*Speaker

# [P083] Study of the impact of ion beam analysis on the molecular preservation of mummy hair by a proteomic approach

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Hair is a powerful bioindicator used in toxicological and environmental studies since it can incorporate toxic elements during its growth. As part of a project on the characterization of mummy hair, elemental analyses by particle-induced X-ray emission (PIXE) were carried out using the AGLAE accelerator from the C2RMF. The objective was to detect traces of metals such as Fe,Pb, Hg or As that could be correlated to mummification rituals or to a specific environment.

Analytical techniques based upon ionizing radiation are widely employed in the study of heritage materials especially because they are considered as non-destructive. But the highly focused ionizing beams used for imaging can induce modifications in the most sensitive materials, notably the organic ones. The objective of this work is to determine the impact of this analytical technique at the macroscopic and molecular levels on hair from mummies weakened by alteration. In previous work, markers of degradation of proteins of these ancient capillary fibers have indeed been highlighted.

Hair (modern and ancient) were studied following irradiation with 3-MeV protons at a fluence of 1x10(exp15) to 2x10(exp15) protons/cm2 required for trace element mapping. Modifications were characterized following a proteomic "bottom-up" approach specifically dedicated to the study of hair from mummies [1]. The study of specific PTMS such as deamidation of asparagines and glutamines allowed for the first time to show the influence of the initial molecular state of preservation on the resistance of samples to irradiation.

Fresnais *and al.*(2017), *OMICS A Journal of Integrative Biology*, 21,361-370.

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# [P084] Structural Insight into an FMN-Dependent Ene-Reductase Through Ion Mobility Mass Spectrometry

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In the last decades, an increasing tendency to apply biocatalysis in organic synthesis was observed. Among redox enzymes, the ene-reductase from *Gluconobacter oxydans* (GOX-8) turned out to represent a versatile biocatalyst, useful for the reduction of different types of activated C=C double bonds. Encouraged by the success of ion mobility mass spectrometry for studying protein structures in the absence of bulk water, we chose this methodology to obtain information about the folding of this protein in the gas phase, and therefore also in the condensed phase.
The ene reductase GOX-8 was analysed using *off-line* nanoESI-Q-IMS-ToF mass spectrometer (Synapt G2Si, Waters Corp., Manchester). Data analysis was performed using and DriftscopeTM and BioLynxTM (Waters, Manchester). Previous results hinted on the presence of different folding states in the gas phase, as revealed by ion mobility spectrometry. These findings were to be reviewed, as other results showed a uniform behavior of the protein during ion mobility separation.
When GOX-8 is analysed using denaturing conditions, the non-covalent bound co-factor FMN was not bound to the protein, as expected. However, when native conditions were employed, GOX-8 was detected exclusively in complex with the co-factor FMN. The data indicates, that binding of the cofactor FMN by GOX-8 is complete. The ion mobility data of the protein analysed using denaturing conditions revealed a uniform distribution of the unfolded protein. This was also the case, when the protein GOX-8 was analysed using native conditions. Several acquisition parameters were evaluated, if they influence the results of the ion mobility separation.

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# [P085] The Mass spectrometry Analysis of Insoluble Proteins in the Spinal Cord of ALS-linked (hPFN1G118V) Mouse Model

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The PFN1 gene, encoding Profilin1 (PFN1) protein, has been reported as one of the genes implicated in motor neuron disease. Accumulating reports suggest that formation of aggregates containing PFN1 may be a mechanism of motor neuron death. We found that mutant PFN1 forms aggregates in the spinal cord, motor cortex and brain tissues isolated from mutant (PFN1G118V) transgenic mice. To gain insight into the neurotoxicity mechanism of mutant PFN1, we conducted a proteomic analysis using a LTQ-OrbitrapVelos on the insoluble fraction of spinal cord lysates of wild-type and mutant PFN1 transgenic mice. Quantification of mass spectrometry data using a label-free MS1 intensity based approach in MaxQuant led to the identification of a total of 2904 proteins at 1% FDR, of which 177 proteins had a statistically significant altered expression level. Additionally, several pathways were detected in the mutant mice. Interesting pathways and networks from basic biochemical pathways e.g. proline biosynthesis 1 and cellular signaling pathway, e.g. EIF2 signaling to pathways that are implicated in neurodegeneration, e.g. cell death and survival were identified. In order to block the up- and down-regulation and inclusion of these proteins in the insoluble fraction, blocking the aggregation of mutant PFN1 was a primary goal. We tested our ROG-102 compound derived from Crocus plant and observed that treatment with ROG-102 decreased the aggregation of mutant PFN1 in vitro. In conclusion, our mass-spec analysis revealed the identity of proteins in aggregates, and we showed that Crocus plant compounds have anti-aggregation properties.

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# [P086] Proteomics applied to the study of archaeological ceramics (IInd century) preserved in submarine context

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Ceramic material represents essential witness of human activities through years. The objective of this study is to identify protein residues trapped into archaeological amphorae preserved in submarine context during several thousand years. The focus was given to the study of Dressel 14 amphorae from shipwrecks (IInd century). These amphorae were supposed to store and transport fish derivate products such as *Garum* and *Liquamen*. The proteomics workflow was firstly adapted to the study of *Garum* model fish sauces prepared from various fish species (e.g. sardines, mackerels). After the optimization of extraction, digestion and data-treatment procedures, both muscle proteins (e.g. tropomyosin, myosin) and blood proteins (e.g. hemoglobin) were successfully identified. Due to the lack of fish protein sequences in proteomic/genomic databases, most of the proteins were identified using sequence homologies to other fish species (e.g. *Danio renio*, etc.). D*e novo* sequencing allowed the identification of new un-sequenced peptides from muscular and blood proteins and several peptides could be used to discriminate the different fish species. The developed protocol was applied on several hundreds micrograms of archeological amphorae. For the first time, 157 proteins were successfully identified among which 19 muscular proteins (e.g. myosin light chain 1,2 and 3). Based on the current databases that include 21299 fish myosin sequences (19 for *Thunnus* genus, 21 for *Scomber* genus) and internal databases (fresh *Thunnus* and *Scomber* muscles), 4 myosin peptides could be attributed to *Scombridae* family. This result represents the first analytical confirmation of the use of Dressel 14 for *Scombridae* fishes transportation.

*Speaker

# [P087] Mass spectrometric investigation of degradation products of peptides derived from the C-terminal Tail of integrin beta 3

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Integrins are a large family of cell surface receptors. They have large extracellular domains and small cytoplasmic domains that mediate signal transduction. [1, 2]
The direct interaction of peptides derived from the cytoplasmic domain of integrin beta3 [748-788] with paxillin-LIM 2 / 3 is currently being characterized by various NMR experiments by our research group. [3]

In this study, we describe the mass spectrometric investigation of degradation products which occurred during the expression and purification (detected with *Size exclusion chromatography*) of two short peptide constructs (45 and 37 aa) from integrin beta3.

Mass spectrometry results of the two peptide constructs from integrin beta3 confirmed that both are proteolytically heavily degraded.

The various hydrolysis products of the two peptides from integrin beta3 were characterized by positive electrospray ionization (ESI) mass spectrometry. Specific sequences of hydrolyzed peptide fragments were identified by calculation of their exact masses.

In the ESI-MS spectra of the peptides from integrin beta3, various series of the ions clearly indicate that the C-terminus was the primary site of hydrolysis. Based on these results we propose that different types of enzymes are responsible for the hydrolysis.

Mass spectra were recorded using an ESI-Q-TOF mass spectrometer maXis (Bruker Daltonik Bremen). Sequences of individual peptides were determined using the sequence editor algorithm, incorporated into Bruker D.A. software. Biotools was applied to correlate the MS/MS spectra with amino acid sequences.
These studies should help to improve the expression and purification protocol of the proteins of interest by adding a specific Serine protease inhibitor.

*Speaker

# [P088] Peptidomics of the nematode Haemonchus contortus

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The nematode *Haemonchus contortus* is an animal endoparasite infecting wild and domesticated ruminants worldwide. It is a very common parasite and one the most pathogenic nematodes of ruminants. Existing anthelmintics predominantly target elements of the helminth classical neurotransmission network (GABA, Glu, ACh...) and act by compromising nematode motor function. However, the increase of parasite resistance to existing chemotherapeutics warrants identification of antiparasitics with novel modes of action. Neuropeptide signaling has been proposed as a promising target for novel drugs against helminthes which still has not been exploited. Obviously, detailed knowledge on the neuropeptide sequence and their post-translational modifications is required to understand the in vivo physiological role and function of bioactive neuropeptides. We therefore set out a peptide extraction protocol and a peptidomic workflow to biochemically characterize bioactive peptides from both first stage (L1) and third-stage larvae (L3) of *H. contortus*. This strategy allowed the first identification at the peptidomic level of more than 100 endogenous amidated peptides arising from 40 precursors of *H. contortus*.
Furthermore, this methodology allows monitoring eventual changes in peptide expression for example among different animal stages or in response to treatment.

*Speaker

# [P089] Weighted Gene Correlation Network Analysis as Grouping Strategy for Nanomaterial Risk Assessment in Alveolar Macrophages

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The public is increasingly exposed to various engineered nanomaterials (NMs) due to their wide application in e.g. medicine, cosmetics, building materials or packaging, and the continuous development of novel NMs. Since the respiratory tract is considered as the primary target organ for airborne NMs, the toxicological effect of NMs on alveolar macrophages (NR8383) was investigated using shotgun proteomics with the aim to develop grouping strategies to facilitate NM risk assessment.
Obtained protein abundance ratios were analyzed using weighted gene correlation network analysis (WGCNA), where protein abundances are correlated to construct a gene co-expression network followed by hierarchical clustering to identify modules of proteins that are related to certain NM treatments. For this analysis, physicochemical properties of the NMs, e.g. size or coating, were integrated to get more powerful and reliable prediction models.

The results show just a few modules comprising proteins that show significant correlation with the treatments themselves. These modules contain mainly proteins that are connected to inflammatory activity or metabolic processes. Most importantly however, several modules containing proteins correlated significantly with one or more of the physicochemical properties. For example one module containing proteins related to oxidative stress shows significant correlation with the surface area of the NMs, suggesting that these properties influence the oxidative stress response in alveolar macrophages.
In conclusion, these results indicate that WGCNA is a valuable tool to develop NM grouping strategies based on toxicological effects on protein level.

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# [P090] A first glance on the O-glycans of mucin from porcine stomach

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Mucins are heavily *O*- and *N*-glycosylated proteins, which are the main constituents of mucus. Mucus covers the epithelia of e.g. the respiratory and the gastrointestinal tract. Due to the dense decoration with glycans mucins protect the underlying epithelium from pathogenic and biochemical attack, i.e. toxins, enzymatic digestion etc., as well as from chemical damage by, for example, extremely low pH values. As a result these protective properties of mucins impede their analysis with respect to glycan structures and amino acid sequences. In the present study we performed an elimination/alkylaminylation procedure [1] to get hands on the *O*-glycans of mucin from porcine stomach.
Elimination of glycans was essentially performed as described previously [1]. Briefly, the mucin was incubated with aqueous methylamine and ethylamine solutions at 50 ∘C for 6 h and 16 h, respectively. After protein precipitation by use of ethanol the glycans were analyzed by mass spectrometry using a Synapt G2S instrument.

Surprisingly, the elimination/alkylaminylation reaction did not only take place at the peptide backbone but also at the glycan moieties. Besides free glycans *N*-alkylamino glycosides and glycans after elimination of the aglycon were found. The main *O*-glycan species detected were HexNAc(Fuc)HexHexNAc, HexNAcHexHexNAc, and FucHexHexNAc.

Although we are well aware, that the de-*O*-glycosylation was far from being complete (the protein still resisted proteolytic digestion) the elimination/alkylaminylation procedure allowed to detect a representative set of *O*-glycans from the mucin under inspection.
1. Hanisch, F.-G., Jovanović, M., and Peter-Katalinić, J. (2001) Anal. Biochem. 290, 47-59.

*Speaker

# [P091] Cross-linking/Mass Spectrometry of Nuclear Receptor complexes

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**Strategy :** It is now well established that proteins carry out their function through their interactions with other partners. Therefore, structure determination of protein complexes is one key in understanding protein action and in the resulting modulation of cellular dysfunction. Our project aims to develop a global strategy to extend the potential of cross-linking approaches to the characterization of heterodimer interfaces of Nuclear Receptors/DNA complexes. In particular, we want to investigate whether the dimerization interface is modified upon partner (-DNA or coactivator) interactions. For this, we will elucidate the molecular architecture by cross-linking – Mass Spectrometry (XLMS).
**Methodology** : Cross-linking relies on the introduction of a covalent bond between functional groups of amino acids that are nearby in space. Cross-linking has been performed on Retinoid Acid Receptor/Retinoid X Receptor. Taking advantage from our experience, bis[sulfosuccinimidyl]suberate (BS3) has first been used for performing the cross-linking reaction. The reaction conditions were optimized to minimize perturbation on structure and the cross-linking rate was followed by MALDI equipped with a high mass detector. A bottom-up approach was then used to look for the cross-linked peptides and establish a linkage map (through identification of the spatially close lysines).
**Results** : Although salt conditions required for the protein complex stability interferred with our protocol, few inter-protein cross-links could be assigned. We will now perform integrative modeling from a synergetic point of view. While cross-linking data can be employed to guide computational protein structure refinement, modeling will help for the optimization of the most appropriate cross-linkers.

*Speaker

# [P092] Identification of marker peptides in Tuna species with Isobaric Labelling

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The aim of this project is to identify and characterize valid peptide biomarkers for the differentiation of Tuna species. Marine food and especially Tuna species are often affected by adulteration and mislabelling due to price differences and limited availability. As methods like polymerase chain reaction (PCR) and isoelectric focusing (IEF) are not sufficient for species differentiation, mass spectrometric methods are becoming of significant interest.
For this project, the identification of marker peptides by labelling with isobaric Tandem Mass Tags (TMT) is implemented. TMT reagents are differently labelled stable isotopes which consist of an amino reactive group, a mass normalizer and a reporter ion group with a specific m/z-ratio which is generated during the MS/MS fragmentation. The main advantage of this approach is the simultaneous analysis of two or more Tuna species.
Using isobaric labelling, the peptides of each Tuna species are labelled with a different TMT reagent first and then combined. During the separation using HPLC, identical peptides of all combined Tuna species elute together and during MS/MS fragmentation of the labelled peptides, the peptide fragment ions and the specific reporter ions of each Tuna species are obtained. As each Tuna species is labelled with different TMT reagents, the reporter ions can be used to identify peptides which only occur in one Tuna species and therefore can be used as a specific peptide marker.

*Speaker

# [P093] Charge dependence in peptide and protein sequencing using UVPD at 213 nm

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Tandem mass spectrometry (MS) is a powerful tool for the structural investigation of peptides and proteins. Collision-based tandem MS methods such as collision-induced dissociation (CID) or high energy collisional dissociation (HCD) are routinely used to fragment peptides/proteins in proteomics. Fragment ions in these experiments are mainly formed after redistributing the energy acquired in gas-phase collisions, into the vibrational heat bath of the activated analyte molecules, yielding thermodynamically/kinetically controlled fragmentation. However, sequence scrambling, loss of labile groups, internal fragmentation or inefficient fragmentation of protein ions can complicate sequence determination with collision-based tandem MS methods.

Ultraviolet photodissociation (UVPD) tandem MS has been shown to outperform CID/HCD in terms of sequence coverage in proteomics studies. This is mainly due to the high activation energies per absorbed UV photon and short dissociation times, minimizing a loss of labile groups, homolytic bond cleavages or internal fragmentation. The photoproduct yield (PY) in UVPD, however, is inherently limited by the availability of chromophores, resulting in typically lower fragmentation efficiencies in UVPD compared to CID/HCD.

We investigated the dependence of UVPD PYs of peptides/proteins as a function of ion charge. A home-built 213 nm UVPD tandem MS setup, together with supercharging electrospray ionization was used to dissociate MRFA, angiotensin II, bradykinin, lysozyme and ubiquitin as a function of ion charge. First results show that UVPD PYs are increased, especially for supercharged peptides/proteins compared to low charge states. Hence, this method offers an easy means to increase PYs in UVPD for peptide/protein sequencing.

*Speaker

# [P094] Standardizing and harmonizing multiple TripleTOF® systems for DDA and DIA using a dedicated Performance Kit

Joerg Dojahn * [1], Nicholas Morrice [2], Christie Hunter [3], Robert Graham [4], Brazzatti Julie [4], Whetton Tony [4]

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**Background:** To get consistently high quality, reproducible quantitative proteomics data, proper controls are required. This is particularly important as the proteomics field begins to embrace larger and larger studies where the integrity of the entire workflow must be ensured over many samples, multiple instruments, multiple users, and over long periods of time. A validated kit has been assembled to assess the performance of the TripleTOF® systems for IDA and SWATH® proteomics workflows to aid in performance monitoring and instrument harmonization.
**Method:** A series of standards and tests have been developed to test the performance of the various LC-MS modes. A set of 20 synthetic peptides of varied mass and retention time attributes has been developed for use as an infusion and LC-MS standard. Once confirmed, a standard complex tryptic digest (human) is used to test the performance of the instrument in both IDA and SWATH modes.
**Conclusions:** The Stoller Biomarker Centre in Manchester has eight TripleTOF® 6600 systems for IDA and SWATH analysis operating in microflow mode to help discover novel biomarkers for many different diseases. The eight systems were benchmarked using the newly developed kit in microflow after the systems were installed and also after 6 months of operation. The SWATH analyses showed good instrument harmonization, with only a 5% variation in the number of proteins and 12% variation in the number of peptides quantified across all eight machines. With the current setup over 20 samples can be run per day per instrument.

*Speaker

# [P095] Study of Manganese Superoxide Dismutase Mimic Properties and its Effect on Cellular Model of Inflammatory Bowel Diseases by Mass Spectrometry and Proteomics

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Superoxide Dismutases are metalloenzymes involved in the cellular antioxidant protection pathway controlling reactive oxygen species. Antioxidant defenses, and SODs in particular, are weakened in epithelial cells from inflammatory bowel diseases, leading to an increase in ROS. The manganese complex Mn1, mimicking SOD, is a promising metallodrug against IBDs. It has an intracellular anti-superoxide effect in activated macrophages and an intracellular anti-inflammatory activity in intestinal LPS-stressed epithelial cells, model of oxidative stress and inflammation. Mn1 remains at least partially coordinated with diffuse distribution over the whole cell.

Taking a step further, we investigate the effect of Mn1 in the same cellular model on the protein level, using bottom-up redox proteomics. By using a modified SILAC (Stable Isotope Labeling by Amino acids in Cell culture) strategy, we quantify the changes in protein expression, as well as the oxidation state of cysteines, the main targets of protein oxidation.

In parallel, in order to better understand the metabolization and speciation of Mn1 inside cells, we study the properties of the complex by mass spectrometry. To do so, we characterized the MS/MS fragmentation spectrum of Mn1 and determined the exact mass of isotopes by ultra high resolution. We also studied its behavior in hydrophilic interaction chromatography. We develop a method of separation and detection of SOD mimic metal complexes in cell lysates in a metal-free environment. We finally investigated the intracellular speciation of Mn1, found its signature in cell lysates and quantified the overall manganese content.

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# [P096] Comparison of precursor and fragment based label-free protein quantification methods using pre-defined protein fold changes

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Label-free protein quantification is the most frequently used quantification strategy in proteomics, but is commonly performed on the precursor level in data dependent acquisition (DDA). Recent developments in data independent acquisition (DIA) enabled large scale fragment based label-free quantification. Several studies already showed higher sensitivity using fragment information but the quantification accuracy has not been assessed.
We generated samples with defined fold changes by spiking human cell lysate digests with 5% and 10% (1:2 ratio) of a Saccharomyces Cerevisiae digest and with 15% and 10% (1.5:1 ratio) of an Escherichia Coli digest. Technical triplicates were analysed in DDA, fixed window DIA and WiSIM DIA mode on a Fusion Orbitrap and in variable Window SWATH mode on a 6600 TripleTOF.

DDA results were searched with MaxQuant in label-free quantification (LFQ) mode against a combined database of human, S. Cerevisiae and E. Coli sequences. The output file was also used as a spectral library in Skyline for DDA mode precursor based and DIA mode fragment based data extraction. DDA mode Precursor based extraction with both software resulted in approximately 50% overestimation of the expected fold changes. Fragment based precursor extraction approach used in WiSIM performed better but also overestimated the fold changes (_˜30%) and had more false positive results. Best performed fixed window DIA and variable window SWATH with the closed estimation of fold change (_˜10% higher) and least number of false positives.
DIA mode fragment based quantification estimates more accurately protein fold changes then conventionally used DDA mode precursor based quantification.

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# [P097] Structure Identification and Affinity Characterization of Soybean Agglutinin-Carbohydrate Interaction Epitopes using Proteolytic affinity- mass spectrometry (PROTEX-MS)

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**Introduction**

The study of lectin–carbohydrate interactions is particularly challenging because of the structural complexity of glycans, the permissiveness and multiplexity of lectins, and the relatively low affinities involved. Previously, proteolytic affinity – mass spectrometry has been successfully employed as a molecular approach for identifying carbohydrate-binding peptides and to analyze glycan–protein interactions, thus motivating experiments with native soybean agglutinin, a lectin resistant to digestive enzymes in the GI tract and with the unique property to bind to several carbohydrate-containing molecules.

**Experimental**

As an essential first step for to the identification of carbohydrate binding sites, the primary structure of Soybean Agglutinin was characterized by proteolytic peptide mapping/ mass spectrometry. Galactose and N-acetylgalactosamine (GalNAc) were then immobilized on a gold surface chip, respectively, through divinyl sulfone (DVS) activation and through a tailor-made glycoprobe peptide displaying glycans. The affinities of intact SBA with Galactose and GalNAc were then assessed by surface acoustic wave biosensor analysis (SAW). Proteolytic extraction has then been performed by using these glycan-displaying gold surfaces and DVS-activated Sepharose as an affinity column for the capture of the epitopes peptides.

**Results and discussion**

Binding affinities in the micromolar range were obtained, as expected for lectin-carbohydrate interactions. Several peptides including the key amino-acids Asp88, Ala105, Asn130 and Leu214, involved in the SBA-sugar complex, were identified. These results provide insights for a rapid molecular approach to decipher glycan–protein interactions.

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**Novel aspects**
Glycan-displaying gold surfaces combining kinetic and structural information of lectin-carbohydrate interaction.

# [P098] Assessing the Hydrophobicity of Amino Acids via Ion Mobility – Mass Spectrometry

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During electrospray ionization (ESI), amino acids assemble into large metastable clusters that can be transferred intact into the gas phase. These clusters have been extensively studied using ion mobility-mass spectrometry (IM-MS), a method, which provides information about the ions' size by measuring its rotationally-averaged collision cross-section (CCS). Recently, IM-MS exemplarily showed for a few amino acids that cluster formation strongly depends on the polarity of the molecule's side-chain. Hydrophobic amino acids form more extended structures than the predicted theoretical isotropic growth of an idealized spherical assembly ($=\sigma 1 n \hat{} (2/3)$; with $\sigma 1$ being the monomer CCS and $n$ the cluster size number). Polar amino acids, on the other hand, assemble into more compact oligomers.
In this study, we used IM-MS to systematically investigate cluster formation of all canonical amino acids and implemented a new correction factor $\alpha$ to account for the deviation from the theoretical isotropic growth ($=\sigma 1 n \hat{} (2/3) \alpha \hat{} (2/3)$). The correction factor $\alpha$ reflects the hydrophobicity of each side chain and is used to implement a new hydrophobicity scale for amino acids. A comparison to already established hydrophobicity scales showed in general a good agreement. However, compared to other scales our new method does not depend on instrumental parameters such as chromatographic columns, gradients, temperature, or derivatization. As such the here-presented approach has the advantage that it is based on an intrinsic property and thus allows a more reliable comparison and classification of amino acids.

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# [P099] Towards higher sensitivity in targeted proteomics using nanoLC columns with a reduced inner diameter.

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**Introduction:** nanoHPLC is a frequently employed separation techniques for the sensitive detection of peptides in bottom-up proteomics. Columns of ≥75 $\mu$m inner diameter (ID) allow for the detection down to the amol range. However, sensitivity is still not sufficient to target the vast of low abundant proteins in a complex biological sample. Here we present a systematic comparison of a 50 $\mu$m and a corresponding 75 $\mu$m reversed phase column for targeted analysis of peptides in proteolytic digests towards an improved coverage of low abundant peptides.**Experimental:** nanoLC-MS was performed using an Ultimate RSLCnano online-coupled to an LTQ Orbitrap Velos Pro operated in PRM mode. Samples were loaded onto an Acclaim PepMap pre-column (75 $\mu$m x 2 cm, 5 $\mu$m, 100 A) in 0.1 % TFA at flowrate of 10 $\mu$L/min. Separation was conducted using a linear ACN gradient in the presence of 0.1 % FA using an Acclaim PepMAP main column with 50 $\mu$m or 75 $\mu$m x 50 cm, 2 $\mu$m, 100 A using a flow rate of 100 nL/min and 250 nL/min, respectively. **Preliminary Results:** Using the 50 $\mu$m column, targeted detection of 11 stable isotope labeled-peptides (30 amol – 30 fmol) spiked into 750 ng HeLa digest revealed on average a 2-fold improvement in intensity. Hence, reduction of the inner diameter improves the sensitivity towards the detection of low abundant proteins.

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European Mass Spectrometry Conference

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# Thurday, 15 March

# Thurday Morning
# Plenary Lectures

Ambient Laser Desorption Ionization – instrumentation and applications

**Zoltan Takats, Imperial College London**

Prof. Zoltan Takats has obtained his M.Sc. degree in Chemistry from the Eotvos Lorand University, Budapest, in 1998. He has carried out his Ph.D. research at the Chemical Research Centre of the Hungarian Academy of Sciences and at Purdue University. He has obtained his Ph.D.degree in analytical chemistry in 2003. He has been doing mass spectrometry-related research for more than 15 years, with a primary focus on the development of novel atmospheric pressure ionization methods. He is the primary inventor of electrosonic spray ionization, desorption electrospray ionization, jet desorption ionization and rapid evaporative ionization mass spectrometry methods. Besides pursuing a scientific career, he has been deeply involved in the introduction of mass spectrometry-based neonatal screening programme in Hungary and served as the head of one of the national screening laboratories. Following a couple of years spent at Justus-Liebig-Universität in Gießen, Germany, he currently works as Professor of Analytical Chemistry at Department of Surgery and Cancer, Faculty of Medicine, Imperial College London. Present research interests include the application of ambient ionization methods in surgical metabonomics and development of mass spectrometric imaging techniques for the rapid phenotyping of cancer patients.

## Abstract:

Laser desorption ionization was introduced in the early 1970s and was subsequently used for MS imaging. In contrast to the versatility of the approach, the LAMMA (laser microprobe mass analyser) technique did not become popular in the broader analytical community and the original laser desorption was quickly superseded by the significantly more sensitive MALDI which also allowed the analysis of arbitrary molecular species regardless of their chemical nature or molecular weight.

Atmospheric pressure laser desorption ionization received attention from 2010 as a potential method for the in-vivo analysis of biological tissues, as an alternative (or alternative way of implementation) for the rapid evaporative ionization mass spectrometry (REIMS). The electric current used for tissue aerosolization in case of REIMS exerts a purely thermal effect on tissues, which can also be achieved by low-fluence infrared laser irradiation. The inherent problems with ambient laser desorption ionization are identical to those with REIMS; low sensitivity and contamination of the instrument by large aerosol particles. These phenomena can also be perceived that the ALDI process generates an aerosol with sub-optimal particle size distribution and majority of analyte molecules end up as contaminants instead of undergoing ionization.

In order to enhance ionization efficiency and minimize the precipitation of organic debris in the ion optics, we have devised a special atmospheric interface setup featuring a heated declustering element. The heated metal part is positioned in the free jet expansion region

following the first conductance limit of the atmospheric interface. Our hypothesis was that particles accelerated by the free jet expansion will undergo surface induced dissociation, which is further enhanced by the heat transfer between the heated surface and the particles. The novel atmospheric interface setup was tested using model aerosols and aerosols obtained by the laser ablation of tissues using 337 nm UV laser and 2.94 μm and 10.6μm wavelength infrared lasers for ablation. The concept solved instrument contamination problems and provided a factor of 20-50 signal enhancement. The dramatic ionization efficiency difference observed between model aerosols and aerosols obtained by tissue ablation suggested that the analyte (largely complex lipid) content of tissue originated aerosols exceeded the upper limit of the dynamic range of the method, which hypothesis was supported by calibration experiments. In order to further enhance the ionization efficiency, we have developed an atmospheric interface setup which mixes organic solvent with the sample aerosol, resulting in significantly lower in-droplet concentration. Various organic solvents were tested and significant signal enhancement was found in case of aliphatic alcohols. Infusion of isopropanol into the interface gave an additional 20 times enhancement and also provided a means to infuse internal calibrants into the instrument.

The system comprising a $CO_2$ laser has successfully been tested for the analysis of bacterial cells, human cell cultures and human tissues. The novel setup gives comparable results to electrosurgical REIMS, with 3-5x better signal-to-noise ratios, resulting in better classification performance.

# Mass Spectrometry in Forensics

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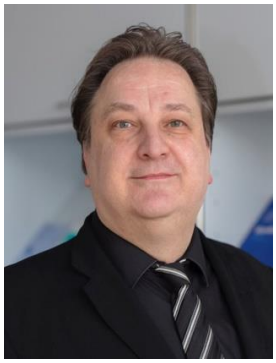

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**Abstract:**

According to The American Board of Forensic Toxicology (ABFT), forensic pharmacology and toxicology encompasses the measurement of alcohol, drugs of abuse, prescription drugs and other substances in biological specimens and interpretation of such results in a medico-legal context. While Sherlock Holmes did not need more than a magnifying glass to solve cases, highly sophisticated mass spectrometric equipment is a prerequisite for successful forensic casework in the modern forensic lab. Confirmation of immunoassay results is routinely done by simple hyphenated MS techniques. However, more sophisticated MS techniques are needed for the very specific problems in forensic routine which are unknown to the normal user of mass spectrometry: Postmortem samples which can be anything from fresh to severely putrefied can cause surprising matrix effects. Sometimes only alternative matrices are available (tissues, organs, hair, nails, oral fluid, respiratory air). In the last years, hundreds of new psychoactive substances (NPS) have entered the market, for which reference standards are rarely available. Mass spectrometry has been used to encounter all these problems. Postmortem tissue samples are investigated by MALDI-MS imaging (MALDI MSI) techniques using multiple reaction monitoring mode for screening and MS/MS or $MS^3$ for confirmation. Consumption behavior of illegal drugs can be monitored in single hair using MALDI MSI or even TOF SIMS. Intake of drugs or medicaments can be detected even in exhaled air employing the most sensitive LC-MS/MS equipment. High resolution mass spectrometry allows for identification of new drugs. Finally, simultaneous QUAL/QUAN analysis using liquid chromatography high resolution MS and employing new Data Independent Acquisition (DIA) approaches (MS/MSALL with SWATH Acquisition) will make forensic toxicology fit for future. Finally, combination of LC-MS/MS and GC-MS/MS methods will open the world of metabolomics and proteomics for forensic purposes.

# Proteomic, PTM and structural biology 3

# [Keynote] Site-specific N-glycosylation analysis of the HIV-1 glycan shield

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The HIV-1 envelope glycoprotein trimer is covered by an array of N-linked glycans that shield it from immune surveillance. The high density of glycans on the trimer surface imposes steric constraints limiting the actions of glycan processing enzymes, such that multiple under-processed structures remain on specific areas. These oligomannose glycans are recognized by broadly neutralizing antibodies (bNAbs) that are not thwarted by the glycan shield but, paradoxically, target it.

We performed quantitative, site-specific N-glycosylation analysis of a soluble, recombinant trimer (BG505 SOSIP.664) using a combination of ion-mobility mass spectrometry, MALDI-TOF and in-line electrospray mass spectrometry on glycans as well as glycopeptides resulting from protease digestions. This orthogonal approach allowed us to confidently quantify the N-glycosylation composition of individual glycan sites and displays the first quantitative site-specific N-glycan study on a native-like Env trimer.

Our analysis allows us to map the extremes of simplicity and diversity of glycan processing at individual sites and reveals a mosaic of dense clusters of oligomannose glycans on the outer domain of Env. We confirm the remarkable overall dominance of oligomannose-type glycans and reveal a mosaic of glycan microclusters bearing under-processed glycans. The new knowledge we present on the site-specific glycan composition of bNAb epitopes should inform us about the types of immunological response that Env vaccine candidates may need to induce.

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# [O1] Displacement Chromatography Mode – High protein sequence coverages and identification rates for low $\mu$g-range proteomics using online 2D-LC-MS

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The complexity of proteomes is still a challenge in bottom-up proteomics. Deep proteome profiling generally requires large initial protein amount and multi-dimensional separation strategies. Here, we report on an online 2D-LC-MS/MS approach applying strong cation exchange chromatography (SCX) in displacement chromatography mode (DCM) as the first separation dimension. This method enables comprehensive proteome profiling of low starting amounts of a few $\mu$g (5 $\mu$g). The first detailed comparison of DCM with conventionally used gradient chromatography mode (GCM) highlights a significantly better separation efficiency in DCM. Especially for peptides with a net-charge state (NCS) of +2, which represents the majority of tryptic peptides in mammalian proteomes, DCM provides a significantly better separation. These peptides were separated over several fractions, which is not possible to achieve in GCM due to theirs low affinity towards the SCX column. The better separation in DCM results not only in a considerably higher reproducibility (Pearson's r=0.93), but significantly increases identification rates of both peptides (2.6-fold) and proteins (1.7-fold). The better separation and identification rates in DCM results in a significantly higher number of identified unique peptides per protein and protein sequence coverages, thus providing more reliable quantitative results. The higher sequence coverage are beneficial for applications such as proteogenomics, where it is important to have high protein sequence coverages to identify single amino acid variants and splice-junction peptides.

*Speaker

# [O2] Protein and proteoform profiling of saw-scaled viper, Echis carinatus, venom using a top-down approach

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Identification and characterization of venom-based components continue to play an essential role in human health, either in drug discovery or in the treatment of envenomation. Venomous snakes, in particular, include the largest of venomous animals, their venoms being deadly cocktails, each comprising of unique mixtures of peptides and proteins with incredible specificities. The combination of different chromatographic separation methods with high-resolution, accurate-mass (HRAM) mass spectrometry platforms is essential to get a precise depiction of the astonishing complexity of venoms. The most widely used technique for venom analysis is bottom-up proteomics, where enzymatically digested venom components are subjected to tandem mass spectrometry for *de novo* peptide sequencing. However, the major challenge for the bottom-up protein identification is a limited sequence coverage, which results in the loss of information about proteoforms (genetic variations and post-translational modifications). Here, we report top-down venomics of the medically significant saw-scaled viper, *Echis carinatus*, which is responsible for the most snakebite envenomation mortality and morbidity worldwide. Native and reduced forms of proteins were separated and identified using a hybrid quadrupole orbital trapping mass spectrometry (Q Exactive HF-X, Thermo Scientific GmbH, Bremen, Germany). We identified more than 120 proteins and peptides in intact mode from different protein families such as snake venom metalloproteinases (SVMP), L-amino acid oxidases (LAAO), phospholipase A2 (PLA2), and cysteine-rich secretory proteins (CRISPs) with multiproteoform complexes plus proteolytic cleavage peptides (bradykinin-potentiating peptide and SVMP-inhibitors).

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# [O3] Cross-Linking and Mass Spectrometry Give Insights into the bMunc13-2/Calmodulin Interaction

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bMunc13-2 is a brain-located isoform of the Munc13 protein family, which is essential for vesicle priming in synaptic short-term plasticity. During synaptic vesicle priming, bMunc13-2 is regulated by the highly conserved Ca2+-binding protein calmodulin (CaM). The complex between CaM and bMunc13-2 is involved in the formation of the SNARE complex that drives synaptic vesicle fusion with the membrane. So far, only sparse information on the CaM/bMunc13-2 interaction is available and complex conformation had been exclusively observed with short bMunc13-2 peptides comprising the proposed CaM binding sites. Hence, a bMunc13-2 domain (aa 367-780) was employed for cross-linking and native MS experiments to obtain more detailed structural information on the CaM/bMunc13-2 complex. Cross-linking experiments between CaM and the bMunc13-2(367-780) domain were based on the incorporation of photomethionine as well as on a variety of chemical cross-linkers. Cross-linked amino acids were identified by LC/MS/MS (Orbitrap Fusion Tribrid, Thermo Fisher Scientific). Cross-linking/MS revealed an interaction between both proteins and confirmed the expected 1:1 stoichiometry of the bMunc13-2/CaM complex, which was confirmed by native MS (High Mass QTOF 2, Waters Micromass). For gaining more detailed insights into the CaM/bMunc13-2 interaction, a bMunc13-2(704-742) peptide containing the C-terminal CaM binding site was docked with CaM by Rosetta. The resulting models of the CaM/bMunc13-2 interaction displayed an $\alpha$-helical structure of the bMunc13-2 peptide, interrupted by a loop allowing a slight flexibility of the peptide's N-terminus. Additional cross-linking/MS and isothermal titration calorimetry measurements with a bMunc13-2(703-742) peptide verified the proposed interaction.

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# Ionization

# [Keynote] Mass spectrometric analysis of disease triggering amyloids: combining ESI-IMS and LILBID-MS

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Spontaneous aggregation of proteins can be the reason for a diversity of diseases. Prime examples are e.g. Alzheimer's Disease (AD) and Parkinson. The proteins being involved in those disease (beta-amyloid (A$\beta$42) and $\alpha$-Synuclein, respectively) show the ability to spontaneously aggregate via oligomers through to fibrils. Investigations revealed the oligomers as the toxic species in the emergence of those diseases. Mass spectrometry (MS) is well-suited to investigate the evolvement of oligomers of those amyloidogenic proteins. It is a very useful tool to analyze the kinetics of the oligomerization process. Combining MS with ion mobility spectrometry (IMS) can additionally correlate structural changes of those peptides to the oligomerization process.
Laser-induced-liquid-bead-ion-desorption (LILBID) MS is an ionization technique which has the ability to conserve non-covalent interactions; even those challenging to observe otherwise as the oligomers of A$\beta$42 up to the 16mer. Therefore, it is possible to use LILBID to study the kinetics of the oligomerization by time-resolved measurements and the effect of molecules which influence the oligomerization. Additionally, electrospray-ionization (ESI) combined with IMS makes it possible to correlate the oligomerization and the impact of influencing molecules to structural changes of the amyloid peptide.
We present the differences, advantages and disadvantages of both methods for studying amyloids. By combining the advantages it is possible to analyze oligomerization, ligand binding and structural changes in dependence of different ligands for a better understanding of the amyloid's behavior.

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# [O1] Silicon nanostructures for versatile SALDI-MS peptide analyses

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Surface Assisted Laser Desorption Ionization-Mass Spectrometry (SALDI-MS) represents a very attractive alternative to conventional MALDI technology for the analyses of small organic compounds and biomolecules as demonstrated by the variety of inert surfaces have been investigated during the last decade.Up to now, disposable ready-to-use target plates (MassPrep DIOSTM, Quick Mass, NALDI and more recently REDIChip) can be purchased for SALDI analysis of small molecules. In order to overcome the limitations encountered with the use of such probes (surface pollution affecting detection sensitivity and reproducibility, purchase of a specific target holder, overall cost), we explored various silicon-based materials exhibiting different physico-chemical properties. Both material architecture (amorphous powders, nanostructured particles and surfaces as well as ordered three-dimensional constructions) and material hydrophobic character tuned by specific chemical derivatization were probed as crucial parameters for achieving efficient and robust detection. An home-made array of model peptides covering a wide structural and mass diversity allowed comparing the performances of all investigated silicon-based supports (peptide detection sensitivity, reproducibility/repeatability, method robustness (easy sample preparation, need for ionization additives, straightforward sample deposit depending on material wetting properties), ionization source tuning using MALDI-TOF/TOF instruments). Finally, we further performed surface functionalization to broaden the scope of applications. MALDI matrix grafting and peptide immobilization were achieved. For the latter, the introduction of an analytical construct bearing a photolinker permitted both covalently anchoring the chosen model peptide and its subsequent photorelease offering an adaptable LDI-platform able to capture/enrich targeted peptides from complex biological samples prior to their sensitive detection and characterization.

*Speaker

# [O2] Combination of 2,5-Dihydroxybenzoic acid and 2,5-Dihydroxyacetophenone Matrices for unequivocal assignment of Phosphatidylethanolamine Species in complex Mixtures

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Unequivocal assignment of phospholipid peaks in complex mixtures is difficult if only the $m/z$ values but no tandem mass spectrometry (MS/MS) data are available. This is usually the case for matrix-assisted laser/desorption ionization time-of-flight (MALDI-TOF) MS imaging experiments. The situation is even more complex since many matrices induce the loss of a methyl group from phosphatidylcholine (PC) and thus render it detectable as negative ion.
Selected lipid mixtures of known compositions were investigated by negative ion MALDI-TOF MS. In addition to established matrices such as 2,5-dihydroxybenzoic acid (DHB) and 9-aminoacridine (9-AA), different binary matrices, including 2,5-dihydroxyacetophenone (2,5-DHAP) as matrix additive to DHB, were tested to probe their performance in both ionization modes. Beside artificial PC and phosphatidylethanolamine (PE) mixtures of known compositions, egg yolk and liver extracts were selected as biologically relevant systems.
The majority of the herein used binary MALDI matrices lead to the loss of a methyl group from PC in the negative ion mode, which makes the clear identification of PE species ambiguous. However, this problem does not apply if a mixture of DHB and 2,5-DHAP is used. Therefore, the application of DHB / 2,5-DHAP as matrix is a simple method to unequivocally identify PEs even in complex mixtures as negative ions and without the necessity to separate the individual lipid classes prior to MS detection.

*Speaker

# [O3] Direct Ionization and Reaction Monitoring via Acoustic Levitation Ambient Mass Spectrometry

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Acoustic (ultrasonic) levitation, first described in 1933 by B´ucks & M´uller [*Z. Phys.* (1933) 84:75-86] for levitation of small samples, emerged as the most practical method of object levitation. Most liquid samples can easily be levitated if they exhibit sufficient specific density ($\rho$s) and surface tension ($\sigma$s). In our previous work employing DART-MS [E. Crawford *et al. Anal. Chem.* (2016) 88: 8396-8403], it was determined that the heated ionizing gas beam of the DART source could not penetrate the acoustic field of the levitator in order to interact with the sample. Solvent assisted inlet ionization (SAII), a method of ambient ionization for liquid samples occurring directly in the inlet of a mass spectrometer, employs by design a fused silica capillary tube to transfer liquid sample to the mass spectrometer. The fused silica tubing proved optimal for directly extracting droplets from the levitator and simultaneously acted as the ionization transfer tube. In this work, SAII was coupled for the first time to an ion trap mass spectrometer. Optimization of the SAII interfacing was critical for ionization performance and external resistive heating of the capillary inlet showed overall improved analyte signal intensities, as also reported for other commercial mass spectrometer platforms with heated capillaries or customized heated capillary extenders. In addition, positioning of the fused silica capillary in the inlet was key to the ionization efficiency. A derivatization reaction of cholest-4-en-3-one with Gerard's Reagent T consisting of a 6 $\mu$L total reaction volume was compared with bulk (600 $\mu$L) reactions.

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# Imaging mass spectrometry 2

# [Keynote] Fourier Transform Infrared Microscopy guided Mass Spectrometry Imaging of Tissue Morphologies

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Modern preclinical research and clinical practice have been substantially advanced by multimodality imaging that combines multiple sensor types. In this context, matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) has been recognized as a valuable technique to deliver chemically specific distribution patterns of hundreds of biomolecules. For the interpretation of multimodality analyses, most studies so far have focused on multimodal image registration and data fusion which in case of highly dimensional data (like data generated by MALDI-MSI) substantially increases acquisition time and data load. Here, we present an integrated multimodality workflow that combines FTIR microscopy and MALDI-MSI. Based on heterogeneous vibrational bands in mid infrared spectra, deviating cell populations can be identified and used to automatically target MSI acquisition and interpretation to predetermined tissue morphologies, thus circumventing the need for histopathological stains. Moreover, the presented pipeline enables automated, spatially aware retrieval of molecular MSI signatures, as well as a drastic decrease in data load and acquisition time. We exemplify the general applicability of this integrated workflow by unsupervised tumor targeting and marker extraction in CD1 *nu/nu* mice engrafted with U87 glioma tumor cells, Niemann-Pick type C1 I1061T knock-in mice and primary human gastrointestinal stroma tumors and.

*Speaker

# [O1] Towards Higher Sensitivity in MALDI-FTICR Imaging of CNS Drugs Using a Matrix Matching Unique Analyte Properties

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Tissue-specific ion suppression is a form of matrix effect that cannot be entirely avoided in MALDI imaging mass spectrometry since no separation step can be implemented prior to the ionization process. While its negative impact on precision and accuracy in quantitative MALDI-MSI can be diminished (to some extent) by applying isotope internal standards for normalization and matrix-matched calibration routines, the detection capabilities still suffer from this phenomenon, often resulting in significant loss of response for the investigated analytes. One of the MSI applications considerably affected by this phenomenon is the quantitative spatial analysis of CNS drugs. Most of these drugs are low molecular weight, lipophilic compounds, which provide the additional challenge of efficient desorption and ionization using conventional polar acidic matrices (CHCA, DHB). Here, we present for the first time the application of the non-polar DCTB matrix (2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile) for high sensitivity imaging of CNS drugs in mouse brain sections. Since DCTB is usually considered an electron-transfer matrix, we provide a rationale (i.e. computational calculations of gas-phase PA and IE) for an additional proton-transfer ionization mechanism with this matrix. Furthermore, we compare the ionization suppression-caused signal decrease from seven different CNS drugs when employing DCTB *versus* CHCA. The results show a several times lower signal decrease with DCTB in comparison to CHCA and thus provide evidence for MALDI matrix-dependent tissue-specific ionization suppression. Finally, we present the application of DCTB matrix and ultra-high resolution Fourier-transform ion cyclotron resonance to quantitative MALDI imaging of anesthetic drugs (xylazine and ketamine) in mouse brain sections.

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# [O2] Evaluation of non-supervised MALDI MSI combined with microproteomics for determination of glioblastoma heterogeneity

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Gliomas represent 80% of all malignant cerebral tumors and are classified within different malignity grade. Glioblastoma, the most aggressive group, represent more than half of all gliomas but remain a heterogeneous group. Indeed, patient survival can reach from several months to a few years after surgery and chemotherapy.
To study gliomas, MALDI mass spectrometry imaging is an interesting technique, allowing the analysis of tumor heterogeneity. In this study, MALDI-MSI is coupled with spatially-resolved microproteomic within the objective to identify subgroups of glioblastomas patients in order to help diagnosis and prognostic.

At the present time, molecular images were realized on the 50 cases and microprotemic for 6/50 cases. Molecular images are generated from thin tissue section in order to determine the spatial localization of digested peptides. Thanks to unsupervised statistical analysis, we then generated hierarchical clustering of homogeneous molecular regions. According to these regions, microproteomic gave access to large scale protein identification and relative quantification.

Preliminary results show that several groups can be determined. Each group presents specific molecular signature and different median survival: 312 days (n=4), 463 days (n=7), 674.5 days (n=10) and 813 days (n=5). Microproteomic analyses show a panel of proteins specifically overexpressed in one region and associated with division cellular processes.
To conclude, the combination of MALDI-MSI and microproteomic provides new information on these tumors. In the future, these data will permit to build a more precise classification, a better medical care for patients and the identification of potential new therapeutic targets.

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# [O3] A 3-D image of cereal cell walls provided by MALDI MS imaging

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Cells of higher plants are surrounded by a complex composite architecture: the wall, which gives shape and mechanical resistance to the living plant. Mechanical properties of the wall are tuned at the molecular level by the composition and structure of wall components, which differ among plants and/or plant organs. In cereals grains, arabinoxylans (AX) and $\beta$-glucans (BG) are the main wall components. By determining wall properties, they significantly contribute to the quality of the grain for many economically important end-uses. Yet, AX and BG exhibit wide – and so far poorly understood – spatial and temporal structural variations. In that context, our group recently proposed an imaging method based on MS to monitor AX and BG structural heterogeneities in wheat endosperm sections. The originality of our contribution lied in tissue preparation, with an in-situ enzymatic degradation of the walls so to release MS-detectable oligosaccharides at the original location of the AX and BG polymers. In the current work, tissue preparation was improved to preserve the shape of fragile sections by minimizing the handling steps prior to MSI. A series of consecutive cuts sectioned from the brush to the germ of a mature wheat grain were imaged using this improved protocol. Benefiting from the latest developments of SCiLS Lab image analysis software, we then built the first three-dimensional MS image of a wheat grain. The 3D visualization allowed obtaining an overall and simultaneous view of the structural variations of AX and BG along both transversal and longitudinal axes of the grain.

*Speaker

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