DGMS
Deutsche Gesellschaft für Massenspektrometrie

isas Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.

Book of Abstracts

May 14 – May 17, 2023

Kongresszentrum Dortmund, Rheinlanddamm 200, 44139 Dortmund

Exhibitors

Gold Status

BRUKER

ThermoFisher SCIENTIFIC

Silver Status

SCIEX

Agilent Trusted Answers

SHIMADZU Excellence in Science

Bronze Status

Elemental Scientific

Promega

MSVision

PREOMICS

Waters™

EVOSEP

Syft™

Exhibitors

Exhibitor	Booth No.
Bruker Daltonics GmbH & Co. KG	4
Thermo Fisher Scientific GmbH	6
AB SCIEX Germany GmbH	8
Agilent Technologies Deutschland GmbH	10
Shimadzu Deutschland GmbH	5
ESI Elemental Service & Instruments GmbH	3
Promega GmbH	14
MS Vision	7
PreOmics GmbH	12
Waters GmbH	16
Evosep Aps	13
Syft Technologies GmbH	1
Merck KGaA	2
YMC Europe GmbH	15
ionBench	9
LNI Swissgas GmbH	17
Plasmion GmbH	11


15 YMC
2 Merck
9 IonBench
7 MS Vision
14 Promega
13 Evosep
12 PreOmics
3 ESI
17 LNI
Goldsaal Forum
16 Waters
8 AB Sciex
Catering
Catering
6 Thermo
Catering
Catering
4 Bruker
Catering
Catering
Rampe neu
5 Shimadzu
10 Agilent
1 Syft
11 Plasmion
Tür neu
KÜNSTLER
WC
KÜCHE
Projekt
Kongreßzentrum Westfalenhallen
Grundriss Erdgeschoß
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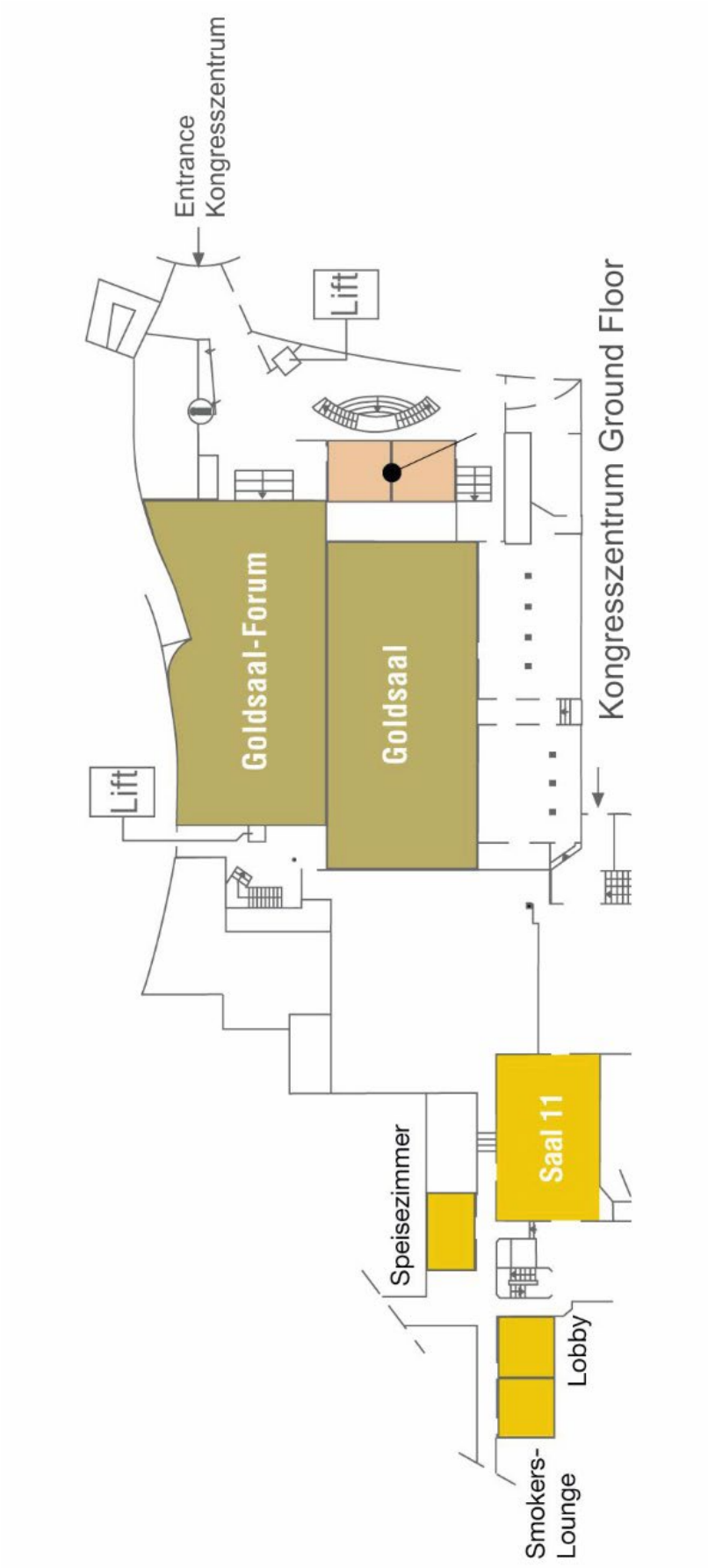
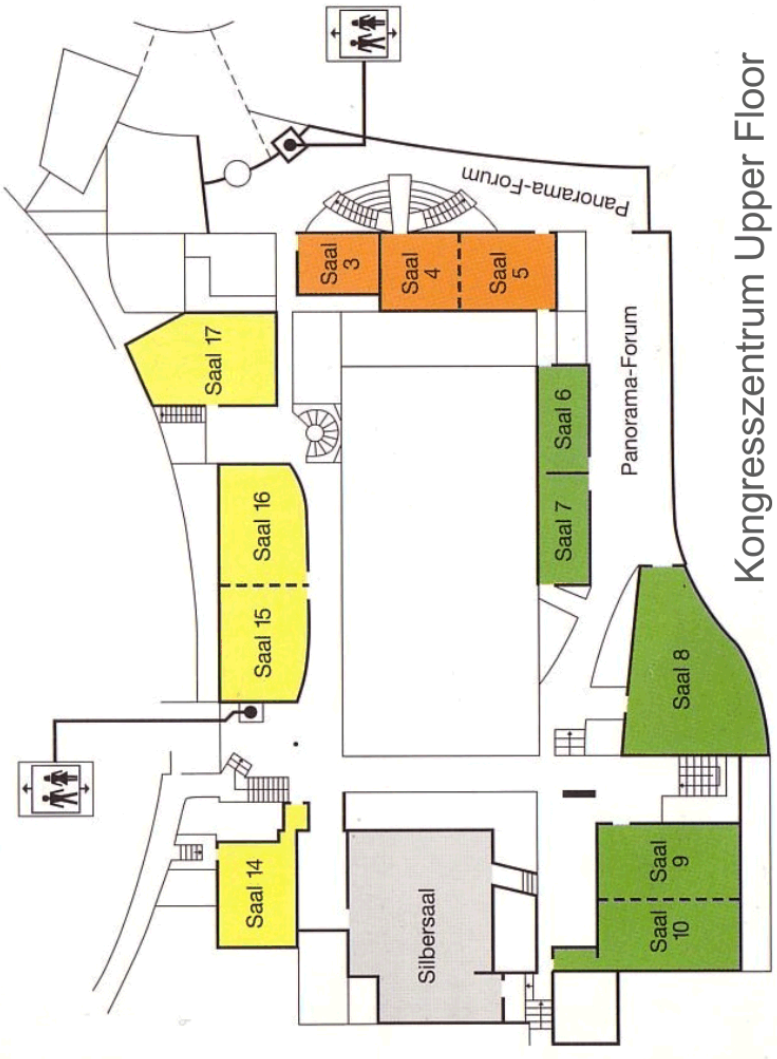
Format
DIN A3
Dateireferenznummer
npkz101.dwg
Westfalenhallen Dortmund

Venue – Site Plan

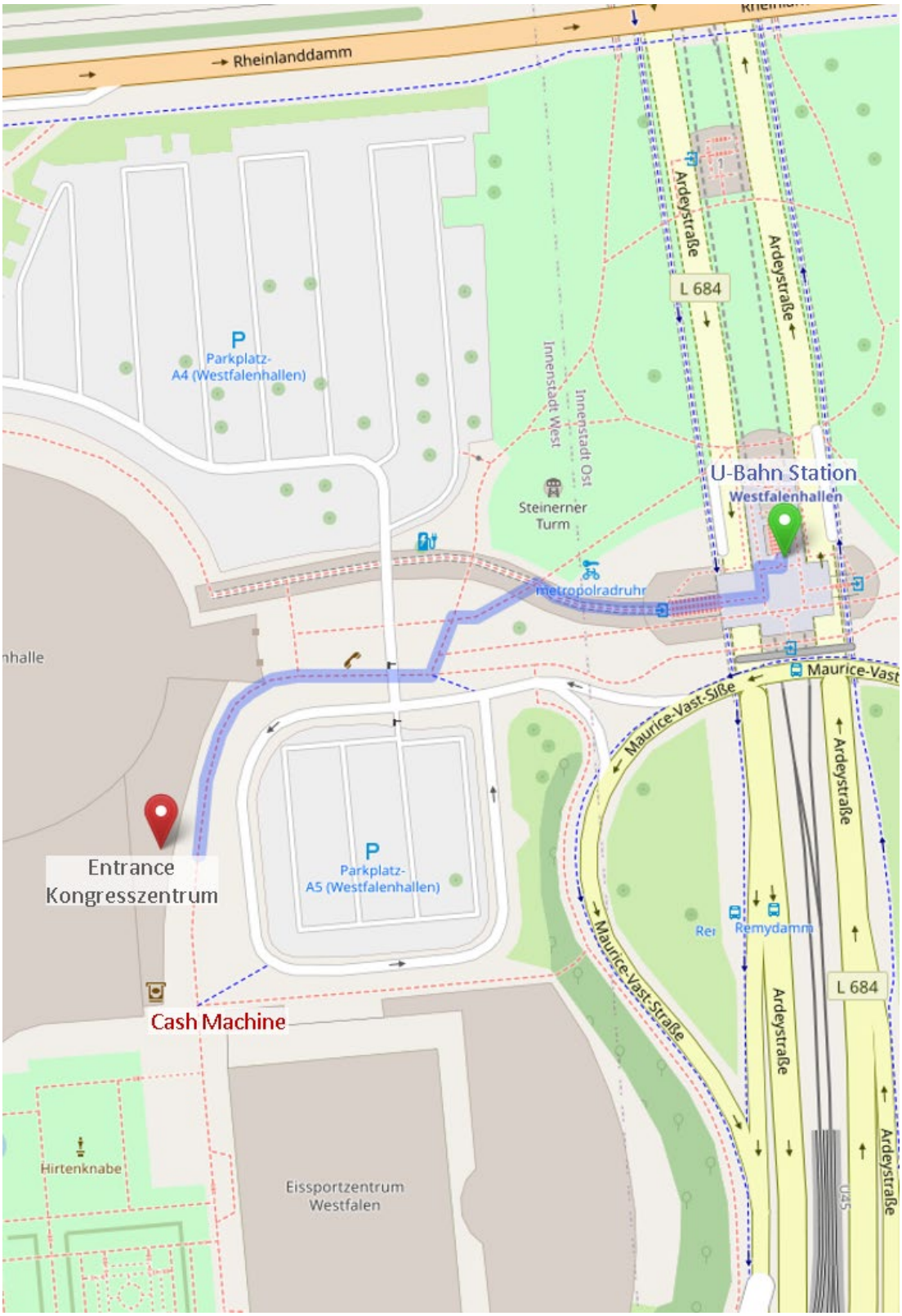


Venue – Site Plan



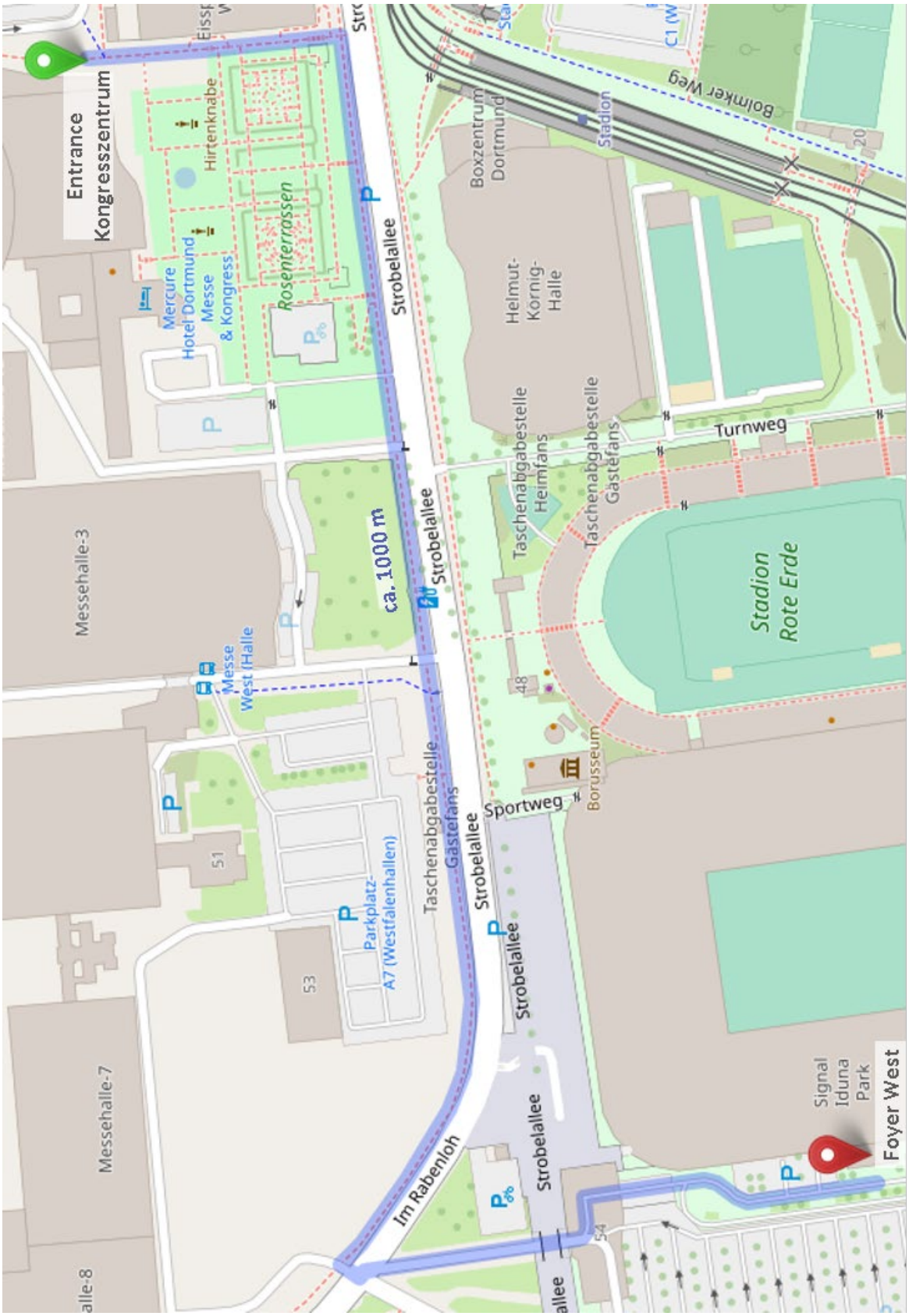
Directions to Kongresszentrum Dortmund

Rheinlanddamm 200, 44139 Dortmund



Directions to Conference Dinner

SIGNAL IDUNA PARK, Strobelallee 50, 44139 Dortmund



Program

Sunday, 14/May/2023

11:00am - 6:00pm	Registration Location: Entrance Foyer Goldsaal					
1:30pm - 2:30pm	DGMS Core Facility Meeting Location: Saal 4 Chair:					
1:30pm - 2:45pm	Skyline Workshop Part 1 Location: Saal 9	Alternative Ionisation Techniques Workshop Part 1 Location: ISAS City 3. OG	Lipidomics Workshop Part 1 Location: Silbersaal	Metrology in Mass Spectrometry - Workshop Part 1 Location: Saal 10	MS Imaging - Workshop Part 1 Location: Goldsaal	Proteome Discoverer - Workshop Part 1 Location: Saal 5
2:45pm - 3:00pm	Coffee Break Location: Foyer Goldsaal					
3:00pm - 4:00pm	**Element MS Member Meeting** Location: Saal 4 Chairs: **Jörg Bettmer, Daniel Pröfrock**					
3:00pm - 4:15pm	Skyline Workshop Part 2 Location: Saal 9	Alternative Ionisation Techniques Workshop Part 2 Location: ISAS City 3. OG	Lipidomics Workshop Part 2 Location: Silbersaal	Metrology in Mass Spectrometry - Workshop Part 2 Location: Saal 10	MS Imaging - Workshop Part 2 Location: Goldsaal	Proteome Discoverer - Workshop Part 2 Location: Saal 5
4:45pm - 5:00pm	Coffee Break Location: Foyer Goldsaal					
5:00pm - 5:15pm	Opening of DGMS 2023 Location: Goldsaal					
5:15pm - 6:00pm	Plenary Lecture I: Metin Tolan: "Shaken, not Stirred!" – James Bond in the Spotlight of Physics Location: Goldsaal Chair: **Albert Sickmann**					

6:00pm - 7:00pm	**Wolfgang Paul Lecture** Location: Goldsaal Chair: **Thorsten Benter**
7:00pm - 8:00pm	**Mattauch Herzog Award Session** Location: Goldsaal Chair: **Bernhard Spengler**
8:30pm - 10:00pm	**Get together** Location: Entrance Foyer Goldsaal

Monday, 15/May/2023

8:00am - 4:00pm	Registration Location: Entrance Foyer Goldsaal			
9:00am - 9:45am	Plenary Lecture II: Jörg Feldmann: ICP-MS as a contributing tool to non-target screening in environmental monitoring Location: Goldsaal Chair: **Johanna Irrgeher**			
9:45am - 11:00am	Wolfgang Paul Study Awards Session Location: Goldsaal Chair: **Michael Mormann**			
11:00am - 11:15am	Coffeebreak, Exhibition, Posters Location: Foyer Goldsaal			
11:15am - 12:45am	Session 1: Proteomics I Location: Goldsaal Chair: **Christoph Borchers**	Session 2: Lipidomics I Location: Silbersaal Chair: **Sven Heiles**	Session 3: Elemental Imaging Location: Saal 4+5 Chair: **Jörg Bettmer**	Session 4: Single Cell Application Location: Saal 9+10 Chair: **Theodore Alexandrov**
12:45pm - 2:15pm	Lunchbreak Location: Foyer Goldsaal	Lunch Seminar: Thermo Fisher Scientific GmbH Location: Goldsaal	Lunch Seminar: Shimadzu Deutschland GmbH Location: Silbersaal	Lunch Seminar: PreOmics GmbH Location: Saal 4+5

2:15pm - 3:45pm	**Session 5: Clinical mass spectrometry** Location: Goldsaal Chair: **Dirk Janasek**	**Session 6: Glycomics** Location: Saal 9 Chair: **Kevin Pagel**	**Session 7: ICP-MS : Nano- /Bioanalysis** Location: Saal 4+5 Chair: **Johanna Irrgeher**	**Session 8: Automation / Instrumen-tation I** Location: Silbersaal Chair: **Michael Lämmerhofer**	**Special Session: „In memoriam session in honor of Michael Przybylski“** Location: Saal 10 Chair: **Brindusa-Alina Petre** Chair: **Michael O. Glocker**
3:45pm - 4:00pm	**Coffeebreak, Exhibition, Posters** Location: Foyer Goldsaal				
4:00pm - 4:45pm	**Plenary Lecture III: Theodore Alexandrov: Spatial single-cell metabolomics reveals metabolic cell states** Location: Goldsaal Chair: **Bernhard Spengler**				
4:45pm - 5:45pm	**Element MS Board Meeting** Location: Silbersaal Chair: **Jörg Bettmer** Chair: **Daniel Pröfrock**				
4:45pm - 6:45pm	**Postersession I** Location: Panorama Forum + Saal 8				
5:45pm - 6:45pm	**DGMS Board Meeting** Location: Goldsaal Chair: **Thorsten Benter**				
7:00pm - 10:00pm	**Conference Dinner**				

Tuesday, 16/May/2023

8:00am - 4:00pm	Registration Location: Entrance Foyer Goldsaal				
9:00am - 9:45am	Plenary Lecture IV: Joachim Richert: „-Omics“ and Beyond – Mass Spectrometry as a Key Enabling Platform in Chemical Industry Location: Goldsaal Chair: **Christian Huber**				
9:45am - 10:00am	Award: Mass Spectrometry in the Life Sciences Award Location: Goldsaal Chair: **Kathrin Breuker**				
10:00am - 10:20am	Mass Spectrometry in the Life Sciences Award 2022 Location: Goldsaal				
10:20am - 10:40am	Mass Spectrometry in the Life Sciences Award 2023 Location: Goldsaal				
10:45am - 11:00am	Coffeebreak, Exhibition, Posters Location: Foyer Goldsaal				
11:00am - 12:30am	Session 9: Pharmaceutical Application of MS Location: Goldsaal Chair: **Joachim Richert**	Session 10: Lipidomics II Location: Silbersaal Chair: **Anne Bendt**	Session 11: ICP-MS: Isotope and Environmental Analysis Location: Saal 4+5 Chair: **Daniel Pröfrock**	Session 12: Young Scientist's Meeting Location: Saal 9+10 Chair: **Lydia Kollhoff**	
12:30pm - 2:00pm	Lunchbreak Location: Foyer Goldsaal	Lunch Seminar: Bruker Daltonics GmbH & Co. KG Location: Goldsaal	Lunch Seminar: AB SCIEX Germany GmbH Location: Silbersaal	Young Scientists Business Meeting Location: Saal 9+10 Chair: **Alexander Köhrer**	Lunch Seminar: Waters GmbH Location: Saal 4+5

2:00pm - 3:30pm	**Session 13: Proteomics II** Location: Goldsaal Chair: **Jens Brockmeyer**	**Session 14: Metabolomics** Location: Silbersaal Chair: **Oliver Schmitz**	**Session 15: Females in Mass Spectrometry (FeMS)** Location: Saal 4+5 Chair: **Anne Bendt**	**Session 16: Data Science** Location: Saal 9+10 Chair: **Robert Heyer**
3:30pm - 4:00pm	**Coffeebreak, Exhibition, Posters** Location: Foyer Goldsaal			
4:00pm - 4:45pm	**Plenary Lecture V: Kathryn Lilley: Mapping dynamic re-localization of the subcellular transcriptome and proteome** Location: Goldsaal Chair: **John R. Yates**			
4:45pm - 5:15pm	**DGMS Members Meeting** Location: Silbersaal Chair: **Thorsten Benter**			
5:15pm - 7:15pm	**Postersession II** Location: Panorama Forum + Saal 8			

Wednesday, 17/May/2023

8:00am - 2:00pm	Registration Location: Entrance Foyer Goldsaal			
9:00am - 9:45am	Plenary Lecture VI: Mario Thevis: Mass Spectrometry in Sports Drug Testing - Advances and Challenges Location: Goldsaal Chair: **Kevin Pagel**			
9:45am - 10:00am	Coffeebreak, Exhibition, Posters Location: Foyer Goldsaal			
10:00am - 11:30am	Session 17: MS-Imaging Location: Silbersaal Chair: **Prasad Phapale**	Session 18: Food analysis Location: Saal 4+5	Session 19: Natural Products Location: Saal 9+10 Chair: **Mario Thevis**	Session 20: Automation / Instrumentation II Location: Goldsaal Chair: **Wolfgang Schrader**
11:30am - 11:45am	Coffebreak, Exhibition, Posters Location: Foyer Goldsaal			
11:45am - 12:45pm	Plenary Lecture VII: John R. Yates, III: Why does deltaF508 CFTR fail to mature and cause Cystic Fibrosis: Interactions, Modifications and in vivo Structure? Location: Goldsaal Chair: **Albert Sickmann**			
12:45pm - 1:30pm	Poster Awards and Closing Ceremony Location: Goldsaal			
1:30pm - 2:30pm	Farewell Reception Location: Foyer Goldsaal			
2:30pm - 5:00pm	Post Symposium "LC-MS" Location: Goldsaal Chair: **Hartmut Schlüter** Chair: **Marcel Kwiatkowski**			
3:30pm - 4:00pm	Coffebreak Location: Foyer Goldsaal			

Plenary Lectures

PL 1

Time: 5/14/2023 5:15:00 PM - 5/14/2023 6:00:00 PM Location: Goldsaal

Chair: Sickmann, Albert

"Shaken, not Stirred!" – James Bond in the Spotlight of Physics

Tolan, Metin

Universität Göttingen, Germany

How do James Bond's X-ray glasses work, the ones he uses to see whether the lady at the roulette table has a pistol concealed in her underwear? Is it really possible to launch oneself into the air and catch up with a plane that is free-falling towards the earth? Or to shoot down a helicopter with a pistol? In this lively and informative book, Germany's boldest physics professor Metin Tolan analyses the stunts and gadgets of the 007 films and even answers the question of all questions: Why does Bond drink his vodka martini shaken, not stirred?

PL 2

Time: 5/15/2023 9:00:00 AM - 5/15/2023 9:45:00 AM Location: Goldsaal

Chair: Irrgeher, Johanna

ICP-MS as a contributing tool to non-target screening in environmental monitoring

Feldmann, Jörg

University of Graz, Austria

The focus of the lecture is non-target screening or chemical fingerprinting of substances in environmental monitoring. Today big data are generated when non-target screening (NTS) for water and environmental samples utilizing LC-HRMS are performed. How do we use these data as community? What are the challenges in NTS?

The challenges how the data are used in compound identification and quantification will be discussed in particular with the focus on how simultaneous element-specific detection can help in both processes the identification and quantification. It will be shown the potential of ICPMS to increase confidence in the determination of both known and unknown POPs and their metabolites when no molecular identical standards are available.

Furthermore, this lecture highlight will explain the concept of mass balances for compounds containing a heteroatoms such as chlorine, bromine or fluorine and what benefit we gain if we determine what we usually miss with target analysis or even with NTS using LC-HRMS. The concept of simultaneously used ESI-HRMS and

ICPMS/MS for LC separation will be illustrated by case studies of PFAS, and mercury compounds in wildlife (pilot whale and wild boars) samples as an early warning sign for humans.

PL 3

Time: 5/15/2023 4:00:00 PM - 5/15/2023 4:45:00 PM Location: Goldsaal

Chair: Spengler, Bernhard

Spatial single-cell metabolomics reveals metabolic cell states

Alexandrov, Theodore

EMBL, Germany

tba

PL 4

Time: 5/16/2023 9:00:00 AM - 5/16/2023 9:45:00 AM Location: Goldsaal

Chair: Huber, Christian

„-Omics“ and Beyond – Mass Spectrometry as a Key Enabling Platform in Chemical Industry

Richert, Joachim

BASF SE, Germany

Mass spectrometry (MS) is an incredibly powerful and versatile analytical technique that is used along all business processes in the chemical industry and is a key enabler for product and process innovation. Starting at the stage of early discovery of biologically actives, identification of molecular targets or increasing the understanding of fundamental biological interactions, hyphenated MS platforms are key to success in the “-omics” sciences, i.e. genomics, proteomics, metabolomics, lipidomics and others that leverage the unmatched sensitivity, selectivity, throughput and discriminatory power of MS. Along the path towards new compounds, novel processes and eventually innovative products, there are many more applications in R&D where high-end MS plays a pivotal role, including on-line analytics in production, quality control or the stringent product registration and regulatory process.

The ongoing transformation of the chemical industry towards net-zero-CO2 emissions must be addressed by more energy-efficient production processes, an increased use of renewable energies and a transition towards a Circular Economy that will allow to decouple growth from resource consumption. Recycling loops, like ChemCycling (recycling to feedstock), will require new MS-based analytical methods and concepts.

The presentation intends to give an impression of the broad and diverse uses of MS in chemical industry today and discusses some of the analytical challenges in the years to come.

PL 5

Time: 5/16/2023 4:00:00 PM - 5/16/2023 4:45:00 PM Location: Goldsaal

Chair: Yates, John R.

Mapping dynamic re-localization of the subcellular transcriptome and proteome

Lilley, Kathryn

University of Cambridge, United Kingdom

There are numerous factors that play a part in increasing the complexity of living organisms over what is provided by protein expression. These factors include non-coding RNA mediated control mechanisms, post-transcriptional and post-translation processing, and the subcellular localisation of both, proteins, and RNA. Importantly, aberrant protein synthesis has been described as a key driver in multiple pathological states including cancers and neurodegenerative disorders. While both the transcriptome and proteome rapidly respond to cellular stress via re-localisation, the rules that govern such events are not fully understood. The dynamic mapping of the spatial relationship of RNA and protein on a cell-wide scale in response to cellular perturbation has remained an open challenge.

In this talk, I will describe the technologies we have developed to capture the spatial relationship between the transcriptome and proteome and their interactions.

First, I will discuss how we map the cellular spatial proteome based on physicochemical fractionation of cellular components (LOPIT) followed by quantitative mass spectrometry, giving examples of how its application within many different biological scenarios and describe associated robust computational workflows. I will also show how we have coupled this approach to determine how the steady location of proteins can be influenced by multiple post-translational modifications.

I will then describe briefly how we can use quantitative mass spectrometry to determine which proteins bind RNA and how their interaction in different conditions.

Finally, I will then introduce a new approach for the simultaneous mapping of the both the spatial proteome and spatial transcriptome on a cell-wide scale. By re-engineering our cell fractionation methods to make it compatible with RNA interrogation, we have been able to combine LOPIT with our new method to interrogate RNA localisation (LoRNA) to create the SuMM workflow (Subcellular Multiomics Mapping). We have applied the SuMM workflow to determine the orchestrated re-localisation of RNA and protein upon the induction of the unfolded protein response (UPR). The resulting data show that endoplasmic reticulum-localised mRNAs are more efficiently recruited to stress granules (SGs) than cytosolic RNAs. These data also give insights into how post transcriptional modification of tRNA through interaction with RNA binding proteins as determined using the method above, may impact codon usage bias within the subset of the transcriptome recruited to stress granules upon UPR.

PL 6

Time: 5/17/2023 9:00:00 AM - 5/17/2023 9:45:00 AM Location: Goldsaal

Chair: Pagel, Kevin

Mass Spectrometry in Sports Drug Testing - Advances and Challenges

Thevis, Mario

Deutsche Sporthochschule Köln, Germany

Analytical approaches in sports drug testing are continuously updated and expanded, exploiting new information on drug metabolism and disposition in humans as well as innovations in sample preparation and analysis, and also novel strategies focusing on marker-based test methods have been assessed, developed, and implemented. The resulting improved detection capability and retrospectivity of sports drug testing approaches has considerably limited the formerly available options of substances and methods of doping. In addition, however, and similar to the general population, elite athletes are exposed to a complex set of environmental factors including chemicals, biological and physical stressors, which constitute an exposome that is, unlike for the general population, subjected to specific scrutiny for athletes due to applicable anti-doping regulations and routine doping controls.

Test methods in sports drug testing, relying largely on chromatographic-mass spectrometric methods, were optimized and applied to newly identified challenges, including e.g. the detection and characterization of superior metabolic products of prohibited as well as non-prohibited substances, aiming at enhancing the analytical data available for decision-making processes in test result management. Additional information, resuling from controlled (microdosed) elimination studies and simulations of contamination scenarios, complements the dataset of routine doping controls.

Drug elimination profiles are an important aspect, contributing to the interpretation of analytical test results and supporting the assessment of drug exposure scenarios concerning their plausibility. By means of examples including ingredients of cosmetics, food potentially contaminated with doping agents such as anti-estrogens, and new anabolic agents (SARMs) contributing to continuously increasing numbers of adverse analytical findings, the particularly important role of chromatographic-mass spectrometric analyses in doping controls is illustrated. Optimized test methods allow for utmost retrospectivity and, at the same time, can offer critical information as to the time point of drug exposure and/or the source of the target analyte in athletes' doping control samples.

PL 7

Time: 5/17/2023 11:45:00 AM - 5/17/2023 12:45:00 PM Location: Goldsaal

Chair: Sickmann, Albert

Why does deltaF508 CFTR fail to mature and cause Cystic Fibrosis: Interactions, Modifications and in vivo Structure?

Yates, John R.

Scripps Research Institute, United States of America

tba

Sessions

SES-01

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:15:00 AM Location: Goldsaal

Chair(s): Borchers, Christoph

Session 1: Proteomics I

SES-01-KN

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:45:00 AM

Surveying organelles in cellular context through multifaceted mass spectrometry

Warscheid, Bettina

Julius-Maximilians-Universität Würzburg, Germany

tba

SES-01-01

Time: 5/15/2023 11:45:00 AM - 5/15/2023 12:05:00 PM

A novel intelligent data acquisition Hybrid-DIA mass spectrometry strategy: enabling data-driven and hypothesis-driven approaches in one go

Xuan, Yue (1); Huang, Min (2); Yang, Xiangyun (2); Fort, Kyle (1); Canterbury, Jesse.D. (3); Martínez-Val, Ana (4); Olsen, Jesper (4); Goetze, Sandra (5); Wollscheid, Bernd (5); Makarov, Alexander (1); Moehring, Thomas (1)

1: Thermo Fisher Scientific, Germany; 2: Thermo Fisher Scientific (China) Co. Ltd, Shanghai, China; 3: Thermo Fisher Scientific, San Jose, CA, USA; 4: Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, DENMARK; 5: Institute of Translational Medicine (ITM), Dep. of Health Sciences and Technology (D-HEST), ETH Zurich, Zurich, Switzerland

Translational scientists face the dilemma to choose between comprehensive profiling and sensitive targeted quantitation, especially with large sample cohorts. Proteomic profiling is commonly used to discover biomarkers, having a great potential for prognostic and predictive biomarkers; however, it still misses the sensitivity to quantify all the markers of interests. Therefore, targeted quantitation experiments of the potential markers are analyzed in the validation phase. This leads to high cost, time losses and more sample consumption. To address

these challenges, we develop a novel intelligent data acquisition “Hybrid-DIA” MS strategy that enables comprehensive proteome profiling via high resolution MS1-based data-independent-acquisition MS and on-the-fly intelligently switching the acquisition mode to parallel reaction monitoring (PRM) for sensitive quantification of the markers, substantially increasing throughput and reducing sample consumption.

The global profiling and quantitation performance of Hybrid-DIA MS have been investigated and benchmarked against the standard DIA MS methods by analyzing HELA cell lysate digest. Comparable number of proteins/peptides are identified and quantified with both methods, while Hybrid-DIA MS significantly improves the LOQ/LOD of the low abundant biomarkers down to attomole range. We then applied Hybrid-DIA to analysis the oncoprotein mutations within the lung cancer cell lines, known clinical markers and drug targets from the melanoma patient tissue samples, and major signaling pathways in the cancer cell phosphoproteomes, respectively. With high precision and reproducibility, Hybrid-DIA MS can quantify the EGFR and KRAS cancer mutations in lung cancer cell lines, capture clinically actionable markers in the melanoma patient tissue sample, as well as accurately quantify 100+ phosphorylation sites covering seven major signaling pathways in cancer cells (EGFR, RAS-MAPK, PI3K-AKT-mTOR, AMPK, apoptosis and stress response), while digitalizing their underlying proteotypes.

This novel Hybrid-DIA MS methodology presents a new capability to combine the data-driven and hypothesis-driven approaches in one go, accelerating the entire biomarker discovery and validation pipeline

Reference:

1 Xuan, Y., Bateman, N.W., Gallien, S. et al. Standardization and harmonization of distributed multi-center proteotype analysis supporting precision medicine studies. Nat Commun 11, 5248 (2020). https://doi.org/10.1038/s41467-020-18904-9

SES-01-02

Time: 5/15/2023 12:05:00 PM - 5/15/2023 12:25:00 PM

midia-PASEF maximizes information content in data-independent acquisition proteomics

Distler, Ute (1); Łącki, Mateusz Krzysztof (1); Startek, Michał Piotr (1,2); Teschner, David (3); Brehmer, Sven (4); Decker, Jens (4); Schild, Thilo (1); Krieger, Jonathan (5); Krohs, Florian (4); Raether, Oliver (4); Hildebrandt, Andreas (3); Tenzer, Ste

1: Institute for Immunology, University Medical Center of the Johannes-Gutenberg University Mainz, Germany; 2: Faculty of Mathematics, Informatics, and Mechanics, University of Warsaw, Warsaw, Poland; 3: Institute for Informatics, Johannes-Gutenberg University Mainz, Germany; 4: Bruker Daltonics GmbH & Co. KG, Bremen, Germany; 5: Bruker Ltd, Milton, Canada; 6: Helmholtz Institute for Translational Oncology (HI-TRON) Mainz, Germany

Data-independent acquisition (DIA) approaches provide comprehensive records of all detectable pre-cursor and fragment ions. Here we introduce midia-PASEF, a novel DIA scan mode using mobility-specific micro-encoding of overlapping quadrupole windows to optimally cover the ion population in the ion mobility-mass to charge plane.

Using overlapping ion mobility-encoded quadrupole windows, midia-PASEF maximizes information content in DIA acquisitions. Notably, in contrast to established DIA approaches, the MIDIA cycle time does not increase with increased overall mass range. MIDIA diagonal scans cover the entire precursor m/z range and the specific design of the overlapping quadrupole windows enhances transmission of the fragments, as each fragment ion is targeted in 3/20 midiaPASEF frames.

To process the resulting high-complexity datasets, we developed the Snakemake-based MIDIAID pipeline. MIDIAID enables fully automated processing and multidimensional deconvolution of midia-PASEF files, and includes machine-learning-based classification of precursor-fragment relationships. This enables the precise determination of precursor ion positions for all detected fragment ions across the entire dynamic range with a precision of less than 2 Th.

We show that the MIDIAID pipeline provides highly specific DDA-like deconvoluted spectra from midiaPASEF data at a spectral quality suitable even for de novo sequencing on dataset level using PEAKS. Resulting MIDIA-MSMS spectra can be searched directly with established tools including PEAKS, FragPipe and Mascot.

In conclusion, midiaPASEF enables to reproducibly generate DDA-quality MIDIA-MSMS spectra for precursors across the entire mass range in the selection parallelogram. In contrast to DDA, midiaPASEF is non-stochastic and thus generates detailed detection profiles of each fragment ion in all dimensions, which facilitates the highly specific deconvolution and scoring of precursor-fragment relationships. We envision that these additional layers of information can be used in next generation database search engines to further improve the performance of midiaPASEF. The Snakemake-based MIDIAID analysis pipeline accommodates various approaches for clustering, deisotoping, graph- and .mgf generation. We envision further increase in identification performance by using advanced multidimensional feature detection and clustering approaches. Using machine learning approaches to refine the MIDIA graph based on initial database results, we significantly improved the specificity of precursor-fragment relationships, thereby surpassing the spectral purity of DDA in our deconvoluted MIDIA-MSMS spectra. This also demonstrates that the specific data acquisition in the MIDIA frames as described here does significantly increase the information encoded therein over that encoded in the classical non-overlapping diaPASEF frames.

To illustrate the performance of our workflow, we analyzed a tryptic digest of HeLa using a 30 min gradient (41 min runtime) using a 150 µm ID column in both DDA-PASEF and midiaPASEF acquisition modes. In this analysis, DDA-PASEF acquisition enabled the identification of approx. 30,000 unique peptide sequences, and reached a maximum identification rate of approx. 1,500 unique peptide sequences per minute. First round search results of deconvoluted midiaPASEF datasets resulted in slightly lower numbers, and machine-learning-based refinement of the precursor-fragment relationships in the MIDIA graph in combination with database search in FragPipe enabled the identification of > 2,400 unique peptide sequences per minute in the most complex region of the gradient, which translates into > 40 identified unique peptide sequences per second. To illustrate the benefit of midiaPASEF for challenging sample types, we also demonstrate the principal applicability to phosphopeptidomic and immunopeptidomic samples.

midiaPASEF thus provides all benefits of DIA acquisitions, including efficient ion sampling, high duty cycle and excellent reproducibility. In addition, midiaPASEF allows to generate high-quality deconvoluted MIDIA-MSMS spectra that can be readily exported to various data formats and efficiently analyzed with existing and well-established database search engines that were developed for DDA. Moreover, MIDIA acquisition preserves elution profiles in retention time and ion mobility space, and thus enable to precisely determine the coordinates of the respective ions. In contrast to DDA, fragment ion information is collected across the entire peak, improving ion statistics and thus mass accuracy of fragment ions. Therefore, midiaPASEF-based workflows display excellent potential similar to DDA or small-window DIA approaches for spectral library generation. We envision that the application of midia-PASEF will therefore not be limited to proteomic or peptidomic samples, but can be readily

optimized for the analysis of peptides, lipids, metabolites or other small molecules in the mass range between 50 and 5,000 Da.

SES-01-03

Time: 5/15/2023 12:25:00 PM - 5/15/2023 12:45:00 PM

A medium-throughput DIA-MS workflow enables pre-clinical and clinical proteome studies in cardiac tissue

Lenz, Christof (1,2,3,4,5); Neuenroth, Lisa (1); Lehnart, Stephan (1,3,4,5); Urlaub, Henning (1,2,4)

1: University Medical Center Göttingen, Germany; 2: Max Planck Institute for Biophysical Chemistry, Göttingen, Germany; 3: DFG Collaborative Research Centre 1002, Göttingen, Germany; 4: DFG Collaborative Research Centre 1190, Göttingen, Germany; 5: DZHK Göttingen Site, Göttingen, Germany

Introduction

In spite of the ready availability of hiPSC-derived cellular models, cardiac disease has to be studied on the organ level to corroborate molecular information to e.g. electrophysiological function and phenotype. In addition, cardiac function is highly diversified and localized, necessitating spatially resolved analysis from minute tissue amounts. To enable routine proteome analysis in pre-clinical and clinical samples under these requirements, we have combined pressure cycling-based lysis, automated SP3 sample processing and diaPASEF LC/MS/MS analysis to a workflow that enables analysis to a depth of 6.000 protein groups from 0.5 mm3 tissue volume, at a throughput of 12 samples per day. We have successfully applied this workflow to a range of pre-clinical and clinical research scenarios, and find evidence both for intricate spatial organization of the cardiac proteome as well as for it key role in cardiac function and disease.

Methods

Cardiac biopsies were lysed using a urea-based pressure cycling protocol (PBI Barocycler 2320 XT), reduced and alkylated, and cleaned up and trypsinized by an automated SP3 protocol (Thermo Fisher Scientific Kingfisher Duo Prime) using Amine-coated beads (Resyn Bioscience). Acidified peptide mixtures were separated on 25x0.075 cm RP-C18 Column with an integrated emitter (Ion Opticks) over 100 min and analyzed using a custom-made 20x2 variable window dia-PASEF acquisition method on a hybrid ion mobility/quadrupole/time-of-flight mass spectrometer (Bruker timsTOF Pro 2). Data were analyzed using directDIA processing in Spectronaut v16 (Biognosys).

Results

As SP3 protocols are compatible to a wide range of detergents and chaotropes, we tested 2% SDS and 1% SDC as alternatives to the established Urea-based PCT lysis protocol in the Barocycler. All lysis buffers provided comparable overall results, however the detergent-based buffer exhibited better coverage of hydrophobic, membrane associated proteins.

Automated SP3 cleanup and digestion was set up on a Kingfisher Duo Prime magnetic bead handler. Among different surface chemistries, a combination of Resyn Biosciences Amine beads and 70% acetonitrile precipitation offered the most consistent results. In addition, tryptic digestion could be performed directly into 25 mM ammonium bicarbonate buffer, which after acidification provided direct compatibility with LC/MS/MS

measurement and allowed us to skip additional desalting and drydown steps, which frequently hamper reproducibility. Overall, implementing automated SP3 digestion also significantly reduced the hands-on sample processing time.

Mass spectrometric acquisition was performed using a customized dia-PASEF acquisition method. Using experimentally observed precursor m/z and 1/K0 distributions, variable window size methods were tested and optimized using the publicly available pyDIAid script. Among the conditions tested, a 20x2 window, 100 ms ramp time acquisition scheme provided the best balance between cycle time and sensitivity, and highest number of precursors, peptides and protein groups under the conditions used.

Finally, we tested our optimized workflow on a range of pre-clinical and clinical sample types and obtained stable quantitation for a range of cardiac and pseudo-cardiac tissues:

Rat left ventricle (LV): 6.177 protein groups, 76.707 peptides, 111.166 precursors

Pig left and right ventricle (LV): 4.761 protein groups, 57.857 peptides, 78.123 precursors

Human engineered heart tissue (EHT): 4.945 protein groups, 54.398 peptides, 56.242 precursors

Overall, the established pipeline allows us to process cardiac tissue samples at a rate of 12 samples per day, a turnaround time of 36-48 hours, with minimal manual sample processing.

We used the developed pipeline to characterize spatial proteome organization in a newly established pig heart model for genetically encoded cardiac disease at a resolution of 60 loci. The results corroborate earlier studies that corroborate a distinct chamber-specific organization of the porcine heart, but indicate a much more finely grained spatial organization of the cardiac proteome, which validates the importance of high throughputprotoem analysis coupled to e.g. microscopic analysis.

SES-02

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:15:00 AM Location: Silbersaal

Chair(s): Heiles, Sven

Session 2: Lipidomics I

SES-02-KN

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:45:00 AM

Keynote: Anne Bendt: Lipidomics – the bumpy road towards clinical translation

Bendt, Anne

National University of Singapore, Singapore

Lipid metabolites beyond triglycerides and cholesterol have vast potential for applications in clinical diagnostics, with substantial societal and economical value. To successfully evolve from the current research-grade methods to assays suitable for routine clinical applications, a harmonization - if not standardization - of these mass spectrometry-based workflows is necessary.

This presentation is intended to provide an overview of international efforts to tackle the issues of workflow harmonization, and to serve as an open invitation for others to join this growing community. Specifically, past and planned interlaboratory ring trials for select lipids with potential for clinical diagnostics will be discussed.

SES-02-01

Time: 5/15/2023 11:45:00 AM - 5/15/2023 12:05:00 PM

Assessment of Phospholipid Turnover in Mice by Shotgun Ultra-High Resolution Orbitrap Fourier Transform Mass Spectrometry

Garikapati, Vannuruswamy (1); Rehm, Ronja (2); Schuhmann, Kai (1); Fornasiero, Eugenio F. (2); Shevchenko, Andrej (1)

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Lipidomics provides quantitative snapshot of the lipidome composition at a given time. However, monitoring rates of synthesis, degradation, and/or inter-conversion of the lipid classes and individual molecular species (lipid turnover) is crucial to understand the lipid metabolism and dynamics within living organisms. Herein, we optimized and applied a shotgun ultra-high resolution mass spectrometry (sUHR MS) method to quantify the absolute abundance and estimate the turnover rates of glycerophospholipids and sphingolipids across ages in

distinct biological specimens of 15N-labelled mice. The pulsed metabolic labelling is performed with a 15N-labelled SILAM mouse diet at several fixed time points. Lipids were extracted from the metabolically labelled mouse plasma and tissue homogenates (liver and brain) using MTBE/MeOH/H20 liquid-liquid extraction protocol, and then the extracts were subjected to a shotgun lipidomics workflow in positive- and negative-ion mode. To attain ultra-high resolution, we coupled an external high-performance data acquisition system (FTMS Booster X2, Spectroswiss Sàrl, Lausanne, Switzerland) in parallel to the conventional data acquisition system (Q Exactive Orbitrap MS, Thermo Fisher Scientific, Bremen, Germany) and recorded the time-domain signals (transients) without compromising the scope, accuracy, and dynamic range of full-lipidome quantitative shotgun profiling. The recorded transients were then processed by Peak-by-Peak and lipids were identified and quantified by LipidXplorer software. Taken together, with the employed sUHR workflow we achieved in baseline separation of the first isotopic peak (13C) of unlabelled and monoisotopic peak (15N) of labelled lipid species (Δm = 6.3 mDa) using a conventional QE Orbitrap (D30) MS system and determined the molar content and turnover rates of nitrogen-containing glycerophospholipids (PC, PC O-, LPC, PE, PE O-, LPE, PS, LPS) and sphingolipids (SM, Cer, HexCer) in mouse plasma and tissues.

SES-02-02

Time: 5/15/2023 12:05:00 PM - 5/15/2023 12:25:00 PM

Subcellular lipidomics towards understanding lipid quality control

Wölk, Michele; Fedorova, Maria

Center of Membrane Biochemistry and Lipid Research, Faculty of Medicine Carl Gustav Carus of TU Dresden, Dresden, Germany

Maintaining homeostasis in one of the key features of cellular organization. Cellular control mechanisms involve dedicated machineries overseeing DNA, protein and lipid qualities. In contrast to DNA and proteins, lipid quality control (LQC) is so far poorly understood and represents an emerging topic. The regulation of the cellular lipid content in response to various stimuli is important to maintain the physiological status of a cell and to allow adaptation to everchanging environment. While many proteins can be generally assigned to specific organelles, the same lipid species are present almost in all cellular compartments and only their relative abundance potentially allow to differentiate particular organelles. Thus, lipidome adaptation at organelle level, and thus mechanisms underlying LQC, may be overlooked using bulk lipidomics approaches. Therefore, it is essential to address the lipidome plasticity in response to the incoming stimuli and stressors at the level of individual subcellular compartments, e.g., mitochondria, nuclei, endoplasmic reticulum, and lipid droplets.

Here, to understand the role of subcellular lipid dynamics in LQC, we isolated nuclei and mitochondrial by differential centrifugation from HT1080 cells (human fibrosarcoma). Lipids were extracted and organelle specific lipidomes were characterized by reversed phase liquid chromatography coupled online to an Orbitrap Exploris 240 (ThermoFisher Scientific, Bremen, Germany) in a cycle time dependent DDA approach. Lipid identification and evaluation of their relative abundances were performed within Lipostar 2.1 (Molecular Discovery LTD, Borehamwood, UK). Organelle specific lipid collectives were compared to the lipidome of the whole cell extract to identify enrichment in particular lipid species. For example, in mitochondria cardiolipins (CL) were detected at much higher relative abundance in comparison to the total cell extract, as expected. Additionally, several phosphatidylcholine (PC) lipids, including mostly saturated and monounsaturated species, showed similar or even higher mitochondrial enrichment. For nuclei we observed significant enrichment of saturated diacylglycerols

(DG), i.e., DG 32:0 and DG 36:0, whereas remaining DGs were significantly lower in nuclei compared to the whole cell extracts. The obtained data are used to define subcellular lipid collectives, as a set of molecular species at a given relative abundance, specific for each organelle. By combining cellular fractionation, lipidomics and machine learning approaches, we are aiming for detailed time-resolved mapping on subcellular lipidome remodeling under different stress conditions and, thus a better understanding on the LQC machinery.

SES-02-03

Time: 5/15/2023 12:25:00 PM - 5/15/2023 12:45:00 PM

Comparison of novel fragmentation modes in HILIC-MS/MS based phospholipid analysis utilizing trapped ion mobility spectrometry

Rudt, Edward (1); Jeck, Viola (2); Korf, Ansgar (2); Meyer, Sven W. (2); Hayen, Heiko (1)

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Lipids play an important role in organisms, especially phospholipids as main component of the cell membrane. A change in lipid composition correlates with distinct neurodegenerative and cardiovascular diseases. Therefore, the demand of lipid analysis in clinical biomarker screening increases. However, lipid analysis is analytically challenging owing to the high complexity of analytes with similar fragments, even when high resolution mass spectrometry (HRMS) and fragmentation experiments (MS/MS) are applied. Therefore, orthogonal separation techniques to reduce the occurrence of chimeric MS/MS spectra, produced from isobaric precursor ions, are needed. Trapped ion mobility spectrometry (TIMS) represents a recently introduced technique to address this complexity. Through mobility-resolved fragmentation, novel approaches for fragmentation can be utilized to provide more confident results, e.g., in parallel reaction monitoring (prm) and data independent acquisition (dia). However, these parallel accumulation – serial fragmentation (PASEF) modes have not yet been evaluated for lipidomics.

In this work, an in-depth comparison of mobility-resolved fragmentation modes utilizing PASEF was performed for hydrophilic interaction liquid chromatography (HILIC)-MS/MS-based lipidomics. More specifically, data dependent acquisition (dda = standard PASEF mode), data independent acquisition (dia) and parallel reaction monitoring (prm) were compared for the analysis of human plasma extracts. In addition to the chromatographic lipid class separation, the orthogonal separation technique TIMS was applied for an unambiguous lipid assignment.

The various PASEF modes differ not only in scheduling, but also in sensitivity and selectivity. While in dda-PASEF precursor ions are selected based on the preliminary MS1 full spectrum, dia-PASEF and prm-PASEF do not require a MS1 scan for precursor picking. In dia-PASEF, a broadband fragmentation occurs via predefined mass/mobility windows. In contrast, in prm-PASEF, fragmentation is performed based on a target list with user-defined mobility and retention time sections. Generally, the mobility-resolved fragmentation via PASEF results in an improved spectral quality. Especially for dia-PASEF, the enhanced spectral quality is of relevance, since dia-PASEF lacks precursor-to-fragment assignment based on the simultaneous fragmentation of multiple, potentially interfering precursors in HILIC-MS/MS. In this study, these PASEF modes were rigorously compared based on MS/MS scheduling, the selectivity to reduce chimeric spectra and the sensitivity in generating MS/MS spectra. This

comparison serves not only for lipid analysis, but could also be transferred to other substance classes containing isobaric interferences.

SES-03

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:15:00 AM Location: Saal 4+5

Chair(s): Bettmer, Jörg

Session 3: Elemental Imaging

SES-03-KN

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:45:00 AM

Multimodal imaging to study the uptake of nanoparticles and their biological effects in tissues

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While nanoparticles continue to expand their range of applications in the materials sciences, health sciences and in many other areas of research and industrial applications, there is increasing concern about their uptake, distribution and possible health effects in the body.

Inductively coupled plasma-mass spectrometry (ICP-MS) in combination with dedicated sample introduction systems is one of the most powerful methods to study nanoparticles in tissues. In our current work, we focus on using laser ablation(LA)-ICP-MS imaging to investigate the distribution of elemental constituents of the nanoparticles as well as endogenous elements in rat lung tissues and on their long-range transport to other organs after particle instillation. Quantification is performed by using gelatine-based matrix-matched internal standards. Total elemental imaging as well as single particle (sp)-based ICP-MS imaging provided valuable complementary information.

Due to the comparably large size of the organ slices, micro X-ray fluorescence (μ XRF) is used to pre-screen the samples, giving rise to the observation of areas of higher concentration of the nanoparticles in the lung tissue. Additionally, for selected types of particles, including silica as well as cerium, aluminium and manganese oxides, μ XRF revealed higher phosphorous concentrations in the areas of exposure with the nanoparticles, indicating a biological response. Infrared (IR) imaging based on a quantum cascade laser (QCL) indicated stronger ester absorbance bands, thus linking the effects observed in μ XRF to elevated lipid concentrations.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) imaging analysis of lipids in the tissue samples confirmed that in tissues affected by the nanoparticles, various lipid classes are elevated, while others are depleted in comparison with healthy tissue, thus indicating fibrotic processes being initiated by the nanoparticles. Fibrosis in the lung tissues was subsequently confirmed by microscopic and immunohistochemical imaging.

In conclusion, it was shown that exposure to and biological effects of nanoparticles in rat lung tissues can be investigated successfully by LA-ICP-MS imaging and a series of complementary imaging techniques based on spectroscopy in different spectral regions as well as mass spectrometry.

SES-03-01

Time: 5/15/2023 11:45:00 AM - 5/15/2023 12:05:00 PM

Application of ICP-MS/MS to study potential environmental impacts of the European energy transition – from trace metals to microplastics

Pröfrock, Daniel (1); Ebeling, Anna (1,2); Hildbrandt, Lars (1); Zimmermann, Tristan (1); Wippermann, Dominik (1,2); Faust, Svenja (1); Klein, Ole (1,2)

1: Helmholtz Zentrum hereon, Germany; 2: Universität Hamburg, Department of Chemistry, Inorganic and Applied Chemistry

Legacy heavy metal and species contamination still represents a major adverse threat for many aquatic and marine ecosystems within highly populated catchment areas because of their inherent toxicity, vast sources and persistence.

Despite the ongoing reduction of emissions of such contaminants into the environment, the coastal zones of the North Sea still belong to the most impacted ecosystems worldwide. In particular, the ongoing evolution of coastal zones into industrialized areas, e.g. due to extensive shipping or the construction of offshore wind parks within the framework of the ongoing energy transition in Europe even boosted the release of either known, but also of various new contaminants into the marine environment. This includes e.g. rare element groups such as the so called TCEs beside other threats such as micro and nano plastics (MP/NP), which gained a strong scientific and public interest during the last years.

The accurate analysis of such contaminants is in particular of significance for public health concerns beside the overall future sustainable development and management of the coastal zones as required by EU wide legislation.

This contribution will focus on the role of ICP-MS/MS as powerful tool to study different unwanted chemical side effects on the marine environment due to the strong development in offshore wind energy production within the context of the ongoing European energy transition.

In particular the application of ICP-MS/MS for interference handling as well as the role of new hyphenation approaches for routine ultra trace analysis of emerging contaminants at ng/L levels, in order to allow a systematically evaluation of the emission load caused by anthropogenic activities within the coastal zone will be highlighted. In addition the suitability of ICP-MS/MS as a complementary tool in micro- and nano plastic research, to study MP interactions with other relevant environmental contaminants will be discussed.

SES-03-02

Time: 5/15/2023 12:05:00 PM - 5/15/2023 12:25:00 PM

Hyphenation of a high-speed laser ablation system to Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for imaging applications

Kutscher, Daniel; Asogan, Dhinesh; Manecki, Marcus; Chrást, Petr

Thermo Fisher Scientific (Bremen) GmbH, Germany

Laser Ablation (LA) coupled with ICP-MS is a well-established way to directly analyze solid samples. The main advantages of laser ablation include the ability to avoid lengthy and potentially contamination prone sample preparation protocols and the ability to obtain information about the spatial resolution of an analyte in a sample. The interest in so-called mapping techniques has increased in recent years, calling for laser ablation systems to develop methods to improve sample transfer and therefore speed of mapping experiments. New laser-ablation systems dedicated to high-speed mapping are commercially available and can be easily coupled to quadrupole ICP-MS for such applications.

With the improvement in sample transfer and washout times from laser ablation systems, the time available to analyze discrete packets of sample from a laser pulse is drastically reduced. The limit for lateral resolution using a sequential ICP-MS, such as a quadrupole ICP-MS, is dependent on the dwell times chosen for each measured m/z channel. This has a direct impact on the signal-to-background ratio achievable for each m/z channel; therefore, a direct impact on the final image contrast for each mapped m/z channel.

In this poster, the authors chose a Granite thick section containing a range of elements in localized crystal distributions to demonstrate the ease of coupling to a commercially available high-speed mapping laser ablation system, ease of dwell-time optimization for best signal-to-background ratio and image contrast across all scanned elements, and finally fast analysis times from sampling to image.

SES-03-03

Time: 5/15/2023 12:25:00 PM - 5/15/2023 12:45:00 PM

LA-ICP-MS based investigation of the fate of hemoglobin-derived iron in a mouse model of intracerebral hemorrhage with stereotaxic injection of isotope-enriched blood

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Intracerebral hemorrhage (ICH) represents a subtype of stroke, which is characterized by the rupture of a cerebral vessel and subsequent bleeding into the brain tissue. The primary damage and initial mortality in patients with ICH is mainly dependent on the mechanic injury due to the mass effect from the hemorrhagic volume. The ICH-related secondary brain injury (ICH-SBI) develops with delay after the initial bleeding. ICH-SBI manifests with delayed neurological deficits and has a major impact on the clinical outcome of patients after ICH. While the development of ICH-SBI is multifactorial, neurotoxicity and oxidative stress arising from the iron containing hemoglobin (Hb) and its metabolites is thought to represent a major driver in this process. Investigating the fate of Hb-derived iron within the brain parenchyma after ICH will provide the basis for further elucidation of the mechanisms leading to SBI. Laser ablation coupled to inductively coupled plasma mass spectrometry (LA-ICP-MS) has been established as a valuable tool for elemental bioimaging and can be used for the spatial resolved quantification of iron in the brain tissue.

As an abundant, endogenous element, iron displays a large background signal in the brain tissue impeding the differentiation between natural occurring iron and iron originating from the hemorrhage, especially at lower concentrations. To address this challenge, we performed an animal study, where mice were held under iron-deficient diet, while simultaneously receiving intravenous 58Fe-ferric carboxy-maltose supplementation. This leads to the formation of 58Fe-enriched Hb in the blood of these animals. The blood was then withdrawn from the donor animals and injected into the brains of a separate set of twelve mice with natural iron isotope distribution. The mice with artificial ICH were then sacrificed at different time intervals ranging from 1 hour to 28 days after the injection. The brains were removed, snap frozen, and cryosectioned at 10 µ m thickness. The sections were thaw mounted onto glass slides and parallel sections were produced for histological staining as well as spatial transcriptome analysis.

In LA-ICP-MS, 56Fe and 58Fe on brain sections were quantified using matrix-matched gelatin standards. Elemental bioimages were generated for the iron isotopes as well as for phosphorous, copper and zinc to display anatomical features. Results show that the presented method provides high sensitivities for both iron isotopes as well as a clear differentiation between iron distribution caused by hemorrhage and natural background at a satisfactory spatial distribution. The 58Fe distribution shows clear correlations with the results obtained from histology and spatial transcriptome analysis, conforming the methods' ability to accurately study the fate of Hb-derived iron in a mouse model of ICH.

SES-04

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:15:00 AM Location: Saal 9+10

Chair(s): Alexandrov, Theodore

Session 4: Single Cell Application

SES-04-KN

Time: 5/15/2023 11:15:00 AM - 5/15/2023 11:45:00 AM

Recent Advances in Single-Cell and Spatial Proteomics

Kelly, Ryan

Brigham Young University, United States of America

Biological tissues are highly heterogeneous, consisting of a variety of cell types, states and subpopulations, and understanding heterogeneity at the single cell level is of great interest for biomedical research. We have developed a platform termed nanoPOTS (Nanodroplet Processing in One pot for Trace Samples) to minimize sample losses normally incurred during sample processing. In combination ultra-low-flow separations and latest-generation mass spectrometry instrumentation, we now achieve in-depth proteome coverage for low-input samples including single cells. To date, we can quantify >3000 proteins from single mammalian cells and achieve similar coverage for frozen and FFPE tissue squares as small as 50 μ m. We will describe advances in cell isolation, sample preparation, ultrasensitive separations, MS acquisition and data analysis that have made this possible, as well as efforts to minimize or eliminate the need for custom instrumentation for such analyses. Finally, prospects for further dramatically improving the measurement throughput, depth of proteome coverage and quantitative accuracy for single-cell and other low-input proteomics studies will be discussed.

SES-04-01

Time: 5/15/2023 11:45:00 AM - 5/15/2023 12:05:00 PM

Linear ion trap (LIT) data independent acquisition (DIA) as a powerful tool for the quantitative analysis of low input samples.

Plitt, Corinna Marie (1,2); Wulf, Maximilian (1,2); Schipp, Lea (1,2); Marcus, Katrin (1,2); Eggers, Britta (1,2)

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Data-independent acquisition (DIA) is a commonly used mass spectrometry method, in which all precursor ions in a defined m/z range are fragmented, resulting in an in-depth identification of peptides. DIA further offers high optimization potential, allowing for sample-specific adjustments. Although DIA has many advantages, it is limited by the number of windows, as the time required for data acquisition in DIA is directly proportional to it, increasing the overall orbitrap (OT) cycle time. Since increasing the cycle time often results in an insufficient number of

data points acquired per peak, especially for low abundant peptides, DIA methods utilizing the OT are still limited in their performance.

Therefore, in recent years, few research groups have revived the idea of using the ion trap (IT) in low-input DIA, as its fast-scanning speed leads to higher peptide identification rates compared to OT-based methods. It therefore can provide an advantage in the analysis of low input samples, such as human tissue, where often only limited material is available, or single cell approaches, despite its lower resolution. A first study by Borràs et al., 2021 demonstrated the effectiveness of DIA-IT for low-input samples. They found a consistent increase in identified peptides with DIA-IT methods compared to DIA-OT methods using serial dilution protein digestion (1, 10, 100, and 1000 ng) with a large number of small isolation windows.

Thus, the objective of this study is to evaluate the suitability of DIA-IT for quantitative and comparative proteomic studies. We aimed to 1) reproduce the results of the initial study using a different cell line, 2) optimize our DIA-IT method for true low input samples such as a excised tissue and single cells 3) determine the suitability of DIA-IT for quantitative and comparative proteomic studies, using a goldstandard, a reference sample set containing spike-in proteins in different ratios, enabling to determine the accuracy of protein ratios obtained with DIA-IT and DIA OT

Our study provided us with valuable insights into the viability and accuracy of using DIA-IT for proteomic research. In summary, we found that DIA IT is indeed superior when analyzing true low input samples such as single cells as it increased the number of peptides identified in such and additionally retains accurate quantitative results.

SES-04-02

Time: 5/15/2023 12:05:00 PM - 5/15/2023 12:25:00 PM

Single cell analysis using a CellenOne and dia-PASEF combined with PaSER identifies thousands of proteins at scale

Adams, Christopher (1); Krisp, Christoph (1); Seth, Anjali (2); Hartlmayr, David (2); Mueller, Torsten (1); Krieger, Jonathan (1); Srikumar, Tharan (1); Rainczuk, Adam (1); Schmit, Pierre-Olivier (1); Tourniaire, Guilhem (2); Lubeck, Markus (1)

1: Bruker; 2: Cellenion, Lyon, France

Enhancements in trapped ion mobility spectrometry (TIMS) has the potential to make important contributions to the Single Cell Proteomics (SCP) field. Automated single cell sorting and sample preparation using the cellenONE platform, with the timsTOF SCP allows for fast and sensitive proteome analyses at the single cell level. Data independent acquisition (DIA) mode data files using deep learning with neuronal networks (e.g., DIA-NN), can lead to further improvements in detectability and quantifiability of proteins from minimal input samples such as single cells. An automated and seamless sample workflow where single cells can be transferred to a proteoCHIP using the CellenOne was tested. Sample processing is performed on the proteoCHIP, allowing transfer directly to the LC autosampler (nanoElute 2). We tested HEK293 and HeLa cells using the CellenOne/proteoCHIP and timsTOF SCP/dia-PASEF workflow. Using a 30-minute gradient, data was acquired by dia-PASEF and analyzed by PaSER (Bruker). For HEK293 cells, the single cell sorted channel corresponded to 837 protein groups form 2877 precursors identified. Not surprisingly, for both cell types more cells corresponded to a linear increase in the

number of proteins identified where 20 HEK93 cells identified 5,001 protein groups from 26,000 precursors. PCA analysis at the single cell level showed clustering and cell type discrimination. Finally, comparing known protein expression profiles of CDK1, CDK2, EEF1A1 and EEF1A2 in the two cell types examined showed preferential expression that is expected for HeLa cells. A start to finish single cell proteomics workflow, including cell sorting, processing, acquisition, and analysis.

SES-04-03

Time: 5/15/2023 12:25:00 PM - 5/15/2023 12:45:00 PM

Investigation of THP-1 Derived Macrophage Polarization using Single-Cell MALDI Mass Spectrometry Imaging

Schwenzfeier, Jan (1); Bien, Tanja (2); Koerfer, Krischan (3,4); Weischer, Sarah (5); Dreisewerd, Klaus (1); Soltwisch, Jens (1)

1: Institute of Hygiene, University of Münster, Germany; 2: Bruker Daltonics, Bremen, Germany; 3: Instute of Psychology, University of Münster, Germany; 4: Otto Creutzfeldt Center for Cognitive and Behavioural Neuroscience, University of Münster, Germany; 5: Imaging Network, University of Münster, Germany

THP-1 is a human monocytic leucemic cell line that is commonly used as a model to mimic human monocyte-derived macrophages (hMDM). By stimulating THP-1 cells with phorbol 12-myristate 13-acetate (PMA) they differentiate into naive macrophages (M0). By exposing the macrophages to different cytokines they can be further polarized into activated (M1) or alternatively activated (M2) macrophages. The aim of our research is to investigate changes in the molecular profile during this differentiation and polarization process on a single-cell level using MALDI-2 mass spectrometry imaging (MALDI-2 MSI).

For our experimental setup the cells were cultured in poly-D-lysine coated 8-well chamber slides, differentiated and polarized using PMA, LPS, IL-4, IFN$_\gamma$ and fixed with 4% formaldehyde. The membranes and nuclei were stained with CellMask Green Actin Tracking Stain and Hoechst33342 respectively and washed with ammonium acetate afterwards. Then, fluorescence and bright field images of the samples were captured using a Zeiss LSM980 Airyscan-2 microscope. Subseqeuently, the slides were sublimated with 2,5-DHAP and measured with a timsTOF fleX MALDI-2 at 5 µ m pixel size.

The single-cell spectra were then generated using a python based in-house built Fluorescence Integrated Single-Cell Analysis Script (FISCAS). In a first step, the script co-registrates microscopy and MALDI images. The aligned region is then used to perfom a cell segmentation based on the fluorescence microscopy using Cellpose. The generated cell masks are then used to assign the mass spectra to their corresponding cells. Data evaluation is then performed using MataboAnalyst or Python.

With that, we were able to investigate changes in the lipid regime of THP-1 cells differentiating and polarizing into different macrophage subclasses (THP-1/M0/M1/M2). With a precise segmentation at hand, we also examined possible correlations between several primary and secondary morphological form factors with changes in the molecular profile.

In a further study we explored changes in the molecular profile of THP-1 dervied macrophages during phagocytosis. For that, we used pHrodo BioParticles which get engulfed by the macrophages and start to

fluoresce during the phagolysosome step of the phagocytosis as a ground truth. Thereby, we were able to study the correlation between the phagosomic activity and several changes we observed in the lipid regime.

SES-05

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:15:00 PM Location: Goldsaal

Chair(s): Janasek, Dirk

Session 5: Clinical mass spectrometry

SES-05-KN

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:45:00 PM

Precision medicine through clinical mass spectrometry - fact or fiction

Ceglarek, Uta

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Precision medicine (PM) aims the prediction, prevention and treatment of diseases considering individual variability in genes, environment, and lifestyle. PM strategy includes decision-making processes based on biomarker-driven omics approaches. During the last two decades mass spectrometry becoming a key omics technology capturing single biomarker but also the whole picture of biological systems. Based on these diagnostic advantages, mass spectrometry paved the way into patient care offering a rapid, effective and economical way to identify and quantify metabolic alterations of pre-defined target metabolites in body fluids. However, for implementation into 24/7 patient care special quality requirements to laboratory diagnostic approaches have to be addressed.

This presentation gives an up-to-date overview of the role of mass spectrometry in diagnostics for prediction, prevention and treatment of diseases in the clinical lab. Current limitations and future challenges will be discussed for the quantification of metabolites and proteins in body fluids under clinical conditions. Recent developments directing to personalized medicine by metabolomic and proteomic approaches will be depicted.

SES-05-01

Time: 5/15/2023 2:45:00 PM - 5/15/2023 3:05:00 PM

Chemical warfare agents react with hair proteins: novel targets for forensic analysis

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Micro liquid chromatography-electrospray ionization tandem-mass spectrometry (μ LC-ESI MS/MS) methods were developed for the forensic analysis of human hair proving exposure to the nerve agents (NA) sarin and VX

and to the blister agent sulfur mustard (SM). For the first time adducted amino acid and peptide biomarkers were detected derived from covalent reaction products with hair keratins (protein adducts) [1,2]. These biomarkers allow evidence of local hair exposure.

Recent incidents in the violent conflict in the Middle East [3,4] as well as attempted murder [5] and assassinations of individuals [6] document the current life- and health- threatening effects of chemical warfare agents (CWA). The deployment of agent vapours and aerosols might lead to inhalational incorporation and to exposed scalp hair of unprotected persons. Due to the chemical reactivity of CWA protein adducts are formed. Adducts results from phosphylation by organophosphorus NA and alkylation by SM both targeting nucleophiles in the side chains of amino acids. A number of adducts with proteins in the circulating blood (e.g., hemoglobin, albumin [3,4]) is well known whereas nearly nothing was known about adducts with hair proteins.

For sample preparation of human hair locally exposed to CWA in vitro, a complex procedure was developed. After lysis reducing cysteine disulfide bridges and thus making proteins soluble, precipitation and washing steps followed. The hair protein pellet was suspended in buffer prior to proteolysis either with pronase (yielding single phosphylated amino acids [2]) or with pepsin (yielding alkylated peptides [1]). Subsequent to ultrafiltration the respective biomarkers were selctively detected by µ LC-ESI MS/MS thus proving the exposure to the agents. Hard keratins representing 65-95% of the total protein content were modified by CWA. Their adducts were shown to be stable at room temperature and contact to air for at least several weeks and months [1,2]. This excellent stability is superior to those of circulating adducts and makes hair an ideal specimen for local post-exposure analysis potentially providing evidence for the break of international law and the chemical weapons convention.

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SES-05-02

Time: 5/15/2023 3:05:00 PM - 5/15/2023 3:25:00 PM

Quantification of Procalcitonin in Human Serum by Immuno-LC-MRM

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Procalcitonin (PCT), a 13kDa protein, is a biomarker for bacterial sepsis; accurate PCT quantification is critical for sepsis diagnosis and appropriate antibiotic-treatment follow-up. In healthy individuals, PCT concentration generally remains below 0.05 ng/mL.1 Immunological PCT quantification methods are routinely used in clinical laboratories, yet there is a need for the standardization of PCT quantification protocols. An orthogonal method to clinical immunological assays, such as LC- multiple reaction monitoring (MRM) method for the quantification of PCT, is required.

In this study, a highly sensitive and robust immunoaffinity LC-MRM quantitative method for procalcitonin detection and quantification in human serum has been developed. Three tryptic peptides from the N- and C-terminal regions of PCT were selected for LC-MRMquantification. For PCT quantification, an LLOQ of 0.25 ng/mL of PCT in human serum was achieved using a sample volume of 1 mL. Targeted mass spectrometric methods, such as LC-MRM-based absolute quantification, combined with deeper structural characterization of intact protein isoforms in standards and biological samples, could provide more direct and deeper insights into PCT levels during sepsis, aiding the development of better diagnostic and treatment methods. The parallel measurement of three PCT peptides renders robustness and reliability of quantification and may allow future differentiation of intact PCT vs. other PCT forms originating from potential degradation, processing or polymorphisms. An established and validated LC-MRM-based quantification of PCT, such as the method presented here, will be relevant as an orthogonal method for harmonization and standardization of clinical assays for PCT in support of the aims of the IFCC WG-PCT.2,3

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SES-05-03

Time: 5/15/2023 3:25:00 PM - 5/15/2023 3:45:00 PM

Development of a Rapid and Sensitive MALDI-TOF Mass Spectrometric Assay for SARS-CoV-2 Detection

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We have developed a rapid, sensitive, and highly specific assay for detecting and monitoring SARS-CoV-2 infections by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). As MALDI-TOF mass spectrometers are available in a clinical setting, our assay has the potential to serve as alternative to the commonly used reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) assays to detect and monitor SARS-CoV-2 infections. Sample preparation prior to MALDI-TOF-MS involves the tryptic digestion of SARS-CoV-2 proteins, followed by an enrichment of virus-specific peptides from SARS-CoV-2

nucleoprotein via magnetic antibody beads. Our MALDI-TOF-MS method allows the detection of SARS-CoV-2 nucleoprotein as low as 8 amol/µ l. MALDI-TOF mass spectra are obtained in just a few seconds, which makes our MS-based assay suitable for a high-throughput screening of SARS-CoV-2 in healthcare facilities in addition to PCR. Due to the specific detection of virus peptides, different SARS-CoV-2 variants are readily distinguished from each other. Specifically, we show that our MALDI-TOF-MS assay discriminates SARS-CoV-2 delta (B.1.617.2) variant from all other variants in patients' samples, making our method highly valuable to monitor the emergence of new virus variants.

SES-06

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:15:00 PM Location: Saal 9

Chair(s): Pagel, Kevin

Session 6: Glycomics

SES-06-KN

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:45:00 PM

Biomedical and biopharmaceutical glycomics

Wuhrer, Manfred

Leiden University Medical Center, Netherlands, The

Glycosylation influences many biological processes in health and disease and modulates the structure and function of biopharmaceuticals. Protein- and lipid-linked glycans are often enormously heterogeneous, and glycomic studies largely rely on mass spectrometry in conjunction with separation techniques to resolve this glycoform complexity. This lecture will present recent progress in integrating structural and functional studies for characterizing the structural diversity and functional impact of glycosylation in a glycoform-resolved manner. The power of mass spectrometry coupled with affinity-based, native-mode separation techniques such as affinity chromatography and affinity capillary electrophoresis will be demonstrated, revealing how minor structural glycomic modifications dictate protein interactions and function.

SES-06-01

Time: 5/15/2023 2:45:00 PM - 5/15/2023 3:05:00 PM

Limited Fun with GAGs: The Reaction of Hypochlorous Acid with monomeric and oligomeric Components of Glycosaminoglycans is surprising

Leopold, Jenny; Prabutzki, Patricia; Nimptsch, Ariane; Engel, Kathrin M.; Schiller, Jürgen

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Introduction

Hyaluronic acid (HA) is a natural polysaccharide composed of alternating N-acetylglucosamine and glucuronic acid residues. HA occurs in many tissues and is particularly abundant in the human joint (synovial) fluid (SF) in concentrations of about 2-3 mg/ml to warrant lubrication of the joints and to reduce friction.

It is commonly accepted that the pathogenesis of many rheumatic diseases is accompanied by a loss of the viscosity of SF and, thus, a reduction of the molecular weight of the HA polysaccharide. There are many potential explanations of this observation: in addition to the effects of HA-depleting enzymes, reactive oxygen species (ROS), which are released by typical inflammation cells such as granulocytes or macrophages, may also play

significant roles [1]. Since considerable activities of the enzyme myeloperoxidase (MPO) could be monitored in inflamed joint fluids, hypochlorous acid (HOCl), as the main product of MPO) probably also plays a major role regarding the oxidative modification of HA. Unfortunately, the so far used analytical methods are rather indirect [2] and do not allow the detection of characteristic products. Surprisingly, there were so far no attempts to monitor the products of HOCl-induced HA degradation by means of mass spectrometry (MS) [3].

Material and Methods

All chemicals, solvents, and the mono- and polysaccharides of interest were purchased in the highest commercially available purity from Sigma-Aldrich (Taufkirchen, Germany) and used as supplied. The tetrasaccharide of HA (HA4) was obtained by enzymatic digestion of the polymer with the enzyme hyaluronidase from bovine testes [4]. The concentration of HOCl was determined by photometry at pH = 12 (ε290 = 350 M-1 cm-1). Carbohydrates were treated with an excess of HOCl and investigated subsequent to dilution with acetonitrile. This also helped to decrease the salt concentration to an amount suitable for electrospray ionization coupled to ion trap (ESI-IT) MS. ESI-IT mass spectra were recorded on an AmaZon SL (Bruker Daltonics GmbH, Bremen, Germany). Separations of selected products were performed by normal phase high performance thin-layer chromatography (HPTLC), which worked much better than different tested high-performance liquid chromatography methods.

Results and Discussion

Carbohydrates were investigated according to increasing complexity: while neither glucose nor glucuronic acid reacted with HOCl, the cconversion of glucosamine into the corresponding chloramine could be easily monitored (Δ = 34). However, it was obvious that no complete turnover could be achieved (even with high excess of HOCl and prolonged incubation times) and there were no further products such as the chloramine, which can be generated by HCl elimination. N-acetylglucosamine, one carbohydrate subunit of HA, is known to react with HOCl under the formation of a transient product (N-chloroamide) that decomposes (under involvement of radical intermediates) slowly into acetate and chlorinated glucosamine. We were only able to detect the N-chloroamide by MS but no further products. Accompanying investigations of the HOCl consumption by UV spectroscopy indicated an elevated reactivity of HA4 and more than the theoretical amount of HOCl was consumed.

Surprisingly, the reaction of HA4 with HOCl resulted in the cleavage of the glycosidic linkages and small amounts of the trisaccharide could be monitored by MS as well as HPTLC. This was an unexpected result since the prevailing opinion is the preferred modification of HA4 by HOCl at the N-acetyl side chain. Cleavage of the glycosidic linkages is normally induced by Fenton chemistry, i.e. by hydroxyl radicals. It has to be elucidated whether HOCl may be converted into hydroxyl radicals at physiological conditions.

New Aspects

It was shown that HA depolymerization may also be induced alone by HOCl.

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SES-06-02

Time: 5/15/2023 3:05:00 PM - 5/15/2023 3:25:00 PM

The Fine Art of Destruction for In-Depth Mass Spectrometry-Based Glycoproteomics: Advances in Measurement and Data Analysis – Exploiting the Diagnostic Potential of Fragment Ions

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The unambiguous mass spectrometric identification and characterization of N- and O-glycopeptides is crucial to elucidate the micro- and macroheterogeneity of glycoproteins, and thus indispensable for in-depth glycoproteomic studies. Such studies can uncover structure-function relationships in various biological contexts, but can also support optimization and quality control in biopharmaceutical production processes. Stepped collisional energy fragmentation, a feature of modern orbitrap and quadrupole time-of-flight mass spectrometers, has been proven very useful in this regard, as lower collisional energy can be used to determine the glycan composition, while higher collisional energy allows the identification of the corresponding peptide. Here, we propose combining lower and stepped collisional energy fragmentation for the in-depth and site-specific analysis of N- and O-glycopeptides [1]. To test and optimize the developed workflow, a set of four representative and bio-pharma-ceutically-relevant glycoproteins was analyzed (IgG, fibrinogen, lacto-transferrin, and ribonuclease B). Using stepped collisional energy fragmentation we identified a conserved fragmentation signature for N-glyco-peptides [Mpeptide+H+0,2X GlcNAc]+, that has rarely been employed in glycoproteomic analyses up to now. This signature represents a valuable indicator for the determination of the correct peptide mass and is particularly important when analyzing glycopeptides obtained after proteolytic digestion with broad or no specificity. Further, we have systematically evaluated the relative abundance of glycan-derived fragment ions, so-called oxonium ions, to retrieve structural information on the glycan moiety of N- and O-glycopeptides, e.g. differentiation of hybrid- and high-mannose-type N-glycans or differentiation between antenna- and bisecting-GlcNAc. Overall, these findings can significantly increase the confidence and comprehensiveness in manual and software-assisted N- and O-glycoproteomics.

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SES-06-03

Time: 5/15/2023 3:25:00 PM - 5/15/2023 3:45:00 PM

Direct Evidence of Glycosyl Cation using Cryogenic Infrared Spectroscopy

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The chemical synthesis of glycoconjugates is very cumbersome due to the unpredictable stereoselectivity in glycosylation reaction. As a result, today only few structures are accessible for functional studies in biology. Despite years of significant progress in method development, no gold standard technique to construct glycosidic bond with well-defined stereochemistry has been reported. Recently, numerous reports highlighted how a better understanding of the reaction mechanism can help to optimize the reaction conditions significantly. However, the impact of the underlying reaction intermediate – the glycosyl cation – is still sparse due to its instability and extremely short lifetime that makes difficult to analyze.

In previous studies we have shown that glycosyl cations can be trapped in a mass spectrometer and probed using cryogenic IR spectroscopy. From the resulting spectra, high resolution structures can be deduced in combination with first-principles theory. First data provided direct structural data of glycosyl cation intermediates bearing prominent features such as neighboring group participation, remote participation and Ferrier-type structures, revealing the origins of stereoselectivity.

Herein, we built on these promising initial results and aim to unravel the mechanistic details of glycosylation reactions. Currently in focus are for example 4,6-O-benzylidene directed glycosylation, which are a promising tool to install highly challenging 1,2-cis glycosidic linkages. Our data reveal an unexpected rearrangement on the 4,6-O-benzylidene acetal group that leads to an energetically more stable anhydro cation. The potential influences of the rearrangement are followed using chemical test reactions. First data indicate that the 1,6-anhydro ring on the intermediate shields the b-face of anomeric carbon and therefore induced nucleophilic addition from opposite a-side. In the future, this brand-new pathway will enable the reliable stereoselective formation of 1,2-cis glycosides.

SES-07

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:15:00 PM Location: Saal 4+5

Chair(s): Irrgeher, Johanna

Session 7: ICP-MS : Nano-/Bioanalysis

SES-07-KN

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:45:00 PM

Facets of Elemental Mass Spectrometry as tools for the characterisation of structures on the nano- and microscale

Clases, David (1); Lockwood, Thomas (2); Sarah, Meyer (2); Xu, Helen (2); Camp, Emma (2); Doble, Philip (2); Gonzalez de Vega, Raquel (1)

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Inductively coupled plasma – mass spectrometry (ICP-MS) is the most versatile element-selective technique which enables ultra-trace analysis across the periodic table. Since its inception about 40 years ago, several technological advances inspired distinct methodological facets promoting analyses beyond the sole quantitative investigation of elements in aqueous solutions. In conjunction with hyphenated techniques as well as dedicated methodologies such as immunochemistry and/or single event analysis, ICP-MS enables chemical speciation analysis, elemental and protein imaging as well as the detection and advanced analysis of micro- and nanoscaled structures.

In this work, a focus is directed on ICP-MS-based technologies for the characterisation of structures measuring only between some nanometres to up to several micrometres. Imaging techniques including laser ablation-ICP-MS are showcased to analyse microparticles as well as bio-integrated structures. For the analysis of discrete particulate elements, a new processing platform is demonstrated to support the analysis of large and complex single event data sets acquired with both quadrupole and time-of-flight mass analysers. Different applications for the detection and characterisation of metallic and carbon-based microstructures are highlighted subsequently.

SES-07-01

Time: 5/15/2023 2:45:00 PM - 5/15/2023 3:05:00 PM

Characterisation of Biogenic SeNPs Produced by Edible Mushrooms

Bettmer, Jörg (1); Suárez-Priede, Andrés (1); Corte-Rodríguez, Mario (1); Mester, Zoltan (2); LeBlanc, Kelly L. (2); Montes-Bayón, Maria (1)

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Selenium (Se) is a chemical element which compounds have been reported as an antimicrobial, antioxidant and anticarcinogenic agent. Its nanoparticulated form (SeNPs) arouses great interest in the field of biomedicine, as its particular physicochemical characteristics confers its lower toxicity as well as greater bioavailability with respect to other selenium species (1). In contrast to the ones produced by means of chemical processes, biogenic SeNPs synthesised by biological systems such as plants, bacteria, or fungi, present a greater biocompatibility and stability. Moreover, their biological origin can confer them special behavior and physiological properties, which grant this type of NPs special relevance from a biomedical point of view (2). In this work, different species of edible fungi, cultivated in a selenite enriched medium, have been studied as potential biogenic nanoparticulated selenium producers.

Following an optimised extraction method to isolate the nanoparticles, single particle analysis by ICP-MS (SP-ICP-MS), which has been already reported for the characterization of individual selenium nanostructures in yeast (3), was applied in this work to address the presence, characteristics, and number concentration of SeNPs in the mentioned fungi samples. Regarding the study about their antibacterial activity, several bioassays were carried out employing both gram-positive and gram-negative bacterial species, and the MIC value (minimum inhibitory concentration) was calculated.

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SES-07-02

Time: 5/15/2023 3:05:00 PM - 5/15/2023 3:25:00 PM

Optimized processing algorithms for the analysis of single particles and cells in inductively coupled plasma-mass spectrometry

Elinkmann, Matthias (1); Reuter, Sarah (2); Bendieck, Niklas (1); Köhrer, Alexander (1); Quarles Jr., C. Derrick (3); Sperling, Michael (1,4); Hippler, Michael (2); Karst, Uwe (1)

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Inductively coupled plasma-mass spectrometry (ICP-MS)-based approaches to analyze nanomaterials and biological cells have experienced a decade of fast development and performance improvement. Through the combination of specialized sample introduction systems and data processing algorithms, the investigation of trace elements in single cells directly from a dilute suspension is becoming increasingly accessible.

In this work, an automated sample introduction system was used to map the endogenous elemental composition in Chlamydomonas reinhardtii green algae that were cultivated under physiological and iron depleted medium conditions. The setup included an on-axis total consumption spray chamber equipped with a microliter low-flow nebulizer. Particular focus was directed to the nebulizer argon gas flow to ensure a mild aerosol generation with minimum cell damage. Further plasma parameters were adjusted for highest signal intensities.

Data analysis was carried out with an in-house software tool written in Java that employs an iterative baseline search and a two step event detection including a split cell event correction. Special attention was given to the recognition and elimination of method-intrinsic measurement artifacts beyond pure statistical models. These cases include background fluctuations, event overlap and deviating signal peak shapes. Based on these observations, useful guidelines to improve overall data quality are presented.

Single cell-based distributions for six naturally occurring elements were separated from the background for both medium conditions. Furthermore, the observed cellular iron and copper content showed a clear differentiation depending on the culture media. A great advantage of this methodology is that no error-prone cell counting required in advance.

SES-07-03

Time: 5/15/2023 3:25:00 PM - 5/15/2023 3:45:00 PM

Detection of Nanoparticles and Microplastics with Elemental and Molecular Mass Spectrometry

Engelhard, Carsten; Escobar-Carranza, Cristian C.; Heide, Maximilian; Schardt, Annika; Schmitt, Johannes; Strenge, Ingo

University of Siegen, Germany

In the first part of the presentation, recent developments in single-particle inductively coupled plasma mass spectrometry (spICP-MS) instrumentation for nanomaterials characterization will be discussed. While millisecond dwell times were used in the advent of spICP-MS, the use of microsecond dwell times helped to improve nanoparticle data quality and particle size detection limits. Further to this development, we could show that a custom-built high-speed data acquisition unit with microsecond time resolution (µ sDAQ) can be used to successfully address issues of split-particle events and particle coincidence, to study the temporal profile of individual ion clouds, and to extend the linear dynamic range by compensating for dead time related count losses. Our next generation DAQ for spICP-MS features nanosecond time resolution. First results of a proof-of-concept study will be discussed.

In the second part of the presentation, we turn to a cooler plasma source, which proved useful in ambient desorption/ionization mass spectrometry (ADI-MS). Specifically, the use of molecular mass spectrometry with a home-built flowing atmospheric-pressure afterglow (FAPA) source (which was first developed in the Hieftje laboratory) for the direct analysis of microplastics will be discussed. In this work, the FAPA source is coupled to a high-resolution mass spectrometer (HR-MS) and used to probe selected microplastics directly on a sample target without a preceding separation step. FAPA-HRMS analysis in combination with principal component analysis is considered an interesting tool for microplastics analysis.

SES-08

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:15:00 PM Location: Silbersaal

Chair(s): Lämmerhofer, Michael

Session 8: Automation / Instrumentation I

SES-08-KN

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:45:00 PM

Why GC-EI-MS when you can do better?

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The high complexity of some matrices such as food, environmental or biological samples could complicate their analytical determination. From an instrumental and data treatment point of view, chromatographic coelutions lead to mixed mass spectra that might difficult the identification of the compounds, especially when hard ionization techniques such as electron ionization (EI) are used. Moreover, depending on the ion source selected, ion suppression effects could become critical, thus hindering the detection of analytes. Thereby, powerful analytical techniques that ensure both a high chromatographic resolution and a selective and sensitive determination are required when dealing with the characterization of complex samples.

Traditionally, GC has been coupled to MS by vacuum ionization techniques like EI, allowing the identification of the ionized compounds by MS libraries (i.e., NIST). However, as mentioned above, these hard ionization sources produce a heavy fragmentation that may hamper the identification of the analytes in complex sample matrices. In this sense, atmospheric pressure ionization (API) sources can offer great potential in GC-MS analyses since these soft ionization techniques usually preserve the molecular or quasi-molecular ion. Moreover, their flexibility for the coupling of GC with advanced high-resolution mass spectrometry (HRMS) systems, usually limited to liquid chromatography applications, enhances the capabilities of these sources for non-targeted applications. One- and two-dimensional GC analyses of complex samples with several API sources (APCI, APPI, APLI and plasma-based sources) will be discussed.

SES-08-01

Time: 5/15/2023 2:45:00 PM - 5/15/2023 3:05:00 PM

Atmospheric pressure field desorption using activated field emitters delivers molecular ions of polycyclic aromatic compounds

Gross, Jürgen H.

Heidelberg University, Germany

Field ionization (FI) and field desorption (FD) are established as soft ionization techniques typically delivering intact positive molecular ions, M+▪, or adduct ions like [M+H]+ and [M+alkali]+ of neutral molecular compounds [1,2]. The modern variant of FD and FI, liquid-injection field desorption/ionization (LIFDI), allows for sample introduction to the emitter under the complete exclusion of moisture and air [3]. All of these techniques, i.e., FI, FD, and LIFDI, are traditionally performed in high vacuum.

Atmospheric pressure field desorption (APFD) mass spectrometry (MS) using standard activated field emitters has only recently been demonstrated [4] as a new contribution to the field of ambient desorption/ionization (ADI) methods. In APFD both ionic and polar analytes delivered positive as well as negative ions even-electron ions depending on the selected polarity of the high voltage applied to the field emitter.

The present work on APFD-MS explores the formation of positive molecular ions, M+▪, from polycyclic aromatic compounds. The molecular ions were formed on and desorbed from standard 13-µ m activated tungsten wire emitters at atmospheric pressure. This was achieved by placing commercial field emitters at about 2 mm distance in front of the atmospheric pressure interface of a Bruker ApexQe Fourier transform-ion cyclotron resonance mass spectrometer. The entrance electrode of the interface was set to 4.5–5.5 kV with respect to the emitter.

Under these conditions, the electric field strengths achieved at the dendritic microneedles were sufficient to allow for the abundant formation of M+▪ ions of polycyclic aromatic compounds such as benzo[a]pyrene and 1,1,4,4-tetraphenyl-butadiene and still of notable abundances when 1-aza-[6]helicene and fluoranthene were employed. In case of the extremely basic 1-aza-[6]helicene as well as with triphenylphosphine protonation strongly competed with molecular ion formation and tended to suppress the field ionization process. All molecular ion compositions were assured by accurate mass-based formula assignments and some additional odd-electron species were obtained via collision-induced dissociation of molecular ion precursors.

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SES-08-02

Time: 5/15/2023 3:05:00 PM - 5/15/2023 3:25:00 PM

Membrane-Inlet Mass Spectrometry as a Tool for the Fast Analysis of Environmental and Security-Relevant Substances in Water

Gehm, Christian (1,2); Streibel, Thorsten (2); Schulz-Bull, Detlef (1); Zimmermann, Ralf (2,3)

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The ongoing development of sensitive analytical approaches for the real-time analysis of marine pollutants is critical for ensuring intact marine ecosystems and, consequently, human health. For many substances, however, low concentrations in marine environments complicate their detection and monitoring. For this purpose, membrane-inlet mass spectrometry (MIMS) has become a versatile technique for fast and direct analysis of a wide variety of volatile and semi-volatile organic compounds dissolved in water. In MIMS, analytes are extracted directly from the water into the gaseous phase using semipermeable membranes. This enables sensitive mass spectrometric detection of organic pollutants without time-consuming sample preparation procedures. Sensitivity can be further enhanced by utilizing selective ionization techniques, such as resonance-enhanced multiphoton ionization (REMPI).

In this conference contribution, we want to highlight the capabilities of MIMS technology for the fast analysis of environmental and security-relevant substances.

First, the results of a field campaign will be presented, in which MIMS was used in combination with REMPI @ 248 nm for the at-line determination of small polycyclic aromatic compounds (PAH) dissolved in the wash water of an exhaust gas cleaning system (scrubber). Within the campaign, diesel fuel as well as three different heavy fuel oils (HFOs) were investigated on a research ship engine equipped with an open-loop scrubber in pilot plant size. For HFOs, the measurements clearly indicate the presence of primarily two - and three – ring PAHs as well as their alkylated species in the wash water at concentrations in µ g/L-range. Moreover, by utilizing REMPI-MIMS, temporal variations of concentrations of individual compounds can be easily monitored. The results of the experiments in this field campaign are supported by well-established GCMS measurements and demonstrate the capability of MIMS as process monitoring tool.

Furthermore, preliminary results are provided regarding the utilization of MIMS equipped with electron ionization for the direct analysis of selected surrogates, metabolites and degradation products of explosives and chemical warfare agents in water. In these experiments, nine different membrane materials (e.g. silicone, latex, polypropylene) were tested, whereas selected materials were additionally investigated in various thicknesses. It can be shown that thin silicone membranes provide the best performance in terms of sensitivity and response time. The presented results are first step towards the development of a new on-line sensor system for the fast and sensitive on-site detection of old munition on the seabed.

SES-08-03

Time: 5/15/2023 3:25:00 PM - 5/15/2023 3:45:00 PM

Structural Insight into Reactive Intermediates by Cryogenic Vibrational Spectroscopy and Computational Methods

Greis, Kim (1,2); Kirschbaum, Carla (1,2); Leichnitz, Sabrina (1,3); Meijer, Gerard (2); von Helden, Gert (2); Seeberger, Peter H. (1,3); Pagel, Kevin (1,2)

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Reaction intermediates in chemistry are often short-lived and can usually not be isolated. As a consequence, their structural characterization is challenging. However, in the vacuum of a mass spectrometer carbocation

intermediates, such as glycosyl cations, can be stabilized. Subsequently, these reactive ions can be probed with cryogenic vibrational spectroscopy in helium nanodroplets.

The ions of interest are generated via electrospray ionization and selected by their mass-to-charge ratio. Then, they are accumulated in an ion trap and afterwards picked up by superfluid helium droplets (0.4 K). The doped droplet beam overlaps with an infrared beam of the Fritz Haber Institute free-electron laser. Resonant photons excite vibrational modes leading to the release of the ions from the droplets, measured by a time-of-flight detector. The ion count plotted as a function of the photon wavenumber leads to an infrared spectrum. The highly resolved spectrum is diagnostic for the ion's structure leading in combination with computed spectra to unambiguous structural assignments.

The results obtained using this method helped to develop a stereoselective building block for α-galactosylations.[1] Furthermore, insight into the fragmentation mechanism of RNA dinucleotides including the role of the RNA autohydrolysis product was obtained.[2] The presented gas-phase approach allows getting unprecedented mechanistic insight into reactions that are usually occurring in solution. In the future, further reactive intermediates will be characterized with this method.

[1] K. Greis, S. Leichnitz, C. Kirschbaum, C.-W. Chang, M.-H. Lin, G. Meijer, G. von Helden, P. H. Seeberger, K. Pagel. J. Am. Chem. Soc. 2022, 144, 20258.

[2] K. Greis, C. Kirschbaum, M. I. Taccone, M. Götze, S. Gewinner, W. Schöllkopf, G. Meijer, G. von Helden, K. Pagel. Angew. Chem. Int. Ed. 2022, 61, e202115481.

SES-SPS

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:15:00 PM Location: Saal 10

Chair(s): Petre, Brindusa-Alina Glocker, Michael O.

Special Session: „In memoriam session in honor of Michael Przybylski“

SES-SPS-01

Time: 5/15/2023 2:15:00 PM - 5/15/2023 2:45:00 PM

Next Generation of Absolute Protein Quantitation in Biological Matrixes using MRM; higher multiplexicity while faster, robust and sensitive

Borchers, Christoph

McGill University / Lady Davis Institute, Canada

The accurate and rapid determination of protein concentrations using multiplexed multiple reaction monitoring (MRM) with isotope-labeled internal standards has the potential to revolutionize biochemistry and medical sciences. The combination of an Evosep One LC with an Agilent QQQ6495C allows a several-fold reduction of analysis time while maintaining high multiplexicity. As an example, we show the use of a multi-protein method for the absolute quantitation of 270 proteins, combined with metabolomics and Artificial Intelligence (AI), to analyze the plasma of a COVID cohort for prediction of survival. Using samples collected on the day of admission to the ICU, a panel of 10 proteins and 5 metabolites was able to predict survival with 92% accuracy. Moreover, optimization of the Evosep/6495C combination allowed adaptation of the 270-protein MRM method to a 24-minute gradient, thus doubling the throughput compared to before.

If additional sensitivity were to be needed for a specific application, immuno-affinity enrichment of targeted peptides can be combined with MRM. We have used this immunoMRM (iMRM) approach for the quantitation of proteins in the immuno-checkpoint pathway, including PD-L1. Currently, PD-L1 expression levels, as determined by immunohistochemistry (IHC), serve as biomarkers for many checkpoint-inhibitor (CPI) therapies and are used to determine the eligibility of subjects for immunotherapy. However, 30-50% of subjects in a PD-L1-positive cohort do not respond to anti-PD-L1 treatment, while some subjects with low or undetectable PD-L1 levels have significantly improved survival with CPI. We have found that IHC-based PD-L1 levels only moderately correlate with PD-L1 iMRM quantification, and that other PD-L1 axis proteins -- including PD-L2, LCK, and ZAP70 -- are good candidates for biomarkers in non-small cell lung cancer.

SES-SPS-02

Time: 5/15/2023 2:45:00 PM - 5/15/2023 3:05:00 PM

Interpretation thresholds 20 ng/mL and 200 ng/mL of the alcohol biomarker phosphatidylethanol PEth 16:0/18:1 in blood and their clinical and forensic applications

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Direct alcohol biomarkers, which are products of ethanol and biomolecules, can be used as evidence for alcohol uptake, for the determination of drinking habits ("social" consumption versus "excessive" consumption) and for abstinence control. They are determined by LC-MS/MS or GC-MS techniques: ethyl glucuronide (EtG) (GC-EI-MS in 1994, GC-NCI-MS in 2001; LC-MS/MS in 1998), ethyl sulfate (LC-MS/MS in 2003), phosphatidylethanol (PEth) (LC-MS/MS in 2009). For quantification in blood, urine or hair samples stable-isotope labelled internal standards are used.

The use of the alcohol biomarkers in blood (PEth), urine or hair (EtG) for abstinence monitoring implies, that they can be detected after drinking alcohol at a certain cut-off concentration used as a threshold for interpretation. Detectability is related to the sensitivity of the detection method. For example, before 2009 PEth was detected by HPLC coupled to evaporative light scattering detection (ELSD). With this technique a sum of PEth analogs (which differ in their fatty acid composition in sn-1 and sn-2 position of the glycerol-backbone) was detected with a detection limit of 35 ng/mL (0.05 µ mol/L). In our routine application with LC-MS/MS currently the limit of quantification is 7.5 ng/mL (0.01 µ mol/L) of a single PEth species (PEth 16:0/18:1) and this can still be optimized.

In an "international consensus" a cut-off for abstinence (or "not in contradiction to abstinence") at 20 ng/mL for PEth 16:0/18:1 was decided on in May 2022. We performed several drinking experiments with different drinking amounts: in one study, single drinking to a blood alcohol concentration of 0.8 ‰ led to PEth concentrations in blood in the range of 25 to 57 ng/mL (from < 20 ng/mL before start of drinking). With lower drinking amounts with target blood alcohol concentrations of 0.3 and 0.5 ‰, respectively, the 20 ng/mL threshold was not reached.

Uptake of lower amounts by traces of alcohol in "alcohol free" nutrition and beverages (with less than 0.5 Vol-% of ethanol), such as sauerkraut, fruit juices, over-ripe bananas, or by accidental inhalation of ethanol from hand-sanitizers or surface disinfectants, may only play a role in abstinence monitoring, if the cut-off limits are lowered. The amount of ethanol ingested or inhaled by these sources remains presumably in the range of 1 to 5 g, which is far below 10 g (which is equivalent to a single drink resulting in a blood alcohol concentration of approx. 0.1 to 0.2 ‰). Therefore, these trace amounts will not result in detectable PEth concentrations in abstinence monitoring as long as a cut-off of 20 ng/mL is used.

In this presentation, formation and detectability of PEth after single and multiple drinking will be presented. Additionally, the elimination of PEth in withdrawal therapy as well as applications for abstinence monitoring and detection of relapses by PEth with a cut-off of 20 ng/mL will be discussed.

SES-SPS-04

Time: 5/15/2023 3:25:00 PM - 5/15/2023 3:45:00 PM

Epitope determination of DNA aptamers as antibody alternatives by affinity-mass spectrometry

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Aptamers are short single stranded DNA or RNA oligonucleotides that exert pronounced selectivity as inhibitors for various signaling pathways, and have recently gained high attention as new therapeutic lead structures vis-à-vis classical IG-type antibodies. In contrast to antibodies, aptamers are chemically synthesized and show a number of unique features in the development of bioassays, drug development, and targeted therapy. As "chemical antibodies", aptamers are non- immunogenic, and do not interfere with cell viabilities, since they specifically bind and release cells, suggesting theirm potential for the evaluation of biomarkers.

In this study we present the molecular identification and affinity determination of specific epitope peptides of the C-Met protein binding to two high affinity aptamers. Two different DNA aptamers binding to a tumour biomarker C-Met protein (tyrosine-protein kinase Met) were tested for the affinity strength and for epitope identification. C-Met is a highly complex protein involved in multiple signaling-pathways. Upon ligand binding, C-Met transmits intracellular signals by a unique multi-substrate docking site. C-Met can be aberrantly activated leading to tumorigenesis and other diseases and has been recognized as a biomarker in cancer diagnosis.

The aptamers CLN0003 (60 bases) and CLN0004 (64 bases) differ by size, structure and interaction strength and they were modified with a C16 amino group to facilitate their immobilization. SPR analysis indicated that the two aptamers have similar binding affinity and previous work shows they have inhibition properties. Moreover, one aptamer (CLN0003) was found to have a structural epitope formed by two peptides (C-Met(524–543) and C-Met(568–576) and the other (CLN0004) a linear epitope (C-Met(381-393).

The molecular comparison of C-Met epitopes to DNA aptamers identified here for the first time opens new perspectives for specific biomarker elucidation and clinical diagnostics. DNA aptamers exert high specificities, stabilities and affinities; moreover, aptamers generally have low immunogenicity. Thus, the determination of DNA aptamer-protein epitopes should open new approaches for peptide-based biomarker elucidation.

SES-09

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:00:00 AM Location: Goldsaal

Chair(s): Richert, Joachim

Session 9: Pharmaceutical Application of MS

SES-09-KN

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:30:00 AM

Hybrid HPLC-MS approaches to the investigation of complex glycosylation profiles in biopharmaceuticals

Huber, Christian

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Gonadotropins are a class of heavily glycosylated protein hormones, thus extremely challenging to characterize by mass spectrometry. As biopharmaceuticals, gonadotropins are prescribed for the treatment of infertility and are derived from different sources: either from pooled urine of pregnant women or upon production in genetically modified Chinese Hamster Ovary cells. Human chorionic gonadotropin (hCG) is sold as a biopharmaceutical under the name Pregnyl® (urinary hCG, u-hCG) and Ovitrelle® (recombinant hCG, r-hCG), and recombinant human follicle stimulating hormone (r-hFSH) is marketed as Gonal-f®. Since glycosylation may greatly impact the efficacy of a drug product, the bioanalytical characterization of glycoprotein biopharmaceuticals is extremely important.

We implement size exclusion (SE) HPLC-MS to automatize the acquisition of native mass spectra of r-hCG dimer, but also u-hCG and r-hFSH, comparing the drug products up to intact heterodimer level. A hybrid HPLC-MS approach was employed for the characterization of r-hCG, u-hCG and r-hFSH drug products at different structural levels. Released glycans were analyzed by porous graphitized carbon (PGC)-HPLC-MS/MS, glycopeptides by reversed-phase (RP)-HPLC-MS/MS, subunits by RP-HPLC-MS and finally the intact native heterodimers by semi-automated online buffer exchange SE-HPLC-MS approach.

The data from different structural levels were integrated using bioinformatic tools, to finally unravel the composition of 1481 co-existing dimeric glycoforms for r-hCG, 1167 glycoforms for u-hCG, and 1440 glycoforms for r-hFSH, and to compare critical quality attributes of the different drug products such as their degree of sialylation and O-glycosylation. The method proved to be useful to compare biopharmaceuticals, e.g. obtained from different sources (recombinant vs urinary) or by different biotechnological strategies (r-hFSHα and r-hCGα). Moreover, the semi-automation of native mass spectra acquisition of gonadotropin non-covalent heterodimer puts the basis for the approach to be implemented in the quality control environment to assess similarity of drug products.

SES-09-01

Time: 5/16/2023 11:30:00 AM - 5/16/2023 11:50:00 AM

Intramolecular disulfide separation and characterization of trastuzumab subunits with CE-MS/MS

Schairer, Jasmin (1,2); Römer, Jennifer (3); Lang, Dietmar (3); Neusüß, Christian (1)

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Introduction

The analysis of antibodies is an ever-evolving field of research. Antibodies can vary, for example, in their glycosylation or amino acid sequence based on the host cell, the purification process, and the storage conditions. To gain more information on the characteristics of the antibody, they can be analyzed using intact or subunit approaches. While intact analysis is primarily used for post-translational modifications, subunits provide additional information. CE can be used to separate subunit moieties, while MS shows the mass differences of the modifications. To couple CE and MS, we have recently introduced the nanoCEasy interface, an easy-to-use, robust, and flexible interface for CE-MS coupling. The interface provides nanoESI properties with low sample dilution and an elegant valving functionality. Here, CE-MS and the nanoCEasy were used to analyze trastuzumab subunits. The MS/MS analysis added information already gained by the CE-MS run.

Methods

For the subunit analysis, trastuzumab was enzymatically digested using FabRICATOR and further reduced using DTT. Samples were additionally stressed using either temperature, low or high pH. An Agilent CE was coupled via the nanoCEasy interface to an Orbitrap Fusion Lumos for the measurement. The separation was done in a 60cm PEO-coated capillary using 20kV separation voltage. An acidic background electrolyte (BGE) containing 10% isopropanol was used for the separation. The MS and MS/MS parameters were set for each subunit individually.

Results

Generating the mAb subunits is easy and done within 1.5 hours. The sample was injected after digestion and reduction without further sample preparation onto the CE capillary. In CE, the subunits are separated based on their electrophoretic mobility in the liquid phase leading to several signals. The MS analysis revealed mass shifts of -2 Da and -4 Da for each subunit compared to the fully reduced subunit, indicating closed disulfide bridges within the molecule. For example, a light chain of trastuzumab can have two intramolecular disulfide bridges. CE can separate the molecules based on the number of intramolecular disulfide bridges, especially when only one is open. For example, four signals are detected in the EIE of the light chain, two of which show the same mass. To determine the exact location of the intramolecular disulfide bridges, MS/MS experiments were done. The first peak in the EIE of the light chain shows two closed disulfide bridges, and the last peak is the completely open form of the molecule. Peaks two and three, showing the same mass, can be distinguished because a different disulfide bridge is closed. The closed disulfide bridge for the first peak is open in the second peak and vice versa. The same separation and peak identification can be obtained for the Fd part, and similar separation and peak identification was obtained for the (partially) reduced Fc part.

In conclusion, it can be shown that CE can separate isomeric proteins with different disulfide bridges. In combination with MS/MS analysis, the exact location of the disulfide bridge in the molecule can be determined.

Moreover, various proteoforms, such as glycoforms, carbamylated, or deamidated forms, can be characterized in great detail. The separation power of this CZE-nanoESI-method, in combination with top-down MS/MS characterization, will be demonstrated and discussed in detail.

References

[1] Johannes Schlecht, Alexander Stolz, Adrian Hofmann, Lukas Gerstung, and Christian Neusüß, Analytical Chemistry 2021 93 (44), 14593-14598

SES-09-02

Time: 5/16/2023 11:50:00 AM - 5/16/2023 12:10:00 PM

Mass spectrometry-based applications in development of biopharmaceuticals

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Biopharmaceuticals (therapeutic proteins, monoclonal antibodies, fusion proteins and alike) are the fastest growing drug market and an increasing number of products are available targeting to treat numerous diseases. During development it is essential to understand potential product variants and degradation pathways to ensure safe and efficient drugs are available to patients. Therefore, mass spectrometry is one of the most important technologies. Its application is ranging from intact molecules up to 200 kDa to subunit as well as peptides after proteolytic digestion. Intact and subunit level is preferred due to faster sample preparation and analysis as well as minimized risk of introducing artefacts by extended sample preparation. Also, insight into the combinatorial appearance of modification can be gained. Further to this also structural information might be gained using ion mobility as a further dimension. Also, different front-end separation techniques beside classical reversed phase chromatography might be useful to separate and online identified the primary structure of e.g. size or charge variants. Another great advantage of mass spectrometric analysis is that after proteolytic digestion site-specific information of post-translational modification can be obtained. By this it can be evaluated how important a modification is depending on its location in e.g. CDR regions or other effector relevant positions. One challenge are modifications that are either isobaric like IsoAspartate or not stable during default sample preparation like Succinimide.

In this presentation I will introduce challenges and workflows aiming to solve these using mass spectrometric applications for the development of biopharmaceuticals.

SES-09-03

Time: 5/16/2023 12:10:00 PM - 5/16/2023 12:30:00 PM

Intact Transition Epitope Mapping – Force differences between Original and Unusual Residues (ITEM- FOUR): A Mass Spectrometric Method to Identify

Antibody Recognition Motives with Single Amino Acid Residue Resolution – Towards Next Generation Heart Attack Diagnostics

Röwer, Claudia (1); Ortmann, Christian (2); Neamtu, Andrei (3); El-Kased, Reham F. (4); Glocker, Michael O. (1)

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Single amino acid polymorphisms (SAPs) can be decisive for insufficient antibody-based diagnosis of life-threatening conditions, such as myocardial infarction. Analysis of antibody recognition motives is the key for tailoring accurate diagnostic tests which form the base of personalized therapy concepts. ITEM-FOUR is a newly developed rapid and robust mass spectrometric method by which binding strengths between antigens and antibodies can be fine-mapped with amino acid residue resolution, thereby enabling determination of influences of exchanged amino acid residues which are important for the antibody´s binding effectiveness. Our mass spectrometric investigations on a human cardiac troponin I (hcTn I) epitope and seven non-synonymous single nucleotide polymorphisms (nsSNPs) which exist in the human population tackles a medically relevant but hitherto mostly neglected problem of current antibody-based point-of-care (POC) diagnostics.

Eight synthetic 15-mer peptides which encompass the hcTn I epitope sequence and its SAPs were tested to determine their binding characteristics to a monoclonal anti-hcTn I antibody which had been deemed relevant for POC diagnostics of myocardial infarction. Immune complex binding strength was measured in solution by isothermal titration calorimetry (ITC) and in the gas phase by off-line nanoESI-MS using a Synapt G2S instrument (WATERS Corp.). Immune complex ions were dissociated by CID and proportions of freed ligands were determined as fractions of total ions at given collision cell (ΔCV) settings. From the mass spectrometry data apparent gas phase binding strengths, apparent thermodynamic properties (ΔH#m0g; KD#m0g) and apparent kinetic characteristics (kD#m0g) were determined after extrapolation to room temperature. In other words, quantitative values of immune complex ion dissociation reactions were determined regarding mean charge states (m) of immune complex ions without additional energy contributions from multiple charging and acceleration (0) in the gas phase (g). Wild-type and - based on nsSNPs - mutated epitope peptides, eight in total, were ranked according to their experimentally determined gas phase dissociation enthalpies (ΔH#m0g), thereby revealing which SAP caused weakened, impaired, or abolished antibody binding. According to ITEM-FOUR analysis, four group-representing peptides were identified: wild-type peptide ENREVGDWRKNIDAL (“very strong binder”; group I), peptide ENREVGDWLKNIDAL (“strong binder”; group II), peptide ENREVGDWPKNIDAL (“unorthodox and weak binder”; group III), and peptide ENREVGDWPENIDAL (“non-binder”; group IV). Ranking of these peptides was mirrored by the in-solution ITC results. Yet, in solution peptides ENREVGDWPKNIDAL and ENREVGDWPENIDAL did not bind to the anti-hcTn I antibody. Interestingly, the binding strength of peptide ENQEVGDWRKNIDAL was consistently ranked as the strongest. Surprisingly, peptide ENREVGGWRKNIDAL which by ITEM-FOUR was ranked to belong to the “very strong binders” (group I) was by ITC determined to be an even weaker binder than peptide ENREVGDWLKNIDAL (“strong binder”; group II). This comparison uncovered solvation-related structure dependencies of peptide ENREVGGWRKNIDAL as likely cause of deviating rankings. And for binding of all the epitope peptides to the anti-hcTn I antibody in an orthodox fashion, biocomputational methods pointed to the importance of adopting and maintaining helical structures. Adopting a helical conformation was particularly important within the peptides´ epitope core regions. Molecular dynamics showed that strongest binders (peptides ENREVGDWRKNIDAL and ENQEVGDWRKNIDAL; group I) remained almost completely helical, and strong binders (peptides ENREVGDWHKNIDAL, ENREVGDWLKNIDAL, and ENREVGDWCKNIDAL; group II) kept helical structures at least in the epitope core regions throughout the simulation time periods. Weak binders or non-binders

(peptides ENREVGDWPKNIDAL and ENREVGDWPENIDAL; groups III and IV) rather quickly abandoned their helical structures particularly in the epitope core regions according to molecular dynamics simulations. Rankings of gas phase binding strengths and in-solution binding affinities matched best when in solution the epitope peptides had adopted conformations which were close to the bound conformation and these conformations remained mostly helically folded over time. In sum, ITEM-FOUR fine-mapped binding structures of wild-type and mutated epitope peptides with amino acid residue resolution.

By investigating an hcTn I epitope and its nsSNP-caused SAPs, we chose to tackle a medically relevant but hitherto unsolved problem of current anti-body-based POC diagnostics. For "next generation" diagnostic kits, it is advised to interrogate mutual SAP-related interferences on antibody binding to answer the question whether or not the diagnostic antibody´s capability of abundance difference determination of a selected marker protein is in fact reliable in every real-world scenario. If not, it may be requested to accompany a POC assay's result with an additional SNP analysis of the individual patient, e.g., through commercial SNP analysis suppliers, to exclude potential SAP-related diagnostic biases. Alternatively, updated diagnostic kits might have to be equipped with more than one highly specific antibody to cover at least the most abundant SAPs of relevance within a respective antigen´s epitope region of interest to cope with modern precision medicine and individualized / personalized therapy requests.

SES-10

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:00:00 AM Location: Silbersaal

Chair(s): Bendt, Anne

Session 10: Lipidomics II

SES-10-KN

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:30:00 AM

Deep drive into human lipidome: discovery of tissue specific lipid collectives

Fedorova, Maria

Center of Membrane Biochemistry and Lipid Research, Faculty of Medicine Carl Gustav Carus of TU Dresden, Dresden, Germany

Modern lipidomics provide access to hundreds of lipid species routinely identified in various biological matrixes. Considering high translational potential of lipidomics phenotyping for biomedical and clinical studies, we often look at the relative differences between healthy and disease conditions skipping in-depth description of the lipidome in question. To provide mechanistic understanding of tissue lipidome organization and it response to various stimuli and stressor, qualitative and quantitative inventory of tissue lipidomes are required. Furthermore, capturing alterations in lipid metabolism might be as important as identifying static lipid signatures resistant to certain (patho)physiological stimuli. Over the last years, we invested in developing and optimizing analytical workflows for in-depth characterization of lipidomes from different murine and human tissues. Using combination of various types of chromatographic separation, advanced tandem mass spectrometry and tuned bioinformatics tools, it is now possible to identify and quantify up to thousand of lipid molecular species spanning over eight orders of magnitude in their concentrations. Clearly, each tissue is characterized by specific constellation of lipids species which we call a “lipid collective”. Such lipid collectives, described here as a set of particular lipid molecular species at a given relative abundances, provide unique snapshot of specialized lipidomes and support our endeavour in linking tissue lipid compositions to its functional specialization. Moreover, understanding the dynamics of tissue specific lipid collectives remodelling in health-disease continuum will provide deeper and more mechanistic understanding into pathology of dysmetabolism related disorders.

SES-10-01

Time: 5/16/2023 11:30:00 AM - 5/16/2023 11:50:00 AM

Short-term Stability of Lipids in Murine Tissue Homogenates

Dorochow, Erika (1); Gurke, Robert (1,2); Rischke, Samuel (1); Geißlinger, Gerd (1,2); Hahnefeld, Lisa (1,2)

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Lipids are ubiquitous biomolecules involved in (patho-)physiological processes and their analysis in tissue samples represents a highly relevant research topic as tissue types exhibit distinct lipid profiles that can be individually altered by diseases. However, pre-analytical factors are seldom considered in lipidomics studies in tissue and remain hardly explored to this day although lipidomics has been present for about 3 decades.

To elucidate the influence of pre-analytical factors during the processing of tissue homogenates, homogenized samples from four murine tissues (kidney, liver, spleen, heart) were stored at room temperature as well as in ice water for up to 120 min, representing usual processing times in lipidomics studies. Homogenization was performed using wet grinding with 25% ethanol and the addition of 10 µ M indomethacin. After the respective incubation time, the samples were extracted and subsequently analyzed via ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS). Lipid stability was assessed by calculating lipid class ratios (e.g. Cer/SM, LPC/PC, LPE/PE, LPI/PI, LPG/PG, DG/TG). Additionally, the changes in lipid profiles induced by the storage at -20 °C and -80 °C for up to 24 h were investigated to clarify if intermediate storage of homogenates is acceptable, e.g., to apply an alternative extraction protocol.

We found that lipids in tissue homogenates were generally stable when samples were kept in ice water for a short time as more than 90% of investigated lipid class ratios remained unchanged after 35 min. In contrast, only approx. 40% of lipid class ratios were unchanged when stored at room temperature. After 120 min, this portion further decreased to 25%, while 53% of lipid class ratios were still stable after the same time for storage in ice water. Additionally, differences between the investigated tissue homogenates could be detected, indicating individual lipolytic activities. Furthermore, intermediate storage of homogenates at -20 °C for 24 h led to more prominent alterations in lipid profiles compared to storage at -80 °C.

Our findings demonstrate that pre-analytical factors in lipidomics research demand more attention, accurate description, and careful handling since they are crucial to generate reliable and reproducible results. We conclude that tissue homogenates should be processed under cooled conditions and as fast as possible (≤ 35 min). If tissue homogenates require intermediate storage, storage at -80 °C is recommended.

SES-10-02

Time: 5/16/2023 11:50:00 AM - 5/16/2023 12:10:00 PM

Systemic proteome response to unsaturation of membrane lipids

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Organisms respond to dietary and environmental challenges by adjusting the compositon of glycero- and glycerophospholipids. This may favorably alter membranes properties (e.g. increase membranes fluidity at low temperatures), but how it affects the membrane proteome and, eventually, organs physiology is an open question.

Drosophila melanogaster is not able to synthesize sterols and also polyunsaturated fatty acids, but can acquire them from the food. We developed a series of semi-synthetic foods to manipulate the lipid composition of flies organs and singled out the impact of lipid metabolism, organ morphology and, specifically, unsaturation of membrane lipids. By using shotgun lipidomics and a combination of label-fee and targeted proteomics we

demonstrated that the lipid class composition of organs is conserved and this is a general principle of lipid homeostasis in eukaryotes, while the variability of length and unsaturation of fatty acid moieties is a part of generic compensatory response. Lipid metabolism pathways responded to the content of dietary TAG independently of their unsaturation. Unexpectedly, in membrane-rich organs (e.g. eyes) the bulk of membrane proteins was unaffected by lipids unsaturation with the notable exception of proteins involved in light sensing and fine muscle organization. Altogether, this prompted us to revise some basic principles of the interplay between diet, lipidome, proteome and physiology during dietary interventions.

SES-10-03

Time: 5/16/2023 12:10:00 PM - 5/16/2023 12:30:00 PM

Mass spectrometry based profiling of Ricinus communis and Abrus precatorius seed extracts - A forensic approach

Scharrenbroch, Lisa (1,2); Kirsch, Dieter (1); Schäfer, Thomas (1); Ahrens, Björn (1); Lermyte, Frederik (2)

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Biological toxins are a focus of concern by public health and law enforcement on national and international levels due to the increasing threat of their deliberate release in a bioterrorist attack. Among these biological toxins the plant toxins ricin, which is present in seeds of the castor bean plant (Ricinus communis) and abrin, which is present in seeds of the rosary pea plant (Abrus precatorius) are of particular interest owing to their worldwide availability, ease of preparation, high toxicity, and the lack of medical countermeasures. Over the last decade, they attracted much attention in the context of threatening letters containing unknown "white powders" and terrorist threat scenarios, culminating in the prevention of a bioterrorist attack using ricin in Cologne, Germany, in 2018. The topicality was again underlined in the beginning of the year 2023, when an anti-terror operation in Castrop-Rauxel, Germany was suspected to involve ricin.

Analytical methods for the detection of ricin and abrin have already been established. However, toxin containing seed extracts also comprise a significant proportion of other biomolecules such as carbohydrates, small molecular weight metabolites and lipids. The content of each biomolecule in the final extract is influenced by the preparation process resulting in a unique pattern characteristic for the agent. Recognition of such patterns provides not yet available forensic information by potentially establishing correlations between crime scenes, preparation sites and suspects.

To raise the evidential value of forensic investigation in this field, mass-spectrometry based methods for the characterization of seed extracts prepared from castor beans and rosary peas have been developed combining lipidomic and metabolomic approaches. Known procedures were applied for sample preparation and analytical methods were based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Separation of the complex biological system was achieved on a C8 analytical column using gradient elution to optimize the sensitivity of the system. An Orbitrap Fusion mass spectrometer, fitted with an electrospray (ESI) source and operated in positive ion mode at a scan range between 150-2000 m/z, was used for detection. High resolution FTMS analysis allowed for biomolecule identification as well as semi-quantitative analysis of the seed extracts, while the subsequent multi-stage fragmentation analysis using both, ion trap-based resonant excitation collision-induced dissociation (CID) and quadrupole-based higher energy collisional dissociation (HCD)

provided more in-depth structural information about the molecular species. The acquisition of complementary CID and HCD fragmentation patterns and low abundant structure-specific fragment ions allowed the detection and identification of structurally diverse molecular species at different concentrations with high mass accuracy despite the mixture complexity of the original seed extracts.

The developed method was successfully applied to differentiate between seed extracts prepared from Ricinus communis and Abrus precatorius. Semi-quantitative information about molecular species was obtained with the help of FTMS analysis and MSn-experiments using CID and HCD fragmentation were performed to identify specific biomarkers within the seed extracts and confirm distinct structural elements. Various characteristic lipid species, including glycerolipids, glycerophospolipids, and sterols as well as low molecular weight metabolites such as alkaloids were identified and semi-quantified with the help of obtained chromatograms and MSn-spectra.

In addition, the influence of different seed extract preparation techniques on the biomolecule composition and resulting chromatographic peak profile was studied. Obtained chromatograms demonstrated that the developed method can not only provide plant specific information but also variations in the peak profile between extracts of the same plant were observed which depended on the solvent and volume of the solvent used for extraction. Subsequent hierachial cluster analysis based on a set of identified biomarkers suggests that molecular forensics can help linking a biotoxin found at a crime scene to a specific plant species and preparation method by identifying unique, identifiable patterns characteristic for the agent. Obtained results were converted into a database which allows the search of specific monoisotopic masses of identified biomolecules. Such databases and chemometric models can readily be used in forensic investigations to determine the composition and classification of an unknown “white powder” suspected to be a preparation of a toxic plant extract.

The developed mass-spectrometry based method for the profiling of Ricinus communis and Abrus precatorius seeds provides comprehensive information about the composition of plant extracts. This information can be implemented in profiling strategies and is of high value for forenisc intelligence in terms of sample comparison.

SES-11

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:00:00 AM Location: Saal 4+5

Chair(s): Pröfrock, Daniel

Session 11: ICP-MS: Isotope and Environmental Analysis

SES-11-KN

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:30:00 AM

Established and novel isotope tools in the environmental and life sciences: From tracing mammoth migration to metabolic turnover

Irrgeher, Johanna

Montanuniversität Leoben, Austria

In this talk, an overview of the potential of modern isotopic analysis based on MC-ICP-(CC)-MS and TIMS in environmental- and life science applications will be given. The variety of applications of the natural variation in isotopic composition and the use of enriched isotopes will be discussed. The latter approach highlights the importance of data processing based on isotope pattern deconvolution.

A recent example on the use of the well-established tracer isotopes of strontium in combination with oxygen and nitrogen isotopes will be presented to reconstruct the movements of an Arctic woolly mammoth that lived 17,100 years ago, during the last ice age. In this collaborative project led by the University of Alaska, Fairbanks, sequential analyses of isotope ratios with high temporal resolution along an entire 1.7-meter-long tusk were used. The ultra-high 87Sr/86Sr variations record monthly, weekly and potentially sub-weekly mobility patterns. An isotope-guided random walk approach to compare the tusk's strontium and oxygen isotope profiles to isotopic maps was used. The modeling reveals patterns of movement across a geographically extensive range during the animal's ~28-year life span that varied with life stages. The findings will be discussed in view of today's climate challenges.

The use of enriched stable isotopes to monitor environmental or metabolic processes has become indispensable. The potential of enriched Pb (204Pb) was assessed to monitor pathways of trace levels of Pb in humans in a clinical study under the patronage of Glock Health, Science and Research GmbH. This study aimed to assess the effect of purified clinoptilolite tuff on enteral lead uptake in adults using stable lead isotope 204Pb as a tracer. In this randomized, placebo-controlled, double-blind, parallel-group study, 42 healthy participants were randomized. Isotopic pattern composed of a blend of a large quantity of the element with a natural isotopic composition and an enriched stable isotope at orders of magnitude lower levels pose a nontrivial analytical problem. Isotope pattern deconvolution (IPD) was successfully applied as mathematical tool based on multiple linear regressions. The method allowed for deconvolving isotope pattern from measured isotope ratios without knowing the quantities of different isotope sources incorporated and mixed into the sample at levels of < 1 pg 204Pb/ g blood.

SES-11-01

Time: 5/16/2023 11:30:00 AM - 5/16/2023 11:50:00 AM

Exploiting the Urban Mine - Methodology for TCE determination in electronic scrap material using ICP-MS/MS

Klein, Ole (1,2); Zimmermann, Tristan (1); Pröfrock, Daniel (1)

1: Helmholtz-Zentrum Hereon, Institute of Coastal Environmental Chemistry, Inorganic Environmental Chemistry, Max-Planck Str. 1, 21502 Geesthacht, Germany; 2: Universität Hamburg, Department of Chemistry, Inorganic and Applied Chemistry, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

Technology-critical elements (TCEs), represent fundamental components in today's high-tech industry. Besides high demand, many TCE (e.g. Ga, Ge, In, Nb, rare earths, and Ta) are also subject to very high supply risks. The scarcity in the supply chain of almost all TCEs often results from a highly dysfunctional recycling economy. Due to insufficient recycling, TCEs are also regarded potential new pollutants, as little is known about their input and fate in the environment. Under these aspects, especially end-of-life electronic parts, such as printed circuit boards (PCBs), light-emitting diodes (LEDs), or lithium batteries, have attracted great attention from the scientific community. Those three materials stand out primarily because of the potential environmental impact of increasing e-waste streams, but also because of their potential of using e-waste as a so-called "urban mine."

In this study, TCE mass fractions of end-of-life PCBs, LEDs, and lithium batteries were determined by ICP-MS/MS. This involved the development of a suitable digestion method for each of the three materials and the subsequent ICP-MS/MS analysis. The digestion method was developed by testing a total of six acid mixtures in both open and microwave-assisted digestion approaches. The certified reference material (CRM) BAM-M505a (e-waste) was used for method validation. To optimize the measurement of digested e-waste samples an approche of ICP-MS/MS with N2O as reaction gas was used. Here, it was shown that N2O results in better oxide formation rates for many TCEs, when compared to the commonly used O2. Thus the usage of N2O leads to lower LODs but also eneble the mass-shift mode to analytes with do not form oxides wen O2 is used.

Applying the optimized digestion protocol (microwave-assisted, 1 mL HCl, 3 mL HNO3, 1 mL HBF4) and the improved ICP-MS/MS method, all certified elements, except Cr, can be analyzed with recoveries ranging from 76% to 123%. End-of-life PCBs, LEDs, and lithium batteries showed mass fractions ranging from 10 µ g kg-1 ± 30 µ g kg-1 (Ho) to 310 g kg-1 ± 120 g kg-1 (Al). Comparing the element mass fractions with their commercially mined primary ores, it becomes evident that mass fractions e-waste can contain oders of magnitude higher mass fractions of TCEs resulting in enrichmentfactors of up to 500 (Pd). Thus illustrating the high economic value of the urban mine

SES-11-02

Time: 5/16/2023 11:50:00 AM - 5/16/2023 12:10:00 PM

A new technique to study nutrient flow in host-parasite systems by carbon stable isotope analysis of amino acids and glucose

Hesse, Tobias; Nachev, Milen; Khaliq, Shaista; Jochmann, Maik A.; Franke, Frederik; Scharsack, Jörn P.; Kurtz, Joachim; Sures, Bernd; Schmidt, Torsten C.

University Duisburg-Essen, Germany

Stable isotope analysis of individual compounds is emerging as a powerful tool to study nutrient origin and conversion in host-parasite systems. We measured the carbon isotope composition of amino acids and glucose in the cestode Schistocephalus solidus and in liver and muscle tissues of its second intermediate host, the three-spined stickleback (Gasterosteus aculeatus), over the course of 90 days in a controlled infection experiment. Similar linear regressions of δ13C values over time and low trophic fractionation of essential amino acids indicate that the parasite assimilates nutrients from sources closely connected to the liver metabolism of its host. Biosynthesis of glucose in the parasite might occur from the glucogenic precursors alanine, asparagine and glutamine and with an isotope fractionation of –2 to –3 ‰ from enzymatic reactions, while trophic fractionation of glycine, serine and threonine could be interpreted as extensive nutrient conversion to fuel parasitic growth through one-carbon metabolism. Trophic fractionation of amino acids between sticklebacks and their diets was slightly increased in infected compared to uninfected individuals, which could be caused by increased (immune-) metabolic activities due to parasitic infection. Our results show that compound-specific stable isotope analysis has unique opportunities to study host and parasite physiology.

SES-11-03

Time: 5/16/2023 12:10:00 PM - 5/16/2023 12:30:00 PM

Analysis of dissolved trace metals in North Sea water: How to obtain reliable data using ICP-MS/MS?

Przibilla, Anna (1); Iwainski, Susanna (1,2); Zimmermann, Tristan (1); Nantke, Carla (3); Pröfrock, Daniel (1)

1: Helmholtz-Zentrum Hereon, Germany; 2: Fachhochschule Aachen, Faculty of Chemistry and Biotechnology, Germany; 3: Leibniz Institute for Baltic Sea Research, Germany

Trace elements play a major role in biogeochemical cycles and oceanographic processes. The analysis of dissolved metals in the low ng L-1 concentration range is only possible by recent advances in ICP-MS/MS instrumentation. Especially for seawater analysis, coupling the seaFAST© (Elemental Scientific) pre-concentration system to an ICP-MS/MS lowers the detection limits to the sub-ng L-1 range (e.g., <0.005 ng L-1 for Gd, <0.8 ng L-1 for Cu and <0.03 ng L-1 for Cd, Ce and Co) by automatically removing the salt-matrix from the sample. However, analytical results can only be as good as the analyzed sample and the applied sample preparation methods. Seawater samples are taken onboard ships with limited lab space and cooling capacities

during cruises that often last up to several weeks. Here, either sample contamination by the necessary filtration process or bias that might be introduced to unfiltered samples by desorption and adsorption during storage, e.g. from the contained particulate matter or microorganisms, is possible.

We studied the impact of cooling and freezing on aliquots of an unfiltered seawater sample over nine weeks and filtered all aliquots with two different methods in parallel (pressure filtration with NucleporeTM polycarbonate filters and vacuum filtration with recently available DigiFILTERTMs). Analysis of the filtrates by seaFAST© ICP-MS/MS revealed that cooled storage of unfiltered seawater samples is the worst choice for dissolved trace element analysis, especially for the analysis of Co, Mn and Pb, as their dissolved concentrations decreased by 64 %, 93 % and 63 % within nine weeks, respectively. If filtration is not possible directly after sampling, frozen storage of samples is an option, but also in frozen samples, the dissolved Mn concentration decreased by 20 % within nine weeks. Comparing both applied filtration methods regarding their blank values, repeatability and comparability, we obtained similar results from both methods for Cd, Cu, Mo, U, V, W, Zn. For other elements like La, Mn and Pb, the obtained dissolved concentrations differed significantly, leading to 180 % and 440 % higher concentrations for Ce and Fe after pressure filtration compared to vacuum filtration. This indicates that it is important to report not only the filter pore size but also the whole filtration method and monitor filtration blank concentrations instead of analysis blanks.

SES-12

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:00:00 AM Location: Saal 9+10

Chair(s): Kollhoff, Lydia

Session 12: Young Scientist's Meeting

SES-12-KN

Time: 5/16/2023 11:00:00 AM - 5/16/2023 11:30:00 AM

Quantitative Sphingolipidomics in Caenorhabditis elegans via SFC-TIMS-MS

Scholz, Johannes; Rudt, Edward; Hayen, Heiko

Institute of Inorganic and Analytical Chemistry, University of Münster, Germany

Amongst the sphingolipids, sphingomyelins (SMs) represent an abundant lipid class with key functions in the formation and chemical protection of plasma membranes. Especially the functionalisation of defined parts of the cell membrane, so called lipid rafts, is induced by SMs. Recently, SM was identified as a biomarker for various diseases e.g. non-alcoholic fatty liver disease (NAFLD) and is strongly connected to the sphingolipid metabolism as a source for bioactive sphingolipids such as sphingosine-1-phosphate (S1P). To investigate diseases, but also for studies concerning senescence, aging or the toxicity of drugs the nematode Caenorhabditis elegans (C. elegans) is often employed as a model organism. Its' simple and fast cultivation, the limited number of cells and the transparency are some advantages of the worm and reasons for the frequent usage.

In this study, we present a method for the quantification of SMs in C. elegans. This was achieved by the hyphenation of supercritical fluid chromatography (SFC) and trapped ion mobility spectrometry (TIMS) in combination with a quadrupole-time of flight-mass spectrometer (Q-ToF-MS). SFC, using a mobile phase of compressed and heated CO2 and an organic solvent as modifier as well as a polar stationary phase can carry out a fast separation according to the lipid class to ensure the coelution of the internal standard with the lipid class. This coelution minimises differences in matrix effects, e. g. ion suppression, between the internal standard and the analytes to ensure a reliable quantification. The separation within the class was achieved after the chromatographic dimension using TIMS, a postionisation technique for separation by size and shape. This technique is based on an electric field gradient trapping the ions in a funnel while a nitrogen flow is pushing them forward. The separation, which is achieved by lowering the electric field gradient, gives information on the mobility of the ions and thus the collision cross section (CCS) as well as the three-dimensional structure of the analytes. This additional dimension of separation helps with the identification of complex mixtures and supports the quantification. In addition, structural information was generated through Parallel Accumulation Serial Fragmentation (PASEF) MS/MS experiments. For the first time, this 4D-Lipidomics method enables the quantification of lipids via a hyphenation of SFC and timsTOF technology.

SES-12-01

Time: 5/16/2023 11:30:00 AM - 5/16/2023 11:50:00 AM

Formation, Characterisation and Disassembly of Metallosupramolecular Complexes in vacuo

Geue, Niklas (1); Bennett, Tom S. (1); Arama, Alexandra A. (1); Ramakers, Lennart A. I. (1); Timco, Grigore A. (1); Whitehead, George F. S. (1); McInnes, Eric J. L. (1); Armentrout, Peter B. (2); Burton, Neil A. (1); Winpenny, Richard E. P. (1); Barran, P

1: The University of Manchester, United Kingdom; 2: University of Utah, USA

The interest in metallosupramolecular complexes has rapidly grown in the last decades, along with a simultaneous increase in the complexity of the synthesised molecules. Their characterisation can be difficult, as X-Ray crystallography or reliable computations are often not feasible due to the complex size, and solution phase techniques are limited in the information they provide.[1] Ion mobility mass spectrometry (IM-MS), coupled with tandem mass spectrometry and supported by DFT calculations, are not only uniquely qualified to structurally characterise these complexes, but also to investigate the properties of their fundamental building blocks. We showed that we can a) evaluate and tune their stability by substituting metals, ligands and charge carriers, b) discriminate competing disassembly mechanisms, c) assess the complexes' topology, conformational flexibility, atom density and cavity size, and d) identify novel target molecules for bulk phase synthesis.[2-4]

In the last years we have been studying a range of polymetallic complexes using IM-MS, including a homometallic {Cr8} ring, open {Cr6} horseshoe oligomers, a series of heterometallic {Cr7M} rings (M = MnII, FeII, CoII, NiII, CuII, ZnII, CdII) and their related rotaxanes, as well as {CrxCu2} hourglass and {Cr12Ln4} lanthanide clusters. The common feature of all these complexes is their ability to encapsulate or coordinate secondary ammonium cations [NH2RR']+ via non-covalent interactions, making these supramolecular polymetallic complexes interesting targets for e.g. quantum computing.

Our observations, taken together, provide an empirical framework to follow in for the future design and characterisation of self-assembled polymetallic complexes. We highlight the advantages and opportunities of using IM-MS for synthetic molecules, yielding a comprehensive overview of how this technique can study various properties of the same compound family.

[1] Geue et al., Structural characterisation methods for supramolecular chemistry that go beyond crystallography, Chem. Soc. Rev. 2022, 51, 8-27.

[2] Geue et al., Disassembly Mechanisms and Energetics of Polymetallic Rings and Rotaxanes,

J. Am. Chem. Soc. 2022, 49, 22528 – 22539.

[3] Geue et al., Adduct Ions as Diagnostic Probes of Metallosupramolecular Complexes using Ion Mobility Mass Spectrometry, Inorg. Chem. 2023, ASAP.

[4] Geue et al., Formation and Characterisation of Polymetallic Rings in vacuo, under review at Nat. Synth., ChemRxiv 2022.

SES-12-02

Time: 5/16/2023 11:50:00 AM - 5/16/2023 12:10:00 PM

The reactive glucose metabolite dimethylglyoxal mimics neurological complications of diabetes and its levels in the brain are influenced by the gene ILVBL

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Introduction: Reactive glucose metabolites are involved in neurological complications of diabetes by generating advanced glycation end products (AGEs). AGEs are formed by the reaction of dicarbonyls with proteins and induce oxidative stress, tissue damage and inflammation. Moreover, high levels of blood glucose worsen the outcome of ischemic stroke. So far, only glyoxal (GO), methylglyoxal (MGO) and 3-deoxyglucosone (3-DG) have been recognized as reactive glucose metabolites.

Materials & methods: Native dicarbonyls were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS; TSQ Endura, Thermo Scientific). 13C-labeled dicarbonyls generated from 13C6-labeled glucose or 13C3-sodium pyruvate were analyzed using a high-resolution accurate mass platform (Q-Exactive, Thermo Scientific). Deuterated methylglyoxal (d4-MGO) was used as internal standard (IS). Dicarbonyls were analyzed after derivatization with deuterated o-phenylenediamine (d8-OPD). Formed quinoxalines were separated on an Ascentis Express C18 or a Cortex®T3, applying a gradient elution with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B with a flow rate of 0.2 ml/min. Diabetic conditions in mice were induced by streptozotocin (STZ) administration. After 12 weeks, behavior was analyzed in an object place recognition test (OPRT). Acute hyperglycemia was induced by 12C6-glucose or 13C6-glucose injection prior to a permanent occlusion of the distal middle cerebral artery (MCAO) to model hyperglycemic stroke. Plasma, brain and liver were analyzed.

Results: When inducing diabetes by injecting STZ, mice were unable to identify the replaced object after a 1-hour delay, indicating a cognitive decline under diabetic conditions. To investigate the underlying mechanisms, we measured α-dicarbonyls by derivatizing them with o-phenylenediamine (OPD) and detecting formed quinoxalines using LC-MS. As IS, we originally planned to use 2,3-dimethylquinoxaline (2,3-DMQ). Surprisingly, we observed higher intensity of 2,3-DMQ in plasma of diabetic mice compared to the control group, suggesting that hyperglycemia has increased levels of either 2,3-DMQ itself or dimethylglyoxal (DMG), the dicarbonyl that generates 2,3-DMQ after derivatization. Therefore, we replaced the derivatizing agent and IS by deuterated o-phenylenediamine (d8-OPD) and by deuterated methylglyoxal (d4-MGO), respectively. The new analysis revealed elevated concentrations of DMG in plasma and brain of diabetic mice. Subsequently, DMG effects were investigated. Long-term treatment with DMG for 14 weeks led to signs of cognitive decline in the OPRT, resembling those observed in the STZ model. Therefore, treatment with DMG mimicked neurological complications of diabetes. Elevated levels of DMG in diabetic mice raised the possibility that it is a product of glucose metabolism. Studies in mouse brain endothelial cells (bEnd.3) showed that DMG is generated from glucose via two molecules of pyruvate. [13C1-2], [13C1-3] and [13C3-1,2,3] pyruvate, but not [13C1-1] pyruvate, enriched half-labeled [13C2] DMG, indicating that C-1 (carboxyl group) of pyruvate is not incorporated in the DMG

structure. Interestingly, we observed higher levels of [13C2] DMG and [13C4] DMG under hypoxic conditions compared to normoxic conditions. Subsequently, we investigated the role of Ilvbl, a gene homologous of acetolactate synthase that mediates DMG formation in bacteria and yeast. Knockdown of Ilvbl lowered levels of [13C4] DMG in cells, under hypoxic conditions. In our diabetic animal model, Ilvbl was involved in the generation of DMG in the brain. Here, DMG levels were higher in Ilvbl +/+ mice compared to Ilvbl -/- mice. However, Ilvbl did not influence levels of DMG in plasma or liver. In acute hyperglycemic mice, hypoxic conditions generated by MCAO increased levels of labeled and non-labeled DMG in the ipsilateral ischemic hemisphere compared to the contralateral hemisphere of the brain. In both hemispheres of the brain, non-labeled DMG levels were higher in Ilvbl +/+ than in Ilvbl -/- mice. Only in the ipsilateral hemisphere of the brain, [13C2] DMG levels were significantly reduced in Ilvbl -/- mice compared to Ilvbl +/+ mice. However, DMG generation was not influenced by ILVBL in acute hyperglycemic mice without ischemia, possibly due to overall lower levels of DMG in the brain.

Conclusions: Our data highlight the importance of DMG as reactive glucose metabolite. Levels of this compound are elevated in plasma and brain of mouse models of diabetes and hyperglycemic stroke. In mice with chronic hyperglycemia and in ischemic mice under acute hyperglycemic conditions, DMG generation in the brain was attenuated by Ilvbl knockout. Importantly, DMG led to cognitive impairment in mice. DMG and ILVBL could therefore be pharmacological targets in diabetes and hyperglycemic stroke.

SES-12-03

Time: 5/16/2023 12:10:00 PM - 5/16/2023 12:30:00 PM

Development of a reference measurement procedure for the quantification of cardiac troponin using inductively coupled plasma mass spectrometry

Kuhfuß, Danja (1); Müller, Marvin (1); Swart, Claudia (1); O´Connor, Gavin (1,2)

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Cardiac troponin (cTn) is a diagnostic and prognostic biomarker of myocardial infarction and acute coronary syndrome. Like many other protein biomarkers, the measurement of cTn lacks standardization. This can lead to routine laboratory measurements generating non-comparable results which can often lead to mistreatment or misdiagnosis of patients. The aim of this work is to establish a reference measurement procedure (RMP), that could be used in the production of matrix calibrators and/or quality control materials, enabling the evaluation of all cTn assays in real clinical settings. The provision of comparable measurement results would inform on appropriate patient reference ranges and enable common decision-limits, expressed in agreed measurement units, resulting in better patient treatment.

Isotope dilution mass spectrometry (IDMS) is a well-established measurement procedure which has been successfully applied for the accurate quantification of biological analytes. For proteins, this is often achieved via the use of specific proteolytic peptides. Here we investigated a complementary potential RMP for cTn subunit I (cTnI). In comparison to the more common ESI-MS approaches, our approach is based on the combination of metal-based isotope dilution and ICP-MS. To make the peptides detectable via ICP-MS the peptides were first covalently modify with a metal. This was achieved by the selective reaction of cysteine amino acids with a label consisting of a lanthanide incorporated in 2,2′,2”-(10-(2-((2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-Mal). Preliminary

experiments, using synthetic cTnI specific peptides containing the metal tag, without the enrichment step, using LC-ICP-MS resulted in detection limits of 25 nmol/L peptides (corresponding to 600 µ g/L cTnI). As cTn is a low abundant protein, which is normally present in blood at the ng/L range, we investigated an immunoaffinity enrichment method, using magnetic beads, to achieve lower limits of detection.

Metal labeling followed by GluC digestion of a recombinant cTnI yielded fully labeled peptides. These were separated using reversed phase-liquid chromatography and detected with ICP-MS. Enrichment with cTnI specific antibodies bound to protein G magnetic beads from a buffer solution of cTnI resulted in a LOD of 10 µ g/L of cTnI. However, subsequent studies in serum resulted in a LOD of 120 µ g/L due to matrix effects. The method is currently being improved to reduce background noise levels and enhance recovery.

This project 18HLT10 CardioMet has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme.

SES-13

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:00:00 PM Location: Goldsaal

Chair(s): Brockmeyer, Jens

Session 13: Proteomics II

SES-13-KN

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:30:00 PM

Proteomics and phosphoproteomics for uncovering cancer biology and precision oncology

Jimenez, Connie

Amsterdam University Medical Center, Netherlands, The

Next generation quantitative proteomics based on data-independent acquisition mass spectrometry (DIA-MS) enables large scale clinical proteomics. In the oncology context, where personalized treatment requires analysis of single samples, phosphoproteomics coupled to Integrative Inferred Kinase Activity (INKA) analysis has emerged as tool that can prioritize actionable kinases for targeted inhibition (1-3).

In the first part of my talk I will present a multi-laboratory collaborative cancer proteome profiling effort, The DIA-MS-based Cancer Proteome Atlas (TCPA) to better understand cancer biology and to identify core and cancer type enriched molecular therapeutic targets and biomarkers. This pan-cancer proteome landscape consists of 999 primary cancers, yielding a filtered dataset of 9663 proteins. Unsupervised analyses as based on UMAP, hierarchical clustering and ssGSEA of cancer hallmarks show remarkable cancer type clustering despite heterogeneous sample types. Known cancer biology and immune subtypes are rediscovered by various bioinformatic approaches, along with cancer (sub)type associated proteins that may provide novel markers and targets.

In the second part of my talk, I will present our on-going progress in CRC, tALL and PDAC to employ phosphoproteomics coupled to INKA analysis for target discovery and pinpointing personalized kinase inhibitor (combination) treatment in cancer.

1. Beekhof R, Henneman AA, Jimenez CR et al.,. INKA, an integrative data analysis pipeline for phosphoproteomic inference of active kinases. Mol Syst Biol. 2019 May 24;15(5):e8981.

2. Cordo' V, Jimenez CR, Meijerink JPP et al., Phosphoproteomic profiling of T cell acute lymphoblastic leukemia reveals targetable kinases and combination treatment strategies. Nat Commun. 2022 Feb 25;13(1):1048.

3. Piersma SR, Henneman AA, Jiménez CR et al.,. Inferring kinase activity from phosphoproteomic data: Tool comparison and recent applications. Mass Spectrom Rev. 2022 Sep 26:e21808

SES-13-01

Time: 5/16/2023 2:30:00 PM - 5/16/2023 2:50:00 PM

Quantification of site-specific histone acetylation rates combining acetyl-CoA and acetylation dynamics by Proteo-Metabo-Fluxomics.

Egger, Anna-Sophia (1); Rauch, Eva (1); Sharma, Suraj (2); Heiland, Ines (2); Thedieck, Kathrin (1); Kwiatkowski, Marcel (1)

1: Universität Innsbruck, Austria; 2: The Arctic University of Norway, Tromsø, Norway

Histone acetylation is an important reversible modification and a hallmark of epigenetics. To understand the biological function of this reversible modification, it is not enough to simply determine abundance levels, but it is necessary to capture individual acetylation and deacetylation reactions at global level. We developed a quantitative proteo-metabo-flux approach and pipeline for simultaneous quantification of acetyl-CoA turnover and site-specific histone acetylation rates based on combined metabolic and chemical labeling (CoMetChem).

We used histone deacetylase (HDAC) inhibitors (SAHA, MS-275) to demonstrated that our CoMetChem approach allows the activity of HDAC inhibitors to be described by site-specific histone acetylation reaction rates. We were able quantify site-specific histone acetylation dynamics and our results suggested that a pre-existing acetyllysine promotes acetylation of a neighboring lysine. As expected MS-275 and SAHA treatment resulted in decreased deacetylation rates. Interestingly, however, we were also able to show that MS-275 increased the acetylation rate of the doubly acetylated H3 species at both K18 and K23.

We used simultaneous proteo-metabo-flux analysis to investigate whether our proteo-metabo-flux approach allowed to describe the effects of reduced glycolytic activity induced by AKT inhibition (MK-2206 treatment) at the level of acetyl-CoA and histone acetylation dynamics. Our results showed that the correction of acetyl-CoA label incorporation is necessary for accurate quantification of histone acetylation rates, which may otherwise be underdetermined by up to 50%. Reduced glycolytic activity resulted in lower acetyl-CoA turnover and changes in histone acetylation reaction rates.

CoMetChem and simultaneous proteo-metabo-flux analysis allows a comprehensive description of reversible acetylation dynamics by quantifying both acetylation rates and deacetylation rates, and to link dynamics in energy metabolism with histone PTM dynamics.

SES-13-02

Time: 5/16/2023 2:50:00 PM - 5/16/2023 3:10:00 PM

A mass spectrometry detergent for biochemistry

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When drugs are administered to human bodies it is likely that therapeutic effects are caused by drug binding to membrane-bound proteins. Native mass spectrometry and detergents are increasingly used to study the structural organization of membrane proteins in response to environmental factors, such as proteins, lipids, and

salt. Finding a detergent that enables both purification and native mass spectrometry of membrane proteins is challenging. With the aim to design universally applicable detergents, a research alliance has been initiated, including researchers from University of Oxford, Freie Universität Berlin, and TU Dortmund University. Here, our latest progresses will be presented regarding the development of a promising detergent class: dendritic oligoglycerol detergents. The molecular structure of oligoglycerol detergents can be tuned to optimize individual parameters relevant to the purification and analysis of membrane proteins, including protein yields, delipidation, charge reduction, and the ability to analyse membrane proteins from physiologically relevant buffers. Recent case studies identified an optimized oligoglycerol detergent candidate, i.e., first-generation, dendritic oligoglycerol detergent ([G1] OGD). Results obtained from purification and analysis demonstrate its utility for monitoring cooperativity in salt and ligand binding to G-protein-coupled receptors (GPRCs) and the role of lipids in the oligomerization of drug resistance pumps. The GPCR family and drug resistance pumps are currently intensively studied due to their substantial roles in human health and disease. The optimized [G1] OGD serves as an enabling step for capturing membrane proteins and their structural responses to environmental interactions by native mass spectrometry. More broadly, oligoglycerol detergents can facilitate research at the interface of mass spectrometry and biochemistry.

SES-13-03

Time: 5/16/2023 3:10:00 PM - 5/16/2023 3:30:00 PM

Oxidation is an underappreciated PTM in the regulation of immune responses that interferes with protein abundance and other PTMs: integrative analysis of redoxome, phosphoproteome, and proteome in macrophage activation

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Innate immune cells, such as macrophages, play a central role in the first line of defense against xenobiotics and pathogens, and contact with them triggers complex molecular mechanisms. In addition to the proteome, which has been characterized extensively in the past to gain insights into induced molecular mechanisms, the regulation of post-translational modifications (PTMs) is of great importance to understand the regulation of protein function. In the activation of macrophages, PTMs such as phosphorylation, acetylation, and ubiquitination have already been identified to be relevant and investigated using PTM-omics. However, there is as yet no global information on changes in the oxidation status of immune cells during activation, although these can trigger complex signaling cascades.

To assess the relevance of oxidations in macrophages and the extent of their interaction with the phosphoproteome, we analyzed the redoxome, phosphoproteome, and proteome of 4 h or 24 h lipopolysaccharide (LPS)-activated THP-1 macrophages using tandem mass tag (TMT)-based approaches. For the proteome and redoxome, 20 µg protein per sample was used, and for the phosphoproteome, up to 100 µg of protein was used. Samples were prepared using a paramagnetic bead approach that included off-line orthogonal fractionation, resulting in two fractions per sample, which were subsequently subjected to a nano-UPLC system (Ultimate 3000, Dionex, USA) coupled to the mass spectrometer (QExactive HF, Thermo Scientific, USA) via a chip-based ESI source (Nanomate, Advion, USA).

We reliably quantified 4268 proteins, 3407 phosphorylated sites, and 3003 oxidized sites, with 26 %, 27 %, and 34 %, significantly altered (adjusted p-value ≤ 0.05) after 24 h of LPS stimulation, respectively. Next, an integrative analysis workflow including enrichment analyses as well as network-based analyses was applied, revealing the time-dependent regulation of proteins, phosphorylation, and oxidations. Thereby, clear differences in the dynamics of phosphoproteome and redoxome were observed. While protein abundance and phosphosite abundance generally showed the same trends at 4 h and 24 h, the redoxome led to the opposite trends, with increased cysteine oxidation after 4 h and decreased oxidation of the respective sites after 24 h, and vice versa.

Considering Reactome's Toll-like receptor 4 cascade, which represents the activation pathway of macrophages treated with LPS and was significantly enriched after 24 hours, we found 29 proteins associated with this pathway significantly altered. These included 19 proteins that were altered in abundance, 5 regulated phosphosites and 25 regulated oxidation sites. Other enriched pathways included mainly those involved in inflammatory and metabolic processes, for which several proteins showing effects on phosphosites as well as oxidized cysteines were identified, i.e. LCP1, PTPRC, and CD44 as modified proteins in inflammatory processes and FASN, PGAM1, and ENO1 as candidates for metabolic processes.

Overall, this study, based on integrative analysis of proteome, phosphoproteome, and redoxome at two time points, highlights the importance of the redoxome in the investigation of inflammatory processes as well as the benefits of considering multiple omics layers to gain detailed mechanistic insights.

SES-14

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:00:00 PM Location: Silbersaal

Chair(s): Schmitz, Oliver

Session 14: Metabolomics

SES-14-KN

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:30:00 PM

Isomer-selective analysis of the phosphoinositide signaling network by liquid chromatography-tandem mass spectrometry

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Phosphoinositides are regulating key metabolic processes including signal transduction via phospholipase C and phospshoinositide-3-kinase pathways. Arising from dynamic phosphorylation/dephosphorylation on the inositol ring of phosphatidylinositols (PI), phosphoinositides (PIPx) comprise seven classes according to phosphate position and number. Combined with various fatty acid side chains high structural complexity arises. For a comprehensive understanding of the phosphoinositide signaling, analytical coverage of the entire metabolic network is highly desirable including besides above lipid classes also the corresponding hydrophilic inositolphosphate metabolites, such as the second messenger inositol triphosphate (I(1,4,5)P3). However, the analysis of the entire PIPx network remains extremely challenging due to their low abundance, high negative charge density, presence of regioisomers, and extremely wide polarity range of the metabolites involved in the network, including PI and DAG (lipophilic), PIPx (amphiphilic), and inositol phosphates (IPx) (hydrophilic). Here, we describe an integrated workflow for these metabolites using a single sample aliquot. After neutral monophasic lipid extraction, the supernatant is taken for general untargeted lipidomics analysis by reversed-phase liquid chromatography (RP-UHPLC), in which PI and DAG are analyzed amongst other lipid classes. The residue is extracted by a two-layer aqueous-organic acidic solvent mixture. The organic layer containing PIPx is employed for phosphate methylation and RP-type chiral UHPLC to separate their regioisomers. The aqueous phase is lyophilized and after phosphate methylation used for isomer-selective RP-UHPLC separation on a cholesterylether-bonded column. Sequential window acquisition of all theoretical mass spectra (SWATH) is used for untargeted general lipidomics analysis and PIPx analysis to acquire comprehensive MS/MS information. Selected reaction monitoring (SRM) is adopted for targeted detection of IPx. This workflow is isomer-selective, reproducible, accurate, suitable for high throughput, which enables the comprehensive analysis of the PIPx signaling network without bias from regioisomer. Some applications will be discussed as well.

SES-14-01

Time: 5/16/2023 2:30:00 PM - 5/16/2023 2:50:00 PM

Lipidomics and Metabolomics analysis reveals impact of ischemic stroke on brain, liver and plasma

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Ischemic stroke is one of the leading causes of mortality and adult disability worldwide. Although there has been great progress in understanding the mechanisms following an ischemic event, treatment options for patients are still limited. Naturally, the brain as primarily affected organ has been the focus of many studies. However, recent research has underlined the relevance of systemic effects of cerebral ischemia. Therefore, we performed untargeted metabolomics and lipidomics to investigate a mouse model of ischemic stroke using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Additionally, serum samples of ischemic stroke patients were analyzed.

Plasma, brain and liver tissue of C57BL/6 mice were collected 2 hours, 24 hours and 1 week after a permanent distal middle cerebral artery occlusion (pMCAO). Infarct size was determined based on a silver staining. Brain tissue for MS analysis was divided into the ischemic ipsi- and the contralateral hemisphere. A control group with sham surgery was included for every time point. Additionally, serum samples collected from ischemic stroke patients one, two and three days after the ischemic event were analyzed. High-resolution accurate mass (HRAM) MS in combination with LC is the method of choice for untargeted approaches due to outstanding sensitivity and high-confidence identification based on exact mass, isotopic pattern, fragmentation and retention time. Plasma, serum and tissue samples were analyzed with two different approaches optimized for either metabolomics or lipidomics. Following a one-phase extraction, hydrophilic metabolites and lipids were separated via hydrophilic interaction LC (HILIC), and reversed-phase (RP-) LC, respectively. Data-dependent data acquisition was performed using a hybrid quadrupole-Orbitrap mass spectrometer (Q-Exactive, Thermo Fisher). Software-based data analysis (TraceFinder 5.1, Compound Discoverer 3.1; Thermo Fisher) included compound identification supported by an MS2 in-house library. Statistical analysis was performed using MetaboAnalyst (5.0).

In total, we were able to detect over 170 metabolites and over 350 lipid species covering several pathways and lipid classes. Numerous changes in metabolite and lipid levels were observed in mice undergoing pMCAO at all three time points. Multivariate analysis (sparse partial least squares discriminant analysis, sPLS-DA) of the brain resulted in a good distinction between pMCAO and control group, with better discrimination based on the metabolite dataset compared to lipids. Same applied to the distinction between ipsi- and contralateral side. For both datasets, the 2-hour time point could clearly be distinguished from the other time points, with greater proximity to the control groups. In the brain, the arginine-, methionine- and other amino acid-related pathways as well as the phosphatidylcholine and carnitine metabolism were altered. In the plasma, changed metabolites were also related to arginine and carnitine pathways and to the energy metabolism. Changes in plasma lipid levels were most pronounced 24 hours after pMCAO. Strikingly, the metabolomic profile of the liver was dramatically changed by the pMCAO already after 2 hours, persisting for up to 1 week, while lipidomic changes in the liver were

only prevalent after 24 hours. Predominantly affected were the purine pathway, and again, the arginine-, methionine- and carnitine pathways as well as the phosphatidylcholine metabolism. Additionally, numerous metabolites and lipids in both tissue and plasma correlated with the infarct size allowing assumptions about metabolite levels, stroke severity and the potential outcome. To underline the clinical relevance of our results, we also investigated serum samples of ischemic stroke patients. Whereas the metabolomic changes showed only little overlap, lipidomics results of human serum and mouse plasma showed similarities concerning the carnitine and phosphatidylcholine metabolism, especially when the outcome of patients was taken into account.

Our untargeted approach based on a metabolomics and a lipidomics screen allows the detection of a broad range of substances. High-confidence identification based on online and in-house libraries enables a straightforward biological interpretation of the results. The mouse model of ischemic stroke provides the opportunity to investigate the effects of cerebral ischemia in different organs, like brain and liver, and excludes confounding clinical parameters. However, the incorporation of a human study is essential for translational research. Our explorative study leads to new hypotheses concerning ischemic stroke and potentially new treatment options. However, further studies are necessary to confirm those findings and to better understand the interaction of brain and periphery in ischemic stroke.

SES-14-02

Time: 5/16/2023 2:50:00 PM - 5/16/2023 3:10:00 PM

Two-Dimensional Liquid Chromatography Tandem-Mass Spectrometry reveals the Deep Metabolome of Marine Dissolved Organic Matter

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Dissolved organic matter (DOM) is one of the most complex natural mixtures. Besides its ubiquity, DOM plays a major role in biogeochemical cycling. Especially in marine systems, where it is of fundamental importance for nutrient retention, trace metal complexation, long term carbon sequestering and further aspects. However, DOM remains poorly understood on the molecular level, which hampers our understanding of ecosystem functioning and potential feedback loops under climate change scenarios. Although multiple studies investigated the chemical composition of DOM with different ultrahigh resolution mass spectrometry or tandem mass spectrometry (MS/MS) approaches, a high degree of non-resolved isomeric complexity is still suggested. To resolve those isomeric compounds, improved separation of molecular features must be achieved before detection by (tandem) mass spectrometry. To further improve the chromatographic separation of DOM we used a 2D-LC-MS/MS approach and employed subsequent molecular networking (MN). Our method consists of a 2D-separation with a first, high pH (1D) offline fractionation and second (2D) low pH reversed-phase separation, followed by negative and positive mode MS/MS. Our results show a reduced sample complexity while enhancing MS2 spectra coverage, leading to more library hits, more networked nodes and a higher annotation rate. In summary, our data provides an unprecedented view into the molecular composition of marine DOM, and highlights the potential for 2D LC-MS/MS approaches to decipher ultra-complex mixture.

SES-14-03

Time: 5/16/2023 3:10:00 PM - 5/16/2023 3:30:00 PM

EMBL-MCF 2.0: A high-resolution LC-MS/MS library and workflow for untargeted metabolomics using low-adsorption surface chromatography

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Metabolomics Core Facility, EMBL Heidelberg, Germany

Driven by steady developments in instrumentation, software, protocols and study design, metabolomics is maturing to become a standard technology, which is already routinely applied in various scientific fields. Despite the growing demand and availability, there are still remaining challenges (e.g., sensitivity, selectivity, and accurate metabolite identification) that the metabolomics community needs to overcome in order to maintain its relevance. With the introduction of different confidence levels for compound annotation, the Metabolomics Standards Initiative has established clear reporting guidelines to ensure the quality of metabolomics datasets.1 Accordingly, to reach highest level confidence in metabolite identification, a matching orthogonal analytical characteristic, like chromatographic retention time, has to be reported in addition to the mass spectrometric data. Unfortunately, this requirement is often not met by researchers.2

In this work we present a new metabolomics workflow together with an LC-MS/MS library,3 covering MS/MS spectra and corresponding retention times for ~250 biologically relevant metabolites. High-resolution data was acquired on an Orbitrap Exploris 240, using authentic standards and a novel

low-adsorption surface column (Atlantis Premier BEH Z-HILIC) in combination with a biocompatible UHPLC system (Vanquish Horizon). By reducing non-specific adsorption, peak broadening and tailing are minimized, in particular for challenging phosphorylated compounds that are prone to interference with metal surfaces in analytical systems. The improved chromatography contributes to enhanced sensitivity and peak capacity and enables the separation of many isomeric compounds (e.g., Leucine/Isoleucine, AMP/cGMP, Sarcosine/Alanine/β-Alanine etc.).

Ultimately, the method was applied to various sample matrices (e.g., plasma, cells, tissue, bacteria, yeast) and it could be demonstrated that a high coverage of metabolites with excellent precision was achieved. Overall, the proposed workflow and publicly available library provide a promising tool, which is compatible with open-source software (e.g., MS-DIAL) and can be easily transferred to and utilized by other laboratories.

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2 G. Theodoridis et al., Ensuring Fact-Based Metabolite Identification in Liquid Chromatography–Mass Spectrometry-Based Metabolomics. Anal. Chem, 2023, 95, 8, 3909-3916

3 P. Phapale et al., Public LC-Orbitrap Tandem Mass Spectral Library for Metabolite Identification. J. Proteome Res., 2021, 20, 4, 2089-2097

SES-15

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:00:00 PM Location: Saal 4+5

Chair(s): Bendt, Anne

Session 15: Females in Mass Spectrometry (FeMS)

SES-15-KN

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:30:00 PM

FeMS network - current overview

Bendt, Anne

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Females in Mass Spectrometry (FeMS) is a group that aims to promote and support women in the field of mass spectrometry. Founded in 2019 by Dr. Anne K Bendt and other mass spec scientists, FeMS has since grown to become a leading community of female scientists in mass spectrometry, with a focus on providing mentorship, networking, and career development opportunities to its members.

During the 90-minute session, Dr. Anne K Bendt will provide an overview of the history and mission of FeMS. Dr. Bendt will also share some of the key initiatives that FeMS has undertaken to support women in mass spectrometry, such as the FeMS mentorship program and the FeMS virtual seminar series. Prof. Kathryn Lilley, an accomplished academic in mass spectrometry, will discuss her academic path. She will share her personal experiences, including the challenges she faced and the strategies she used to overcome them. Dr. Maria Fedorova will discuss the importance of building a professional network. She will share strategies for developing and maintaining professional relationships, including how to identify and connect with potential collaborators and mentors. Dr. Fedorova will also discuss the role of networking in her career development and the benefits of being part of a supportive community like FeMS. Dr. Olya Vvedenskaya will share her experience in volunteer work. She will discuss the benefits of volunteering, both for personal growth and professional development. Dr. Vvedenskaya will also provide advice on how to identify volunteer opportunities that align with your interests and goals, and how to make the most of your volunteer experience. Dr. Anne K Bendt will talk about her unique perspective on scientific research and innovation, drawing on her experiences in both academia and industry. Dr. Bendt will share her experiences and insights on building a successful scientific career outside of the traditional academic path, and offers advice and guidance to those who are interested in pursuing similar paths.

Following the presentations, there will be a networking activity where attendees will have the opportunity to connect with other members of the FeMS community. The networking activity will provide a chance for attendees to share their own experiences, learn from one another, and build lasting professional relationships.

https://femalesinms.com/

SES-16

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:00:00 PM Location: Saal 9+10

Chair(s): Heyer, Robert

Session 16: Data Science

SES-16-KN

Time: 5/16/2023 2:00:00 PM - 5/16/2023 2:30:00 PM

The future has arrived, now try to enjoy it - AI meets MS

Martens, Lennart

Ghent University, Belgium

Tba

SES-16-01

Time: 5/16/2023 2:30:00 PM - 5/16/2023 2:50:00 PM

Unraveling the Complexity of Metabolite Transformations: Using ChemProp2 for prioritizing Potential Biotransformations in LC-MS/MS-based Untargeted Metabolomics

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Non-targeted metabolomics using liquid chromatography-tandem mass spectrometry (LC–MS/MS) is increasingly used to study biotransformation such as drug metabolism or xenobiotic and natural product biotransformation in the environment. While the wealth of data and potential molecules that can be observed offers great promise, the data analysis and annotating unknown transformation products remain challenging. To annotate unknown compounds, different in silico annotation, and static spectral propagation approaches, such as molecular networking, have been developed. To prioritize potential biotransformations within molecular networks, we have conceptualized a chemical proportionality algorithm termed ChemProp2 that identifies anti-correlating related metabolites and thus putative reaction pairs. ChemProp2 makes use of Feature-Based Molecular Networking and scores the correlation of two structurally or (bio)chemically related compounds that could have derived from one another with respect to sequential data points (e.g.over time or space). The score can be then used to prioritize and visualize the directionality of these potential biochemical alterations directly in the molecular network. We validated ChemProp2 with acetylation reactions observed in bacterial drug metabolism, in which we incubated B. subtilis with sublethal concentrations of sulfonamide antibiotics. Taken

together, ChemProp2 facilitates prioritizing potential biotransformations in the untargeted LC-MS/MS based metabolomics.

SES-16-02

Time: 5/16/2023 2:50:00 PM - 5/16/2023 3:10:00 PM

HarmonizR enables the missing value tolerant integration of independently generated proteome datasets across quantification platforms, tissue types and LC-MS setups.

Voss, Hannah Luise (1); Schlumbohm, Simon (2,1); Godbole, Shweta (1); Schuhmann, Yannis (2,1); Dottermusch, Matthias (1); Neumann, Philipp (2,1); Wurlitzer, Marcus (1); Krisp, Christoph (1); Neumann, Julia (1); Schlüter, Hartmut (1)

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Investigating the proteome can add a significant layer of information to biomarker discovery studies, as proteins and their modifications represent the pharmacologically addressable phenotype of cells and tissues. Bottom-up Liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) enables the simultaneous quantification of thousands of proteins from complex samples. In-depth proteome analysis requires long measurement times for individual samples. Together with the general low availability of clinical samples for certain phenotypes this may result in small sample cohorts, limiting the usability and validity of statistical methods.

Integrating in-house data, with datasets from public sources can overcome this limitation but is restricted by the induction of batch-effects trough variable technical setups and high numbers of missing values.

Using the newly developed HarmonizR framework, being able to reduce batch effects without the need for data reduction or missing value imputation, we show –based on in-house and publicly available datasets- the successful integration of proteomic data across different tissue types (Fresh Frozen, Formalin Fixated Paraffin embedded (FFPE)), quantification platforms (DDA, DIA, SILAC, TMT), and Liquid chromatography coupled tandem mass spectrometry (LC-MS) setups.

Applying this strategy, we integrated multiple proteome datasets on different disease. Our results confirm proposed proteomic cancer subtypes from small, independent studies with reliable statistical validity. Additionally, new disease subtypes with relevant clinical features could be identified from integrated datasets. Our framework thus enables the identification of disease subpopulations, associated molecular signatures, and altered signaling pathways in biomarker discovery studies with high statistical validity from small, independent cohorts and might be especially beneficial for the proteomic characterization of rare disease phenotypes.

SES-16-03

Time: 5/16/2023 3:10:00 PM - 5/16/2023 3:30:00 PM

Automated Analysis of Time-Dependent, Big Data for Ultrahigh Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Rüger, Christopher P. (1,2); Celis-Cornejo, Carlos M. (2,3); Maillard, Julien (2,4); Sueur, Maxime (5); Salvato-Vallverdu, Germain (2,3); Afonso, Carlos (2,5); Giusti, Pierre (2,4); Rodgers, Ryan (2,6); Bouyssiere, Brice (2,3); Chacón-Patino, Martha (2,6)

1: University of Rostock, Germany; 2: International Joint Laboratory – iC2MC: Complex Matrices Molecular Characterization; 3: Université de Pau et des Pays de l'Adour, France; 4: TotalEnergies One Tech R&D, TotalEnergies Research & Technology Gonfreville, France; 5: University of Rouen Normandy, France; 6: National High Magnetic Field Laboratory, Florida, United States

In the past decade, the development of novel, more powerful mass spectrometric platforms and their application towards sample materials with ever-increasing complexity revealed bottlenecks in the comprehensive data processing. Ultra-high resolution mass spectrometry, by means of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), has revolutionized complex mixture analysis with unprecedented resolution and mass accuracy that enables molecular formula assignment for tens of thousands of species in a single measurement. Unsurprisingly, hyphenated techniques, such as thermal analysis [1], or chromatographic separation, such as gas [2] or liquid chromatography [3,4], yield extremely large data sets (~10-50 GB) that contain millions of mass spectral peaks. To capture the full potential of time-resolved, high resolution mass spectrometry analyses, automated data processing workflows are needed.

A Python-based framework was developed called PyC2MC (Python for Complex Matrices Molecular Characterization). An advanced software architecture was designed based on our foundation of MATLAB workflows published for the comprehensive treatment of gas chromatographic and thermal analysis coupled to 7 T FT-ICR MS [5]. This framework allows for robust feature detection in multidimensional mass spectrometry data using a heuristic K-means clustering methodology and an iterative method as suggested by Tautenhahn et al. [6], the molecular formula attribution, and two-dimensional mass spectra recalibration.

The software was evaluated utilizing data from three different FT-ICR MS platforms, commercial 7 and 12 T Bruker MRMS systems as well as the world's most powerful 21 T FT-ICR MS platform installed at the National High Magnetic Field Laboratory (NHMFL, Florida, US). Exemplarily, complex data sets of asphaltene samples, a highly aromatic and high molecular weight petroleum fraction, were studied. These ultra-complex organic mixtures are subjected to mass spectrometric detection after online coupling of gel permeation chromatography, resulting in over 900 scans per run with a total of over 2.5 million peaks. As heuristic k-means method ckmeans, an optimized algorithm developed by Wang and Song [7], which compared to the available conda libraries with standard clustering methods (scikit-learn clustering) is twice as fast and gives more consistent results. The approach used here consists of an initial grouping of the data into islands of peaks distributed in each nominal mass along the time axis. A substantial improvement in processing time has been found compared to previous attempts, resulting in comprehensive data treatment in less than five minutes, accomplishing a more time- and resource-efficient feature detection, attribution, validation and input/output data streaming. From a chemical perspective, these advanced processing routines allowed us for a fast and robust attribution of metal-organics (Vanadium/Nickel-porphyrins) within ultra-complex petrochemical and recycling matrices. From the field of

energy transition, we were to able safely attribute Boron-containing oxygenated constituents within biomass pyrolysis oil matrices.

Moreover, we developed a new open-code free Python-based (version 3.9.7, libraries: pandas, NumPy, SciPy) software tool within the framework of PyC2MC dedicated to facilitating data visualization, comparison and statistics. [8] The graphical user interface developed under PyQt5 aims for user-friendly access and high applicability (https://github.com/iC2MC/PyC2MC_viewer). This program can be handled by users with little to no programming experience, but it is also improvable and adaptable for the community with knowledge of Python. The intention of this software is to be useful for anyone treating high-resolution mass spectrometry data of complex matrices.

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SES-17

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:00:00 AM Location: Silbersaal

Chair(s): Phapale, Prasad

Session 17: MS-Imaging

SES-17-KN

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:30:00 AM

Mass Spectrometry Imaging in the Life Sciences: New approaches, current improvements, future trends

Spengler, Bernhard

Justus Liebig University, Germany

Mass spectrometry imaging (MSI) has experienced an enormous gain in attention over the last decade, having entered virtually all areas of the life sciences with much success. Many mass spectrometric technologies with their individual advantages and limitations have evolved or have been adapted for creating molecular images, including LDI, DESI, nano-DESI and other extraction-based methods, SIMS, LA-ICPMS, and, last but not least, MALDI. Having to use a supporting matrix is certainly a disadvantage in terms of easiness and flexibility of a method, but still the advantages of MALDI over the other methods appear to prevail, at least when counting the number of scientific publications. Further improving the “sensitivity” (i.e., the limit of detection) is one of the goals of several developments, for example by employing post-ionization by a second laser or by letting the desorbed molecules cross an electrospray beam. We have combined UV- and IR-MALDI MSI with dielectric barrier discharge (DBD) postionization in this context, with much success especially for non-polar compounds, which are harder to ionize by MALDI. Regarding enhancement of information contents of images, on-tissue derivatisation techniques and automated MS/MS methods were employed, but also statistics, MS/MS and accurate-mass databases, bioinformatics, and big data technologies play important roles here.

Atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) combined with Orbitrap-based mass analysis has demonstrated convincing properties and broad applicability of MALDI instrumentation: The high spatial resolution along with high mass accuracy / mass resolution provides unprecedented insight into biochemical and physiological processes in tissues and cells. Host – guest interactions of, e.g., parasite eggs in infected host liver were molecularly imaged to uncover tissue adaptation gradients and granuloma formation, or to detect release or uptake of substances by host or guest, as defense or as nutrients, respectively. Drug-treated Fasciola hepatica and Schistosoma mansoni were imaged to investigate drug uptake routes and metabolization. On the single-cell level, unicellular parasites in animal skin cysts were imaged and infection markers were determined for various diseases by combining MSI and LC-MS/MS data. Single-cell heterogeneity was described for fibroblast and for microglia cells with laser spot sizes (pixel resolutions) down to 1.4 micrometers.

Besides animal and human tissue analysis, AP-SMALDI MSI has also been applied, for example, to plant metabolomics and fungi fruiting body topography analysis in the context of production of natural flavors. The lecture will give examples of application and describe trends in technological and methodological advancements.

Acknowledgement

Financial support by the LOEWE Center for Novel Drug Targets against Poverty-Related and Neglected Tropical Infectious Diseases (DRUID) is gratefully acknowledged.

Conflicts of interest:

BS is a consultant of TransMIT GmbH, Giessen, Germany.

SES-17-01

Time: 5/17/2023 10:30:00 AM - 5/17/2023 10:50:00 AM

Dataset-dependent MALDI-TIMS-MS imaging: Towards full MS² coverage on biological tissues

Heuckeroth, Steffen (1); Behrens, Arne (2); Wolf, Carina (1); Korf, Ansgar (2); Fütterer, Arne (2); Richter, Henning (3); Jeibmann, Astrid (4); Karst, Uwe (1); Schmid, Robin (5)

1: University of Münster, Germany; 2: Bruker Daltonics GmbH & Co. KG, Germany; 3: University of Zurich, Switzerland; 4: University Hospital Münster, Germany; 5: Institute of Organic Chemistry and Biochemistry of the CAS, Czech Republic

Mass spectrometry (MS) imaging enables the spatial mapping of compound distributions within tissue sections. When applied to biomedical imaging, resolutions in the lower µm range can provide insight into tissues affected by disease states or other conditions. Therefore, MS imaging is becoming more prominent in the life sciences. However, the technique mostly relies on annotations by exact masses only, which leaves a large number of potential isobaric and isomeric compounds. A reason is that most instruments are lacking a data-dependent acquisition (DDA) of fragmentation spectra (MS2). State-of-the-art ion mobility spectrometry (IMS)-enabled mass spectrometers provide collisional cross sections (CCS), improving annotation capabilities for IMS-MS imaging. While the provided CCS values allow more confident annotations, the community still lacks MS2 acquisition strategies.

Here, we present the spatial ion mobility-scheduled exhaustive fragmentation (SIMSEF) workflow to plan dataset-dependent MS2 acquisition and subsequently acquire on trapped ion mobility spectrometry-quadrupole time-of-flight MS (TIMS-qTOF-MS) in an offline approach. An MS1 image is acquired and analysed in the open-source software MZmine 3,[1] creating a comprehensive list of precursor ions and their images. The new SIMSEF module efficiently schedules MS2 experiments across the tissue, based on spatial distribution, intensity, ion mobility, and spectral purity, i.e., a clean precursor isolation window. Multiple precursors are scheduled in a single imaging pixel along the IMS separation, similar to the parallel accumulation serial fragmentation (PASEF) acquisition mode employed in liquid chromatography-IMS-MS. This allows optimal precursor coverage and minimises the required acquisition time. The quality of MS2 spectra is optimised by acquiring multiple collision energies per precursor and scheduling the same precursor multiple times across the whole tissue.

Subsequently, the scheduled SIMSEF experiments were acquired by the parallel reaction monitoring (prm)-PASEF-MALDI prototype supplied by Bruker Daltonics. Downstream data analysis pairs MS2 spectra to their respective precursors in MZmine. The combined CCS, m/z, and MS2 data boost confidence in obtained annotations by spectral library matching, rule-based lipid annotations, and manual MS2 evaluation. The integration of MZmine

with other tools like GNPS and SIRIUS provides advanced workflows, including molecular networking and fragment tree-based compound annotation.

On a sheep brain tissue section, SIMSEF recorded 32646 individual MS2 spectra of 1997 unique precursors with up to 5 different collision energies in just 4468 imaging pixels used for MS2 acquisition on an image with 31,402 pixels. The 300 ms IMS ramp provided separation of lipid species and time to schedule up to 21 precursors (7.3 on average). The analysed sheep brain thin-section yielded annotations to lipids by rule-based annotation and metabolites by spectral library matching.

[1] Article in Press, Nature Biotechnology, https://doi.org/10.1038/s41587-023-01690-2

SES-17-02

Time: 5/17/2023 10:50:00 AM - 5/17/2023 11:10:00 AM

Transmission-mode MALDI-2 Ion Source with In-source Bright-field and Fluorescence Microscopy for Direct Correlative Imaging of Tissue Sections and Single Cells at One Micrometer Pixel Size

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Introduction

Transmission-mode MALDI combined with laser-induced postionization (t-MALDI-2) enables MS imaging (MSI) at sub-cellular resolution and pixel sizes down to about 1 µ m. (Niehaus et al., Nat. Methods, 2019, 16, 925-931.) To advance the method further, a precise co-registration of optical (e.g., bright-field, fluorescence) microscopy and t-MALDI-2-derived MSI data is pivotal. Here we present the integration of slide-scanning microscopy into a t-MALDI-2-MS imaging ion source. By using the same closed-loop piezo-actuated sample stage and microscope objective for material ejection and optical microscopy, both modalities are inherently co-registered with sub-micron fidelity. The ion source was coupled to a state-of-the-art QTOF analyzer system (timsTOF fleX MALDI-2) enabling high-speed data acquisition. We demonstrate the capabilities of our correlative imaging method with selected tissues and cell cultures.

Methods

A timsTOF-fleX MALDI-2 instrument (Bruker) was heavily modified to integrate a piezo-actuated XYZ-stage (SmarAct) and an UV-transmitting objective (Mitutoyo) in transmission mode geometry. The standard timsTOF-fleX smartbeam laser (wavelength: 355 nm, repetition rate: 1 - 10 kHz) was re-routed and used for material ejection, producing ablation marks of ~1 µ m width on the sample. Dielectric mirrors were employed to enable the parallel use of the objective for material ejection at 355 nm and optical microscopy with a CCD camera (The Imaging Source). The sample was front side-illuminated using an LED ring light. Fluorescence images were recorded by inserting filter sets specific to the employed fluorescence stains, each consisting of an excitation filter, a dichroic mirror, and an emission filter, into the beam path. Fluorescence excitation light was generated by fiber-coupled LEDs. Python-based software, developed in-house, was used for autofocusing, acquisition and stitching of optical images. Overlays were generated using flexImaging (Bruker) and SCiLS Lab software

(SCiLS/Bruker). Tissue sections (7-10 µ m thick) and cell cultures (grown directly on glass slides) were coated with different matrices using sublimation and spray deposition protocols.

Results

MALDI-MSI analyses at micrometer spatial resolution require dedicated sample preparation protocols that produce a homogenous microcrystalline or amorphous matrix layer. (Bien et al., Proc. Natl. Acad. Sci. U.S.A., 2022, 119, e2114365119.) Well-prepared samples allowed for the analysis at cellular to sub-cellular resolution. We present MALDI-2-MSI measurements of tissue sections and cell culture at high lateral resolution with pixel sizes as low as 1 µ m x 1 µ m.

With our setup, the MS images and optical images recorded in the ion source are automatically co-registered with a position error < 1 µ m by utilizing the absolute positional accuracy (< 40 nm) of the closed loop stage. No selection of fiducial markers is required for co-registration. On the basis of the optical microscope image, regions of interest for the following MSI measurements can be marked precisely without the need to include notable error margins. This reduces measurement of unpopulated areas at the employed high lateral resolutions and decreases measurement time dramatically. Recording microscope images of different fluorescence channels, produces a high degree of biological and chemical information, complementary to subsequent MALDI-MSI measurements, and enables, for example, generating single-cell mass spectra for specific cells of interest.

The potential of the method is demonstrated with different murine organs, which were coated with HABA matrix using a sublimation protocol. Laser-postionization (MALDI-2) critically increases analytical sensitivity and chemical depth of the MSI analysis. (Soltwisch et al., Science, 2015, 348, 211-215.) We will show overlays of t-MALDI-2-MSI data of different lipid ion species and the microscopy images produced inside the ion source prior to the MALDI-MSI measurement. For example, in mouse cerebellum, small-scale features of the granular layer, the Purkinje cell layer and the white matter regions are all well resolved in the t-MALDI-2 data. Next to tissue sections, we will demonstrate the analysis of cell cultures with sub-cellular resolution and direct co-registration of optical microscopy data. Using automated cell segmentation algorithms based on fluorescence images of Hoechst 33342 (cell nuclei) and FITC coupled WGA (cell membranes) stains, regions of interest for single cells or small groups of cells can be automatically generated for subsequent MSI measurements. By omitting the empty regions between cells, measurement time was decreased by about a factor of five, depending on the population density and cell size.

The presented method could constitute a significant milestone in MALDI-MSI of biological samples because of the additional information gained by the perfectly coregistered high lateral resolution MSI data and fluorescence as well as optical brightfield, images. The ability to record both modalities in one instrument and in one coordinate system opens up new avenues for biomedical and clinical applications.

SES-17-03

Time: 5/17/2023 11:10:00 AM - 5/17/2023 11:30:00 AM

High-Resolution AP-SMALDI MS Imaging of Exogenous and Endogenous Compounds in the Parasite Fasciola hepatica

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Introduction

The parasite Fasciola hepatica (F. hepatica) causes fascioliasis, one of the neglected tropical diseases listed by the WHO.[1] Worldwide, the worm poses a significant threat to various mammals, including humans. High-resolution atmospheric-pressure scanning microprobe MALDI (AP-SMALDI) MS imaging can offer molecular-level insights into the physiology and metabolism of the parasite, which has received little attention until now. As a result, AP-SMALDI MSI findings may aid the understanding of biochemical processes within the worm, contribute to the comprehension of drug operation mechanisms and eventually support the development of new treatment strategies.

Methods

Drug-treated or untreated F. hepatica were embedded in 8% gelatin solution, sectioned (20 µ m thickness) using a cryotome, thaw-mounted onto regular glass slides, and stored at –80 °C until MSI analysis. Before measurement, sections were thawed in a desiccator for 30 min and subsequently covered with DHB, CHCA, or DAN matrix utilizing a pneumatic sprayer system (SMALDIPrep, TransMIT GmbH, Giessen, Germany). Measurements were performed using an AP-SMALDI5 AF ion source, including a 3D autofocusing system (TransMIT GmbH, Giessen, Germany) coupled with a Thermo Scientific Q Exactive HF instrument[2] (Thermo Fisher Scientific, Bremen, Germany). Analyses covered an m/z range of 250–1000 and were conducted at pixel sizes between 15 and 7 µ m in positive- or negative-ion mode. The Mirion software package[3] and the LIPIDMAPS[4] database were used for data analysis and lipid annotation, respectively.

Preliminary results[5][6]

Triclabendazole (TCBZ), Imatinib, and the drug candidate Schl-33.292 were detected in sections of individually treated F. hepatica specimens. Whilst signals of TCBZ and Imatinib were already present in the worm after 20 min of incubation, the Schl-33.292 signal was not found before 12 h of exposure. At the early incubation times, TCBZ was exclusively detected in the outer surface (tegument) of the parasite, whilst Schl-33.292 was only present in the intestine. These findings point towards a tegumental and oral drug uptake, respectively. During further incubation, the drugs spread into other tissues, such as gastrodermis and reproductive organs, as well. Furthermore, signals of predicted Imatinib and Schl-33.292 metabolites were present in the MSI data, spatially superimposable with the signals of the parent drugs.

By imaging sections of the untreated parasite, we investigated the endogenous lipids, present in the parasite's most relevant organs in terms of host contact and reproduction (i.e. gastrodermis, tegument, ovary, testes) and

revealed signals of lipid species that accumulate or even specifically occur in those organs. This points to a specific lipid fingerprint of the different pathogen tissues.

In combination with information obtained from histological staining, these lipid markers also enabled precise localisation of the aforementioned drug compounds within the tissue section.

Novel aspect

Unveiling spatial distributions and metabolic pathways of various compounds in the parasite F. hepatica using high-resolution AP-SMALDI mass spectrometry imaging.

Acknowledgement

Financial support by the LOEWE Centre for Novel Drug Targets against Poverty-Related and Neglected Tropical Infectious Diseases (DRUID) is gratefully acknowledged.

Conflicts of interest

BS is a consultant and SG is a part-time employee of TransMIT GmbH, Giessen, Germany

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SES-18

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:00:00 AM Location: Saal 4+5

Chair(s):

Session 18: Food analysis

SES-18-KN

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:30:00 AM

Understanding Food as biological system – proteomics to tackle authenticity and food safety

Brockmeyer, Jens

Universität Stuttgart, Germany

Food of plant or animal origin can be considered as biological systems with highly complex biochemical composition. Historically, food quality and safety of food has mainly been assessed by detection and quantitation of specific analytes that reflect certain traits while the remaining system has been regarded as mere “food matrix”.

The molecular complexity of food systems can however be exploited to address different relevant aspects of food quality, safety, or authenticity. Mass spectrometry has been one of the central techniques to provide a more systematic understanding of complex foods employing lipidomics, metabolomics, and proteomics.

In this presentation a variety of food proteomics approaches are presented that allow to address relevant and new aspects of authenticity, quality, and safety in different food systems.

SES-18-01

Time: 5/17/2023 10:30:00 AM - 5/17/2023 10:50:00 AM

Cheese metaproteomics unravels microbial dynamics of One-Health relevance.

Tilocca, Bruno; Roncada, Paola

University "Magna Graecia" of Catanzaro, Italy

Cheese is an important component of the Mediterranean diet, commonly appreciated for its health-promoting features and unique taste. Here, among the variety of cheeses, a distinct importance is attributed to raw goat cheese. A pivotal role in the development of these characteristics is attributed to the cheese-associated microbiota and its continuous remodelling over space and time. To date, no thorough metaproteomics study of the raw goat cheese-associated microbiota has previously been conducted and a comprehensive picture of such microbiota, in terms of both composition and activity, is desirable to guide the onset and enhancement of both gustatory and nutraceutical properties. To fulfil this knowledge gap, we employed 16S rRNA gene sequencing and

metaproteomics to explore the functional microbiota of a typical raw goat milk cheese at various ripening time points. Also, structural and functional features of the raw goat cheese microbiota were assessed in the rind and core of the cheese mass. The outcomes of this first explorative survey portrayed a stable architecture of the microbial community over the selected ripening time points, providing evidence of a stepwise, unsteady, fermentation of the cheeses.

The thorough characterization of the composition and activity of the microbiota at different cheese-wheel depths described a rind microbiota struggling to maintain the biosafety of the cheese through competition mechanisms and/or by preventing the colonization of the cheese by pathobionts of animal or environmental origin. On the other hand, the core microbiota was focused on third biochemical processes, supporting its role in the development of both the health benefits and the pleasant gustatory nuances of goat cheese.

SES-18-02

Time: 5/17/2023 10:50:00 AM - 5/17/2023 11:10:00 AM

How processing affects marker peptide abundance in feed ingredients

Stobernack, Tobias; Höper, Tessa; Herfurth, Uta M.

German Federal Institute for Risk Assessment (BfR), Department Food Safety, National Reference Laboratory for Animal Protein in Feed

Feed processing, just as food processing, increases its safety by destroying bacteria. Other pathogens like prions, which induce spongiform encephalopathy in ruminants, cannot be inactivated by standard processing. Therefore, European Regulation 999/2001 imposes strict feeding rules, including ruminant material not being allowed for feed except for milk products and hydrolysed tissue that may be fed to all or just certain species. As current official control methods (microscopy, PCR) are not tissue-specific, several marker peptides have been validated for tissue-specific ruminant detection in feed [1, 2].

In addition to strict feeding rules, animal material with high risk of carrying prions is required to be steam-pressure-sterilized by European Regulation 1042/2011 in order to inactivate potentially present prions. Lower risk material intended to be fed may undergo no treatment at all, be dryed, or pasteurized depending on its origin and classification. Processing techniques are known to impact e.g. allergen quantification by protein modification and degradation by Maillard reaction, oxidation, and lipid peroxidation. This may result in underestimation of up to 80% for milk and nuts in cookies and bread [3, 4]. How, and to which extent, feed processing methods impact mass spectrometric species detection has not been studied to date.

To address this, 37 feed ingredients of bovine origin comprising various tissues and processing levels were collected. Furthermore, bovine meat-and-bone-meals were produced by steam-pressure sterilization according to Regulation (EU) 1042/2011 (20 min, 133 °C, 3 bar; variations: 20 min, 150 °C, 5 bar; 60 min, 100 °C, 1 bar) and subsequent drying. Aliquotes were sampled throughout the processing workflow to be able to trace the impact of individual techniques on the proteins. All samples were analyzed by an extensive bottom-up proteomic workflow using data-dependent analysis on a nanoUHPLC-ESI-Q-Orbitrap mass spectrometer. Three replicate injections were followed by another three injections measured excluding those peptides identified in the first round. Peptide identification was based on regular database search either including 52 post-translational modifications retrieved from food proteomics literature or allowing semitryptic cleavage. Furthermore, an error-tolerant search was conducted, allowing mass shifts from -40 to 210 Da.

Across the entire dataset, 35,092 peptides (including 19,450 modified and 4260 semitryptic) belonging to 4,049 proteins were identified. All 52 searched modifications were detected albeit with a wide range of occurrence spanning from two (imidazolon A) to more than thousand identifications (for each deamidation, single and double oxidation). Error-tolerant search identified 343 additional mass increments that were assigned to mostly amino acid substitutions, physiological modifications, further Maillard products and cation binding. Modifications were unevenly distributed among the product groups. E.g. whereas early Maillard products were predominantly present in milk products, late Maillard products were similarly distributed in milk and blood-based products.

In order to estimate the effect of processing on marker peptide detection and quantification, peak areas of all modified and semi-tryptic versions of a marker sequence were summed up and related to the peak area of the unmodified marker peptide. This estimated modification rate was determined in all samples for all marker peptides long-listed for ruminant detection in feed as well as for hemoglobin β (HBB), serum albumin (ALB), and α-s2-casein (CASA2), which were taken as reference proteins. Within the latter, peptides showed remarkable differences in both susceptibility to modification as well as their span of modification rate across all samples. Consistently and highly modified peptides with modification rates always exceeding 90% were ALB375-386, HBB66-75, and HBB82-94. Other peptides were e.g. consistently low (ALB346-359) or wide-spanning medium (ALB549-557) or highly modified (HBB95-103, CASA248-56). During peptide marker selection, candidate sequences containing amino acids prone to modifications are usually rejected. This was very effectively done for milk markers where out of five marker peptides no modified versions were detectable for two of these peptides (β-lactoglobulin108-116, 165-178) and further two showed low, confined modification rates (CASA138-49, < 5%; CASA2130-140, 12-17%). In contrast, most blood markers showed wide-ranging modification rates. Peptides with these wide-ranging modification rates may indicate the degree of processing that a sample underwent. However, when tracing modification numbers along the meat-and-bone meal production, physiological modifications especially in bone material (hydroxyproline) distorted the results. Further investigation of this dataset will consequently focus on individual, rather processing-induced modifications.

In conclusion, processing-induced modifications do very differently affect peptide markers even though those were carefully selected. It is thus highly recommendable to verify limits of detection with various materials that were subjected to a variety of processing conditions. Furthermore, protein quantitation in processed matrices may have to be improved in future by considering major versions of the marker peptides.

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SES-18-03

Time: 5/17/2023 11:10:00 AM - 5/17/2023 11:30:00 AM

Solving the PFAS Challenge: Comprehensive Screening of 1000s of Relevant Compounds in a Single Run from Organisms at Different Trophic Levels

Gkotsis, Georgios (1); Damalas, Dimitrios E. (1); Nika, Maria-Christina (1); Baessmann, Carsten (2); Galvin, Robert (2); Macht, Martina (2); Thomaidis, Nikolaos S. (1); Ingendoh, Arnd (2)

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Per- and Polyfluoroalkyl Substances (PFAS) are known as “forever chemicals” due to their persistent, bio-accumulative and toxic (PBT) properties and ubiquitous presence in the environment and organisms. Currently, around 5,000 PFAS are marketed worldwide, making their systematic environmental monitoring an extremely challenging task. Adding trapped ion mobility spectrometry (TIMS) to LC-HRMS allows the very comprehensive monitoring of organic micropollutants (PFAS, pharmaceuticals, pesticides and others) in complex environmental matrices such as biota, through targeted and untargeted workflows.

Presented here is LC-TIMS-HRMS as a complete solution for PFAS characterization in environmental samples, combining ion mobility supported target analysis with wide-scope suspect and non-target screening,

Extracts suitable for untargeted screening were generated through a generic sample preparation protocol developed for the simultaneous extraction of 56 PFAS from different sub-groups. The analysis was conducted using LC-TIMS-QTOF. Data independent (bbCID) acquisition and PASEF, an efficient data-dependent acquisition mode, were used for targeted and untargeted workflows, respectively. The target analysis based on four independent criteria was run on the target list of 56 PFAS. In the untargeted data processing workflow, the raw data was transformed into a comprehensive feature table. Then the detected features were lined up by Kendrick mass analysis and annotated using a PFAS suspect list of ca. 5,000 compounds. In-silico prediction of MS/MS spectra and CCS values for the suspected compounds was performed for their identification.

Due to the PFAS structure there is a high probability of isomers, making their separation and structure elucidation very demanding. TIMS capabilities were evaluated to separate coeluting isobaric and isomeric analytes. The ion mobility filtrating resulted in higher sensitivity and lower detection limits of the targeted PFAS as well as significantly higher quality of full-scan MS and bbCID MS/MS spectra. The use of collisional cross sections (CCS) as additional identification criteria enhanced the identification confidence.

A feature table containing thousands of entries was created in the untargeted workflow. Kendrick mass defect analysis filtered features as potential PFAS from the matrix background, based on high fluorine content (repeating CF2 units). Wide-scope suspect screening of 5,000 PFAS using in-silico MS/MS and CCS value prediction was evaluated as a comprehensive approach for a fast and efficient identification.

The proposed workflow provides a comprehensive solution for the characterization of 1000s of PFAS compounds in complex environmental matrices. Therefore, it will assist in understanding the chemical universe of PFAS in the environment and protecting environment, wildlife, and human health.

SES-19

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:00:00 AM Location: Saal 9+10

Chair(s): Thevis, Mario

Session 19: Natural Products

SES-19-KN

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:30:00 AM

Identification of activity-relevant natural products and their modes of action by the correlation of metabolic profiling, bioactivity and genetic data

Wessjohann, Ludger A.

Leibniz-Institut für Pflanzenbiochemie (IPB), Germany

There are three common problems in natural product chemistry: How to find the metabolites relevant for a desired bioactivity? How to avoid replication, i.e. how to avoid cumbersome and costly isolation and structure elucidation of natural products already known for the desired purpose? And how to quickly identify a possible mode of action (target) of a candidate compound?

The interplay of MS-based omics technologies with computational tools allows a faster and more efficient approach to identify both, the relevant candidate natural products without de-replication (i.e. without knowing its structure yet) and the potential mode of action. The general concept of such approaches based on correlation analysis will be presented and a number of examples will be given. These include identification of new natural products in neuroactive plant extracts, pathogen resistance compounds and genetic markers for hops breeding, or the discovery of anticancer modes of action of natural products.

SES-19-01

Time: 5/17/2023 10:30:00 AM - 5/17/2023 10:50:00 AM

Connecting Chemotype to Phenotype - Functional Metabolomics Strategies for Culture-Independent Natural Product Discovery

Petras, Daniel (1); Aron, Allegra (2); Hernandez, Albert (1); Hughes, Chambers (1); Naimi, Amira (3); Pakkir Mohamed Shah, Abzer (1); Reher, Raphael (3); Stincone, Paolo (1); Wagner, Berenike (1)

1: University of Tuebingen, Germany; 2: University of Denver, USA; 3: University of Marburg, Germany

The chemical diversity of natural products represents a tremendous source of bioactive compounds that are fundamentally important for organismal and ecosystem function. Thanks to recent advances in mass spectrometry, bioinformatics, and the massive efforts of the scientific community over the last century, we can identify a wide range of natural products in complex environments.

While such molecular inventories can provide us detailed insights into molecular mechanisms in a given system, the number of known compounds and activities represents only a very small fraction of all natural products. To fully map out and leverage the chemical and bioactivity space of nature, functional assays are needed that scale to contemporary non-targeted metabolomics workflows. To address this need, we develop mass spectrometry-based metabolomics methods that integrate online bioactivity profiling as well as native mass spectrometry. Here we will showcase some of our recent native metabolomics studies and discuss the potential and further development of functional metabolomics strategies for the screening of bioactivity and molecular interactions, which we hope will contribute to a more systematic mapping of the functional roles and fate of small molecules in biology.

SES-19-02

Time: 5/17/2023 10:50:00 AM - 5/17/2023 11:10:00 AM

Pharmacokinetic interaction of rosuvastatin with artichoke (Cynara scolymus L.) leaf extract in rats

Al Masalmeh, Ahmad (1); Mallah, Eyad (1); Mansoor, Kenza (1); Abu-Qatouseh, Luay (1); Darwish El-Hajji, Feras (2); Idkaidek, Nasir (1); Basheti, Iman (2); Haj Issa, Israa (3); Al Meslamani, Ahmad Z. (4); Aws, Saba (1)

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Nowadays, natural products are very common either as complementary therapies in combination with conventional drugs, or as alternative therapies to replace conventional drugs. LC-MS is commonly used in characterization and identification of the relevant plant components. The current study was designed to evaluate the lipid-lowering effect of artichoke leaf extract (ALE) in combination with both low and high doses (10/20 mg/kg) of rosuvastatin, a drug used to treat high cholesterol levels. Artichoke is a plant that is commonly used to treat high levels of cholesterol or other lipids in the blood (hyperlipidemia). Thus, this study was performed to evaluate the drug-herb interaction due to the simultaneous use of artichoke leaf extract (ALE) with both doses of rosuvastatin as well as to the effect of chlorogenic acid (CGA), which is one of the main constituents in artichoke extract. Furthermore, the effect of ALE on the pharmacokinetic profile of rosuvastatin in rat plasma was evaluated by using HPLC.

In-vivo studies were carried out on 56 laboratory rats. Dyslipidemia was induced by intragastric cholesterol intake (2 g/kg) every day for 4 weeks. After inducing dyslipidemia, rats were separated into 7 groups. The first group (control) was given cholesterol solution for another 4 weeks, and the remaining six groups continued to take cholesterol solution for another 4 weeks along with different treatment protocols.

This study has highlighted and supported the effect of rosuvastatin and artichoke both alone and in combination in controlling hyperlipidemia , with several treatment protocols showing significance at the $p \leq 0.01$ level. It would serve as preliminary evidence for both researchers and healthcare providers in combinational therapy between conventional and natural drugs. Furthermore, CGA has proven its efficacy ($p \leq 0.05$) in decreasing lipids, and this indicates that CGA, one of the main components in ALE, may be responsible for reducing lipids. In addition, the pharmacokinetics study showed that coadministration between artichoke extract and a low dose of rosuvastatin

decreased rosuvastatin's bioavailability, and significantly ($p \leq 0.01$) affected Cmax and AUC along with decreasing its concentration in the serum blood from 755 to 388 ng/ml. After the separation of the results into males and females, the gender factor showed an important role in better determining the results and their accuracy.

We hope to take advantage of this approach in the future by studying the combined effect of artichoke with high-dose rosuvastatin using LC-MS techniques.

SES-19-03

Time: 5/17/2023 11:10:00 AM - 5/17/2023 11:30:00 AM

Highly sensitive detection of C20-isoprenyl-phosphates in plant tissues enables detection of changes in substrate availability for the synthesis of vitamin E and vitamin K

Gutbrod, Katharina; Dörmann, Peter

University of Bonn, Germany

Isoprenyl-phosphates are crucial building blocks of a pleithora of plant metabolites, such as carotenoids or phytohormones, but their levels are extremely low and direct detection of isoprenyl-phosphates in plant tissues was difficult. Various indirect techniques have been developed to measure isoprenyl-phosphates, such as radioactive labeling and derivatization. Liquid chromatography-mass spectrometry (LC-MS) methods have improved sensitivity, but still necessitate a considerable amount of sample for extraction.

This research introduces a new procedure that isolates the monophosphates and diphosphates of farnesol, geranylgeraniol, and phytol, using an isopropanol-containing buffer and quantitating them with Q-TRAP LC-MS while utilizing citronellyl-P and citronellyl-PP as internal standards. With a low limit of detection, isoprenyl-phosphates can be precisely quantified in Arabidopsis leaves or seeds, beginning with just 20 mg of fresh weight.

Using this method, we were able to show that the phosphorylation of isoprenoid alcohols is required for the synthesis of vitamin E and vitamin K and characterize the responsible plastidial enzymes.

SES-20

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:00:00 AM Location: Goldsaal

Chair(s): Schrader, Wolfgang

Session 20: Automation / Instrumentation II

SES-20-KN

Time: 5/17/2023 10:00:00 AM - 5/17/2023 10:30:00 AM

Mass Spectrometry-Based Techniques to Elucidate the Sugar Code

Grabarics, Marko (1,2); Lettow, Maike (1,2); Kirschbaum, Carla (1,2); Greis, Kim (1,2); Manz, Christian (1,2); Pagel, Kevin (1,2)

1: Freie Universität Berlin, Berlin, Germany; 2: Fritz Haber Institute of the Max Planck Society, Berlin, Germany

Cells encode information in the sequence of biopolymers, such as nucleic acids, proteins, and glycans. Although glycans are essential to all living organisms, surprisingly little is known about the “sugar code” and the biological roles of these molecules. The reason glycobiology lags behind its counterparts dealing with nucleic acids and proteins lies in the complexity of carbohydrate structures, which renders their analysis extremely challenging. Building blocks that may differ only in the configuration of a single stereocenter, combined with the vast possibilities to connect monosaccharide units, lead to an immense variety of isomers, which poses a formidable challenge to conventional mass spectrometry. In recent years, a combination of innovative ion activation methods, commercialization of ion mobility–mass spectrometry, progress in gas-phase ion spectroscopy, and advances in computational chemistry have led to a revolution in mass spectrometry-based glycan analysis.1 Here we showcase for a couple of examples how complex oligosaccharide structures can be unambiguously identified using ion mobility mass spectrometry and cryogenic ion spectroscopy.

1. M. Grabarics, M. Lettow, C. Kirschbaum, K. Greis, C. Manz, K. Pagel, Mass Spectrometry-Based Techniques to Elucidate the Sugar Code, Chem. Rev., 2022, 122, 7840.

SES-20-01

Time: 5/17/2023 10:30:00 AM - 5/17/2023 10:50:00 AM

Mass Spectrometry Platform as Sample Delivery System for Gas-phase Protein Small-angle X-ray scattering (SAXS) Experiments

Kung, Jocky Chun Kui (1,2); Damjanović, Tomislav (1,2,3); Kierspel, Thomas (1,3); Uetrecht, Charlotte (1,2)

1: CSSB Centre for Structural Systems Biology, Deutsches Elektronen-Synchrotron DESY, Leibniz Institute of Virology (LIV), Hamburg, Germany; 2: Faculty V: School of Life Sciences, University of Siegen, Siegen, Germany; 3: European XFEL GmbH, Schenefeld, Germany

The Mass Spectrometry for Single Particle Imaging of Dipole Oriented Complexes (MS SPIDOC) consortium has designed and constructed a mass spectrometry platform for single particle imaging and related experiments at X-ray light sources. Mass spectrometry and other well-developed gas-phase techniques present an attractive sample delivery and handling method for X-ray strucutral biology experiments. Major advantages include the reduction of background scattering in the solvent free environment, and separation and isolation of particles by their masses and conformations. We have designed a mass spectrometry platform to exploit these advantages and to also include instrumentation that performs dipole orientation of the particles, enabling structural reconstruction from much smaller datasets. The instrument prototype is constructed, tested and recently modified for gas-phase protein small-angle X-ray scattering experiments at the EMBL BioSAXS Beamline of the PETRA III synchrotron. With further experiments at different light sources, the MS SPIDOC platform promises to be a valuable sample delivery system for protein SAXS experiments.

SES-20-02

Time: 5/17/2023 10:50:00 AM - 5/17/2023 11:10:00 AM

Laser Pulse Length Effects in Ultrafast Laser Desorption

Schmidt, Marco (1,2); Irsig, Robert (2,3); Duca, Dumitru (1,2); Peltz, Christian (4); Passig, Johannes (1,2); Zimmermann, Ralf (1,2)

1: Joint Mass Spectrometry Centre, Analytical Chemistry, University of Rostock, 18059 Rostock, Germany and Comprehen-sive Molecular Analytics (CMA) Cooperation Group, Helmholtz Centre Munich, 81379 Munich, Germany; 2: Department Life, Light & Matter, University of Rostock, 18059 Rostock, Germany; 3: Photonion GmbH, 19061 Schwerin, Germany; 4: Institute for Physics, University of Rostock, 18059 Rostock, Germany

Ultrafast laser pulses in mass spectrometry offer new and advantageous opportunities for laser desorption (LD) and ionization of the analytes. By shortening the laser pulses, the ability to ablate any material with reduced thermal damage and without any absorbing matrix is given. However, it is unclear, which pulse lengths are optimal for desorption performance with respect to the key parameters ‘softness’ and ‘efficiency’.

To answer this question, we performed a systematic study on pulse lengths effects in laser desorption, using a two-step laser mass spectrometry approach (L2MS). In general, the L2MS setup allows to optimize each process

individually and study the LD without affecting ionization. By keeping all optical parameters except of the pulse length constant, we directly compare the pulses in the range from femtosecond to nanosecond pulse lengths with respect to fragmentation and desorption efficiency. To exclude interfering effects, e.g. from collisional cooling, we perform all experiments in vacuum. As postionization method we use resonance-enhanced multiphoton ionization (REMPI) with only minimum additional fragmentation.

To enhance the transferability of the study results to real-world applications, we investigate the LD for common medicaments like naproxen and ibuprofen in tablets, and for retene on quartz fiber filter as model of an environmental sample. We perform the fragmentation experiments by placing the liquid nitrogen-cooled tablets directly in high vacuum. There, the tablets are exposed to 800 nm pulses with a pulse length from 50 fs to 6 ns prior to postionization via REMPI with a 266 nm Nd:YAG-laser. For the LD-induced fragmentation, we find moderate benefits already for the picosecond pulses. While the fragmentation pattern does not show any major differences for pulse lengths in the scale of a few fs to low ps scale, the fragmentation tends to smaller fragments for pulses longer than 500 ps and remain at this level for the ns pulse. Depending on the molecule, the survival yield of the molecule decreases for 500 ps and 6 ns pulse length compared to fs pulse length scale by about 30 % (naproxen) and 50 % (ibuprofen).

The LD efficiency is investigated for retene on a quartz fibre filter as a proxy for environmental samples. Retene is known as a marker for forest fires and a REMPI-active substance. The desorbing pulse is focused on one spot of the quartz fiber filter and the signal decay of the parent ion is recorded over the time. In contrast to the fragmentation experiments, major changes in the desorption efficiency can be noticed already in the fs range, pointing on changes in the desorption mechanisms for ultrashort pulses. Comparing 75 fs with 500 fs pulse length, the efficiency increases already by a factor of 3; for 75 fs and 6 ns the efficiency increases by a factor of 17.

This study shows substantial benefits for ultrashort laser pulses over ns laser pulses, especially regarding desorption efficiency. Furthermore, the results are directly transferable to environmental samples, which are often sampled on quartz fiber filters. It can be concluded that fs laser pulses are highly advantageous for sample material economy and for the LD from small molecular structures with low analyte concentrations, e.g. small cell systems, thin cuts of tissue or particles.

SES-20-03

Time: 5/17/2023 11:10:00 AM - 5/17/2023 11:30:00 AM

Surface-Assisted Flowing Atmospheric-Pressure Afterglow Mass Spectrometry (SA-FAPA-MS) for Fast and Direct Quantitative Analysis of Complex Samples

Heide, Maximilian; Escobar-Carranza, Cristian C.; Heinelt, Manuel; Schütz, Désirée A.-M.; Engelhard, Carsten

University of Siegen, Germany

Ambient desorption/ionization high-resolution mass spectrometry (ADI-HR-MS) is an efficient technique for fast molecular analysis. Accurate quantification of analytes in complex, matrix-heavy samples is one remaining challenge in this field [1]. A typical workflow is to add a preceding extraction or separation step to remove the matrix.

In this lecture, it will be demonstrated how functionalized surfaces, specifically thin-layer chromatography (TLC) plates, can be used as sample carriers for the direct analysis of liquids and dried residues. Specifically, cyano- and dimethyl-functionalized silica yielded analyte signals about 100-times higher compared to other surfaces such as glass or metal when probed with the flowing atmospheric-pressure afterglow (FAPA, cf. Hieftje et al. [2,3]) source.

Fundamental advantages of the method, which we call surface-assisted (SA-)FAPA-MS, were demonstrated for basic standards and standard mixtures of selected compound classes as well as for real samples. For example, it was also possible to accurately quantify analytes in matrix-heavy samples (i.e., beverages, electronic cigarette liquids) and validate the results using established methods such as HPLC. The chemical compound class and its physicochemical properties such as proton affinity and enthalpy of vaporization were found to play an important role in the overall desorption and ionization process. Analyte amounts (e.g., nicotine, caffeine) were detectable down to the low fmol range. A direct comparison of both methods showed a significantly higher time efficiency of the SA-FAPA-MS method with minimal sample preparation. SA-FAPA-MS is considered a promising analytical strategy for molecular ADI-MS analyses of complex samples.

[1] J.T. Shelley, S.P. Badal, C. Engelhard, and H. Hayen, Anal. Bioanal. Chem., 2018, 410(17), 4061-4076, https://doi.org/10.1007/s00216-018-1023-9

[2] F.J. Andrade, J.T. Shelley, W.C. Wetzel, M.R. Webb, G. Gamez, S.J. Ray, and G.M. Hieftje, Anal. Chem., 2008, 80(8), 2646-2653, https://doi.org/10.1021/ac800156y

[3] F.J. Andrade, J.T. Shelley, W.C. Wetzel, M.R. Webb, G. Gamez, S.J. Ray, and G.M. Hieftje Anal. Chem., 2008, 80(8), 2654-2663, https://doi.org/10.1021/ac800210s

Workshops

WS01

Time: 5/14/2023 1:30:00 PM - 5/14/2023 4:15:00 PM Location: Saal 9

Skyline Workshop

Heyer, Robert; Reinders, Yvonne; Sakson, Roman

Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Germany

Skyline is a free and open-source academic software that has become the de facto gold standard for targeted proteomics assay development and data analysis. However, applications of Skyline for data-dependent acquisition (DDA) proteomics data evaluation are less common. After presenting a short general overview of the Skyline interface, we will demonstrate how Skyline can be used as a vendor-independent MS data visualization tool to scrutinize DDA data using MS1 filtering. Database search results from various software packages, such as Proteome Discoverer or MaxQuant, can be integrated easily with raw MS data in Skyline as spectral libraries. In the second part of the workshop, we will present the Panorama AutoQC data analysis pipeline. This pipeline allows to automatically monitor LC-MS system suitability data in real-time, visualizing Skyline data via the convenient Panorama online interface accessible from any web browser. To summarize, participants will learn how to visualize their peptide-level DDA data easily using Skyline and Panorama, leading to deeper insight.

WS02

Time: 5/14/2023 1:30:00 PM - 5/14/2023 4:15:00 PM Location: ISAS City 3. OG

Alternative Ionisation Techniques

Franzke, Joachim

Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Germany

Alternative Ionisation Techniques utilising dielectric barriers

Fifteen years ago, the potential of a dielectric barrier discharge (DBD) plasma was presented for the use in analytical element spectrometry. The miniature, planar DBD shows excellent dissociation capability for molecular species having a small size, a low power consumption, and a low gas temperature.

Several years later, a cylindrical DBD was presented by Na et al. applied as an efficient method for molecular mass spectrometry using a small capillary as dielectric barrier. Similar structures result in a variety of methods nowadays commonly termed Ambient Mass Spectrometry (AMS) and the development is speeding up during the last years.

Within this course, different dielectric barrier ionisation techniques for the applied use in analytical chemistry will be presented and functionally characterized. The focus will be on two functional designs delivering robust, stable and efficient alternatives coping with the challenges in modern mass spectrometry: The Flexible Microtube Plasma (Fμ TP) as alternative to APCI, and the DB-(n)ESI as alternative to (n)ESI.

WS03

Time: 5/14/2023 1:30:00 PM - 5/14/2023 4:15:00 PM Location: Silbersaal

Lipidomics Workshop

Bendt, Anne; Heiles, Sven; Hahnefeld, Lisa

1: National University of Singapore, Singapore; 2: ISAS, Dortmund, Germany; 3: Fraunhofer Institute, Frankfurt, Germany

Lipidomics is defined as the comprehensive, systems scale level analysis of lipid metabolites in a biological system. The majority of lipidomic studies capitalizes on the sensitivity and specificity of liquid chromatography mass spectrometry (LC-MS). In recent years, this has enabled the quantitation of the human plasma lipidome comprising hundreds of chemically diverse lipids, from a few microlitres of plasma sample. For spatial resolution, MALDI based workflows are on the rise, depicting for example cancer progression and classification in tissues such as prostate cancer.

So far, lipidomic workflows are firmly anchored in the research space: thousands of research studies analysing various health and disease states have helped elucidate the diverse roles lipid metabolites play in a variety of (patho)physiological processes. Numerous biomarkers have been postulated, primarily for metabolic diseases, various cancers and neurodegenerative conditions.

What would it now take to translate lipidomic research findings into clinical applications?

In this workshop, we will discuss the clinical potential of lipid metabolite analysis and highlight preanalytical challenges for plasma lipidomics by LC-MS and for tissue analysis via MALDI. Community-led initiatives towards harmonization of methods including microsampling technologies will be discussed as well.

WS04

Time: 5/14/2023 1:30:00 PM - 5/14/2023 4:15:00 PM Location: Saal 10

Metrology in Mass Spectrometry - Workshop

Prohaska, Thomas

Montanuniversität Leoben [MUL, MU Leoben], Austria

Metrology in chemistry is defined as the science of measurements. Even if we care about the best measurement results in terms of precision or deviation from “true value”, the validity of these results is determined by their uncertainties. Therefore, a proper understanding of the uncertainty budgets and sources of error is crucial to providing validated measurement results.

This course provides the basic understanding of creating an uncertainty budget and presents different approaches with a focus on Monte Carlo calculations. Using practical examples, the participants should be able to transfer their knowledge to their own measurement results.

WS05

Time: 5/14/2023 1:30:00 PM - 5/14/2023 4:15:00 PM Location: Goldsaal

MS Imaging - Workshop

Römpp, Andreas; Spengler, Bernhard

Universität Bayreuth, Germany

Application areas in mass spectrometry imaging are constantly increasing and include a wide range of analytes and sample types. This often requires adaptation beyond the 'standard' protocols. In this workshop we will discuss new developments and practical aspects of the MS imaging workflow. This includes for example strategies in sample sectioning and preparation. How do I assess and improve spatial resolution? Approaches for the investigation of single cell heterogeneity. How do I analyze my data in terms of quality control and biological interpretation?

WS07

Time: 5/14/2023 1:30:00 PM - 5/14/2023 4:15:00 PM Location: Saal 5

Proteome Discoverer - Workshop

Delanghe, Bernard

Thermo Fisher Scientific, Germany

The Proteome Discoverer workshop will cover new features and improvements of the software. These include a new AI driven search algorithm, CHIMERYS and an enhanced rescoring functionality. Furthermore, workflows and settings for label-free and label based quantification will be discussed. The Q&A session will include troubleshooting common issues and optimizing data analysis. During the workshop, participants will have the opportunity to ask questions and discuss results.

Special Sessions

Mattauch Herzog Award Session

Time: 5/14/2023 7:00:00 PM - 5/14/2023 8:00:00 PM Location: Goldsaal

Chair: Spengler, Bernhard

Mattauch Herzog Award Session

Award 2021

Jens Soltwisch

University of Münster, Institute of Hygiene

Award 2022

Charlotte Uetrecht

CSSB / DESY / LIV / Uni Siegen

Award 2023

tba

Mass Spectrometry in the Life Sciences Award Session

Time: 5/16/2023 9:45 AM - 5/16/2023 10:00:00 AM Location: Goldsaal

Chair: Breuker, Kathrin

Mass Spectrometry in the Life Sciences Award Session

Award 2023

Andrea Sinz

Martin Luther University Halle-Wittenberg, Center for Structural Mass Spectrometry

Institute of Pharmacy

Wolfgang Paul Study Award Session

WPL

Time: 5/15/2023 9:45:00 AM - 5/15/2023 11:00:00 AM Location: Goldsaal

Chair: Mormann, Michael

Wolfgang Paul Study Awards Session

WPL 1

Time: 5/15/2023 9:55:00 AM - 5/15/2023 10:05:00 AM Location: Goldsaal

Wolfgang Paul Study Awards 2021: Bing Peng: Novel strategies for targeted lipidomics in complex biological systems

Peng, Bing

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany

Lipids are fundamental biomolecules deeply involved in numerous biological processes. To elucidate the roles of lipids in biological systems, the study of lipidomics emerged with support from state-of-the-art analytical techniques. However, the high complexity of lipids resulted in many analytical challenges to obtain analyses of whole lipidomes. Therefore, a strategy called targeted lipidomics focuses on scanning and quantifying of selected groups of lipids only. However, one of the main challenges of targeted lipidomics is the lack of suitable bioinformatics tools and workflows to set up a targeted assay for the huge variety of lipid classes. Thus, the aim of this thesis is to improve the targeted lipidomics workflows, including the creation of targeted assays and the parameters for chromatographic separation, and apply and validate developed workflows to different model systems such as the RAW 264.7 cell line (a community model) or platelets as an ex-vivo model.

Firstly, an interim strategy was developed to adapt Skyline (a targeted proteomics software package) for targeted lipidomics analyses. Secondly, LipidCreator was developed, the first open source software fully supporting the targeted lipidomics assay development. LipidCreator not only delivers the computation of fragment masses of over 60 lipid classes, it also provides the functionality to define fragments, introduces stable isotope labeling of lipids for targeted assays, provides an optimization module for collision energy and generates in-silico spectral libraries. This software can either be used as a standalone tool or with Skyline. On the basis of LipidCreator, the high performance chromatography/mass spectrometry-based targeted lipidomics workflows including extraction optimization, lipid category tailored gradients and fragmentation rules in mass spectrometry were established, leading to significantly increased accuracy for the analysis of targeted lipids.

Finally, the established targeted approaches were utilized in conjunction with shotgun lipidomics, enabling us to create a first quantitative draft of the platelet lipidome covering almost 400 lipids species derived from 28 lipid classes and a dynamic abundance range of seven orders of magnitude. The result of the resting platelet lipidome indicated that only 15 lipids comprise 70% of the whole lipid content of platelets. In addition to this, a systematic assessment of the lipidomics network revealed that 80% of the platelet lipidome is unaffected by the change from resting to the activated state, indicating the feasibility of quantitative and differential comprehensive lipidome analyses. The strategies were further applied to a rare disease model (Niemann-Pick A/B) which displays clotting and bleeding issues. For this disease model, it was investigated whether lipids are responsible for this

phenotype. The results showed a strong upregulation of sphingosylphosphorylcholine (SPC) and a direct pathophysiological effect of SPC on the platelet activation and thrombus formation. In conclusion, the established platform has been successfully applied to the quantitative study of biological systems.

WPL 2

Time: 5/15/2023 10:05:00 AM - 5/15/2023 10:15:00 AM Location: Goldsaal

Wolfgang Paul Study Awards 2021: Eike Mucha: Vibrational Spectroscopy of Glycans in Helium Nanodroplets

Mucha, Eike

Bruker Daltonics, Germany

A central theme among the glycosciences is the Janus-faced nature of glycans. Their tremendous structural diversity enables a myriad of biological functions ranging from energy storage to molecular recognition processes. But at the same time, this structural diversity poses a formidable challenge for glycan analysis that impedes the full development of structural glycobiology. In contrast to genomics and proteomics, glycomics lacks generic sequencing methods that allow reliable, high throughput analyses with low sample consumption. Instead, a variety of sophisticated methods is used for glycan analysis, including mass spectrometry. A general challenge using mass spectrometry alone, however, is the unambiguous identification of isomeric glycans. Therefore, it is often coupled to orthogonal techniques, such as liquid chromatography. In the last two decades, the combination of mass spectrometry and gas-phase action spectroscopy emerged for glycan analysis. Various challenges, however, limited gas-phase spectroscopy to smaller glycans. In particular, the thermal activation of ions during the measurement in infrared multiple-photon dissociation spectroscopy leads to significant line-broadening, which limits the amount of structural information that can be obtained by this method. This work overcomes these limitations by combining mass spectrometry and cryogenic vibrational spectroscopy using superfluid helium nanodroplets. The unique low-temperature environment of helium droplets leads to exceptionally well-resolved IR spectra caused by the absence of thermal contributions.

This presentation gives an overview of the original work described in this thesis, as well as numerous subsequent studies that use the concept of cryogenic IR spectroscopy to gain a deeper understanding of glycans.

WPL 3

Time: 5/15/2023 10:15:00 AM - 5/15/2023 10:25:00 AM Location: Goldsaal

Wolfgang Paul Study Awards 2022: Marc Jäger: Optoelectronic Properties of Molecular Semiconductor Systems

Jäger, Marc

TU Darmstadt, Germany

In the present work, optical and geometric properties of semiconductor clusters are investigated experimentally and theoretically. With the help of photodissociation spectroscopy experiments in the UV-Vis spectral range, the clusters were examined in a high-vacuum molecular beam apparatus and experimental absorption spectra were obtained. A wavelength tunable laser system, which was enhanced by a sum-frequency option was employed to probe the optical properties. For a detailed analysis of the optoelectronic properties, suitable cluster structures were determined via a global optimization using an unbiased genetic algorithm with density functional theory while optical absorption spectra were obtained in the framework of time-dependent-density functional theory. Subsequently, a direct comparison between the experimental findings and theoretical predictions were used to discriminate the geometric structure of the particles present in the molecular beam and to interpret the observed light absorptions. The main focus of this work is the systematic experimental and theoretical investigation of cadmium selenide clusters (CdSe clusters) of different compositions and sizes. The results are discussed in connection with larger colloidal CdSe nanoparticles and the solid. In the case of stoichiometric CdSe clusters, the size-dependent development of the optical band gap (starting with the smallest species consisting of a few atoms up to solids) can be divided into two regimes. In the regime of larger nanoparticles, a blue-shift of the optical band gap with decreasing cluster size can be observed, while in the case of small clusters there is no simple correlation with the cluster size. The effect of ligand stabilization and the influence of a net charge were also examined. Structurally, cationic CdSe clusters are similar to their neutral counterparts, i.e. ring structures with exclusively heteronuclear bonds are formed and larger clusters are linked to three-dimensional structures via four-membered and six-membered rings. An exception to this is Cd_2Se^{2+}, which is a rhombus made up of a neutral Se_2 dimer and a Cd^{2+} radical. Beyond that, a significant part of this work includes the development of a novel genetic algorithm called GIGA, which is particularly characterized by its versatility. In addition to the global optimization of isolated clusters in the gas phase, GIGA can also be applied to clusters on surfaces, deposited clusters with adsorbates or clusters in the presence of ligands and is efficiently able to localize the lowest energy isomers. The latter in particular is an important step to enable the connection of isolated clusters in the gas phase to colloidal systems in solution.

WPL 4

Time: 5/15/2023 10:25:00 AM - 5/15/2023 10:35:00 AM Location: Goldsaal

Wolfgang Paul Study Awards 2023: Stefan Wagner: Development and application of diffusive gradients in thin films (DGT) techniques for multi-elemental chemical imaging and isotope ratio analysis

Wagner, Stefan

Montanuniversität Leoben, Austria

In this contribution, an overview on recent progress in the development and application of novel in situ techniques for multi-elemental chemical imaging and isotope ratio analysis of inorganic (trace) compounds will be presented.

The developed techniques are based on diffusive gradients in thin films (DGT), a passive, non-destructive sampling method using thin binding layers containing analyte-selective binding agents, which act as infinite-sink for labile analyte species in aqueous systems. The variety of binding agents allows for the preparation of gels which are selective for a multitude of analytes. When binding layers are stacked, specific analytes (species) can be sampled and separated simultaneously. The binding layer probes can be either deployed directly onto the sample surface for laterally-resolved sampling, or loaded into standardized DGT devices for bulk sampling. Subsequent chemical analysis of the binding layer by mass spectrometric techniques enables the quantification and/or visualization of the sampled analyte fractions.

Here, the potential of novel DGT techniques is shown on selected applications in environmental and materials science. New DGTs were developed and combined with high-end methods of inorganic mass spectrometry, including laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for chemical imaging and multi-collector (MC)-ICP-MS for isotope ratio analysis. These approaches were further complemented by additional advancements in atomic spectroscopy, including planar fluorescent sensors and synchrotron X-ray fluorescence microscopy.

The first study highlights the importance of applying analytical techniques capable of in situ speciation analysis to avoid changes in the species distribution during sampling. Using a novel stacked-layer DGT LA-ICP-MS approach and fluorescent oxygen sensors, distinct patterns of inorganic arsenic (As) redox transformations were observed alongside roots of soil-grown hyperaccumulator plants, providing new insights into biological adaptations to As-contaminated environments.

The second study presents the development of a combined approach using DGT LA-ICP-MS and fluorescent pH sensors for time-resolved mapping of magnesium (Mg) and pH to assess the spatiotemporal dissolution of Mg-based materials applied in medicine. In addition to the first application of these methods in materials science, the developed approach allows to localize and quantify in situ metal solubilization dynamics at high spatial resolution (≤50 µ m), over large workpieces (up to 100 cm^2), and with low uncertainty ($u_{c,rel}$ = 10 %), which makes it a promising new tool to study the degradation and improve the design of technological materials.

In the third study, a selective DGT technique was developed and combined with MC-ICP-MS for the targeted analysis of bioavailable concentrations and isotope ratios of multiple trace elements in soil. This has been demonstrated using two stable isotope systems, strontium (Sr) and lead (Pb), widely used as tracers of archeological remains, environmental contamination sources, and food provenance. The new DGT enables

quantitative sampling of labile Sr and Pb under natural conditions (pH = 4.8-8.2; I ($NaNO_3$) = 0.01-0.1 mol L^{-1}; γ (Ca^{2+}) = 50-160 mg L^{-1} > in synthetic soil solution matrix), permitting the accurate assessment of isotopic variations without significant isotopic fractionation and with low uncertainty ($u_{c,rel}$ = 0.01-0.03 %). The primary advantage of the method lies in the simultaneous analyte preconcentration and matrix separation during DGT sampling, potentially simplifying the resource-intensive sample preparation required for high-precision isotope ratio measurements in complex environmental matrices.

WPL 5

Time: 5/15/2023 10:35:00 AM - 5/15/2023 10:45:00 AM Location: Goldsaal

Wolfgang Paul Study Awards 2023: Stefan A. Pieczonka: Comprehensive characterization of the beer and brewing metabolome

Pieczonka, Stefan A. (1,2); Rychlik, Michael (1); Schmitt-Kopplin, Philippe (1,2)

1: Technical University of Munich, Germany; 2: Helmholtz Munich, Germany

The world around us is in constant flux. We adapt our perspective to changing circumstances and enter undiscovered paths. We test the validity of what seems to be known and seek new insights and approaches in what is still unknown to us. The dissertation of Dr. Pieczonka presents an analytical strategy to make the unexplored complexity and molecular diversity of our food and other biological samples analytically visible and interpretable. It aims to go beyond the numerous metabolomics studies in food analytics, to extend existing hypotheses and, data-driven, to raise new ones.

Foods are considered as complex molecular systems that have a biological (raw materials), chemical (Maillard reaction) and biochemical (fermentation) origin. The basis of the studies is a comprehensive molecular picture of the samples created by the application of ultra-high resolution mass spectrometry. Direct infusion on the FT-ICR mass spectrometer, still rarely used in analytical food chemistry, allows metabolite signals of a wide range of physicochemical properties to be detected. With data processing precisely tailored to the sample matrix, the mass resolution and accuracy of the instrument can be exploited. Looking at the molecular compositions of thousands of molecules simultaneously, a chemical profile of the samples can be drawn. The analytical fingerprint goes far beyond what databases know about our food. Finally, statistical processing of the analytical data provided characteristic signatures for corresponding sample properties. The accurate masses of correlating signals were linked in molecular networks to be able to describe chemical processes in their entirety or, to reveal biochemical relations of potential marker compounds. The hypotheses generated this way were tested and validated by complementary instrumentation, using tandem mass spectrometry or NMR, as well as model systems and reference samples.

The analytical concept was applied in brewing science and research. In the frame of non-targeted authenticity testing, universal metabolite profiles were found for Purity-Law-relevant raw materials and their processing. Molecular networks revealed hundreds of previously unknown derivatives of the dry hopping process, providing insight into their oxidation chemistry. Beers brewed with traditional barley malt could be differentiated from those brewed with proportional use of substitutes. The findings enable the analytical verifiability of the German Purity Law. Starting from a few known plant metabolites, a network of dozens of unexplored derivatives characteristic for certain starch sources such as wheat, maize and rice were revealed. These molecular networks were validated by metabolite identification through chromatography-coupled tandem mass spectrometry. The

statistical correlation of mass signals hidden in the comprehensive molecular view of FT-ICR-MS led to data-driven and molecular network based hypotheses that ultimately could be confirmed - from correlation to causality.

The basic mechanisms of the Maillard reaction are largely well understood. Yet, the incredible complexity of molecules that formed during the chemical interaction of reducing sugars and amino compounds is insufficiently depicted. Hundreds of FT-ICR-MS spectra are the basis of statistical data analysis that contributed to fill this gap. Hidden within the biochemical system of beer, over 3,000 molecules were found that originate from the chemical reaction network. Their molecular composition shared an intrinsic nature that follows Maillard's rules. Again, the combination of ultrahigh-resolution mass spectrometry and network calculations proved to be of great value for investigating new potential reaction cascades.

All of these chemical signatures, patterns, molecular profiles, and ultimate marker compounds were applied in a forensic archeochemical approach. A historical beer sample, dated back to the German Empire era, was subject to the comprehensive metabolomics approach. Even after nearly 140 years, critical production steps of its brewing process could be traced through molecular imprints and markers. The historic beer turned out to be a contemporary witness of a time, when modern industrial food production and food hygiene were made possible by pioneers such as Pasteur, Linde and Hansen.

The in-depth molecular view of our food makes it possible not only to trace production methods and enable authenticity testing. New processes can be developed and guided to gaurantee the quality of our food and agriculture - especially in times of increasing climatic uncertainties.

PS-SYMP

Time: 5/17/2023 2:30:00 PM - 5/17/2023 5:00:00 PM Location: Goldsaal

Chair(s): Schlüter, Hartmut Kwiatkowski, Marcel

Post Symposium "LC-MS"

PS-SYMP-1

Time: 5/17/2023 2:45:00 PM - 5/17/2023 3:00:00 PM Location: Goldsaal

Post Symposium "LC-MS" - Hartmut Schlüter: Soft sampling and homogenizing of tissues with infrared-laser systems for mass spectrometry-based biomolecule analysis

Schlüter, Hartmut (1); Moritz, Manuela (1); Voß, Hannah (1); Kwiatkowski, Marcel (2); Hahn, Jan (1)

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Analysis of biomolecules in tissues require the removal of areas of interest and the homogenization of the selected part. These two steps in the past were performed mechanically by cutting and grinding respectively milling. By these processes molecules will be released from their compartments and dissolved. Unfortunately, also enzymes are released which can convert the analytes into different molecules giving false negative results. A further problem is associated with those parts of the tissues remaining as insoluble particles. Removal of these particles is decreasing the amounts of molecules adsorbing to them. Sampling of tissues with picosecond (1) respectively nanosecond infrared laser systems (PIRL resp. NIRL) is reducing the above-mentioned problems, since the ablation process is very soft – even enzymatic activities are detectable in the tissue aerosol obtained by the irradiation of the tissue with the laser. The conversion of the tissue into an aerosol is induced by the water molecules which are present in tissues. By irradiation of the tissue the water molecules in the tissue adsorb the energy of the laser light. The energy is immediately transferred into translational energy, resulting in an explosion of the water molecules into the atmosphere, thereby completely homogenizing the tissue. In the centrifugate of the condensed aerosol no pellets are visible (2). The condensate can even directly be infused into mass spectrometers without further sample preparation. If the tissue aerosol is immediately condensed, the conversion of biomolecules by enzymes released during ablation, is very limited. The properties of the laser-based sampling of tissues thus are advantageous for the analysis all biomolecules and especially beneficial for the analysis of the original composition proteoforms in tissues prior to sampling (3). In comparison to mechanically homogenization, sampling of tissues by laser ablation is providing higher yields in total amounts as well as in the number of individual molecules, which will be demonstrated for bottom-up proteomics and metabolomics. A further advantage of sampling of tissues with NIRL or PIRL is, that layer by layer of tissues are removable allowing a spatial resolution (4).

(1) Ultrafast extraction of proteins from tissues using desorption by impulsive vibrational excitation. Kwiatkowski M, et al. Miller RJ, Schlüter H. Angew Chem Int Ed Engl. 2015 Jan 2;54(1):285-8. DOI: 10.1002/anie.201407669

(2) Mass Spectrometric Lipid Profiles of Picosecond Infrared Laser-Generated Tissue Aerosols Discriminate Different Brain Tissues. Wurlitzer M, et al. Miller R, Schlüter H. Lasers Surg Med. 2020 Mar;52(3):228-234. DOI: 10.1002/lsm.23096

(3) Homogenization of tissues via picosecond-infrared laser (PIRL) ablation: Giving a closer view on the in-vivo composition of protein species as compared to mechanical homogenization. Kwiatkowski M, Wurlitzer M,et al., Miller RJD, Schlüter H. J Proteomics. 2016 Feb 16;134:193-202. DOI: 10.1016/j.jprot.2015.12.029

(4) Tissue Sampling and Homogenization with NIRL Enables Spatially Resolved Cell Layer Specific Proteomic Analysis of the Murine Intestine. Voß H, Moritz M, et al., Schlüter H, Hahn J. Int J Mol Sci. 2022 May 30;23(11):6132. DOI: 10.3390/ijms23116132

PS-SYMP-2

Time: 5/17/2023 3:00:00 PM - 5/17/2023 3:15:00 PM Location: Goldsaal

Post Symposium "LC-MS" - Kristian Wende: Oxidative modification of proteins by gas plasma-derived reactive species – a general concept in plasma medicine?

Wende, Kristian (1); Nasri, Zahra (1); Minkus, Lara (1); Clemen, Ramona (1); Weltmann, Klaus-Dieter (1); von Woedtke, Thomas (1,2); Bekeschus, Sander (1)

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Non-thermal atmospheric pressure gas discharges (gas plasmas) have entered the medical stage as an alternative means to combat inflammatory disorders, including (chronic) wounds and (pre-) malignant conditions or tumors. The major driver of gas plasma-based effects is the formation of a multi-ROS environment, whose composition can be modulated by technical adaptations of the discharge in a wide range. Both long-lived species and short-lived species (atomic oxygen, singlet oxygen) contribute. While it is accepted that long-lived species such as hydrogen peroxide can penetrate into tissues, the mode of action of short-lived species remains to be elucidated. We hypothesize that the oxidation of biomolecules, especially proteins, and lipids, contributes to downstream physiologic processes. Using amino acids, model peptides, and isolated proteins as target molecules for gas plasma-derived species, we determined target structures and distinctive oxidation products using nanoLC-MS/MS. A site and sub-structure specificity of plasma-derived reactive species was found (1): aromatic and sulfur-containing amino acid residues at the protein surface were preferentially attacked. The newly introduced functional groups are dominated by oxygen (hydroxyl, oxo groups), and – depending on the discharge parameters - chlorination, nitration, or ring-opening reactions (tryptophane). By using stable isotopes, we confirmed the incorporation of atoms from both the gas and the liquid phase, indicative of a significant contribution of gas-liquid interface reactions for the formation of secondary reactive species such as hypochlorite ions that yield from the reaction between atomic oxygen and chloride ions. Subsequently, the oxidized proteins revealed changes in functionality and perception. The example ovalbumin (2), catalase, phospholipase (3), or filamentary proteins, the newly introduced chemical modifications modulated protein activity and recognition and subsequently triggered changes to cell physiology and immune response. In vivo, the same oxidative modifications were detected in gas plasma-treated patient samples.

Accordingly, it can be stated that the oxidative modification of proteins is part of pro-oxidant treatment regimens including gas plasma, and contribute to the respective biological or clinical impact. With respect to medical gas plasma applications, further proof and exploitation of the concept are in progress.

Acknowledgment

This work was supported by the German Ministry of Research and Education (BMBF), grants no. 03Z22DN12 (to K. W.), 03Z22DN11 (To S.B.), and 03Z22Di1 (to K.-D. W.).

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[3] Nasri Z, Memari S, Wenske S, Clemen R, Martens U, Delcea M, Bekeschus S, Weltmann KD, von Woedtke T, Wende K, Chemistry - A European Journal 27 (59), 14702-14710, 2021

PS-SYMP-3

Time: 5/17/2023 3:15:00 PM - 5/17/2023 3:30:00 PM Location: Goldsaal

Post Symposium "LC-MS" - Jörg Reinders: Keeping it simple means keeping it robust: Metabolomic and proteomic analysis of mouse brain

Ullrich, Daniel (1); Rathmann, Bettina (2); Langhauser, Friederike (1); Reinders, Jörg (2)

1: Clinics for Neurology, University Hospital Essen; 2: Leibniz-Institut für Arbeitsforschung, Dortmund, Germany

Multi-omic analyses often require complex sample preparation protocols to facilitate true multi-omic analyses from a distinct sample. Such protocols have not only to take compromises, e.g. in analyte yield and thus sensitivity, but make it also difficult to match the high demands on sample prep reproducibility for omics-analyses, particularly when only minute sample amounts are available.

While sophisticated sample prep methods such as the SIMPLEX protocol facilitated successful analyses in our core facility, they need skilled personal, remain time-consuming and often yield results on omic-levels not initially asked for. Thus, if no lipidomic analyses are intended, we stick to a simple, widely applicable protocol lysing cells or tissues in 80% methanol with respective stable-isotope labelled internal standards for targeted metabolomic analyses and use the protein pellet for an automated tryptic digestion with subsequent µ LC-SWATH-analyses using gas-phase fractionation. This approach facilitated the differential analysis of >6,000 proteins as well as targeted analysis of free amino acids and untargeted metabolomic analysis for mouse brain tissue under hypoxic stress.

PS-SYMP-4

Time: 5/17/2023 4:00:00 PM - 5/17/2023 4:15:00 PM Location: Goldsaal

Post Symposium "LC-MS" - Patricia Prabutzki: Deep lipidomic profiling reveals sex dimorphism of lipid metabolism in calcific aortic valve disease

Prabutzki, Patricia (1); Wölk, Michele (2); Böttner, Julia (3); Schiller, Jürgen (1); Schlotter, Florian (3); Fedorova, Maria (2)

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Calcific aortic valve disease (CAVD) is the most common valvular heart disease in the aging population. Valve replacement or implantation remain the only treatment options, as a pharmacological therapy does not exist. CAVD is characterized by chronic inflammation and lipid accumulation, and is driven by differentiation of valve cell subpopulations, promoting tissue fibrosis and consecutive calcification. Male sex alongside with lifestyle and age are known, prevalent risk factors for the severity of aortic valve impairment. However, the underlying biochemical mechanisms are not well understood. In particular, remodelling processes of the aortic valve (AV) lipidome have never been studied at the molecular level, even though lipid dysmetabolism was proposed to play a significant role in disease progression.

This study aims to unravel the role of the human AV lipidome in CAVD etiology. Since lipids are an extremely diverse class of small biomolecules, state of the art lipidomics methods are required for their analysis. Detailed assessment of cooperative actions of lipids under pathophysiological conditions requires identification and, even more importantly, quantification of tissue and cell type specific lipid signatures. Accordingly, we first determined the composition of the AV lipidome, thereby generating the first human AV tissue reference lipidome, and then proceeded to identify pathophysiological trends in CAVD related lipidome remodelling.

For an accurate identification and semi-absolute quantification of lipid molecular species, we performed deep lipidomic profiling of human three-leaflet (tricuspid) and abnormal two-leaflet (bicuspid) AVs in different pathophysiological stages, including non-diseased, fibrotic and calcified tissues. In order to ensure simultaneous high coverage of lipid classes with different polarities, ionization properties and within a wide range of endogenous concentrations, the analytical methodology had to be optimized for the specific tissue matrix and adequate experimental conditions were carefully chosen. Using a combination of reversed-phase high performance liquid chromatography and high-resolution mass spectrometry (MS), more than 900 distinct lipid molecular species were identified and more than 400 could be quantified across 18 different lipid classes. Lipids were identified with high confidence by accurate mass, MS/MS fragmentation rules and retention time mapping. Quantification was performed by the use of tailored internal standard mixtures, closely reflecting the endogenous concentrations of each lipid class.

Following this methodology, we identified distinct alterations in the AV lipidome, which turned out to be highly sex specific and provide novel insight into the underlying molecular mechanisms of CAVD onset and progression. Among the most pronounced effects, we found an overall upregulation along the sphingolipid synthesis pathway in fibrotic and calcified tissue as well as sex specific trends in neutral lipids remodelling. This study will therefore not only serve as a tissue-specific reference lipidome resource for systems medicine data integration, but will

also provide potentially valuable diagnostic and prognostic markers, which could serve as new targets for clinical applications.

PS-SYMP-5

Time: 5/17/2023 4:15:00 PM - 5/17/2023 4:30:00 PM Location: Goldsaal

Post Symposium "LC-MS" - Stefan Loroch: A Comprehensive Toolbox for Crime Scene Investigation using LC-MS-Based Proteomics Methods

Loroch, Stefan (1,2); Brosch, Tobias (1); Flake, Nina-Mariella (1); Lux, Dominik (1); Roocke, Sascha (1); Stepien, Jenifer (1); Kuhlmann, Stephan (3); Dorn, Annette (4); Birschmann, Ingvild (5); Marcus, Katrin (1); Barkovits, Katalin (1)

1: Medical Proteome-Center, Medizinische Fakultät, Ruhr-Universität Bochum, Bochum, Germany; 2: Protifi LLC, Farmingdale, New York, United States; 3: Landeskriminalamt Nordrhein-Westfalen, Dez. 52.4 - Serologie, DNA-Analysen, Düsseldorf, Germany; 4: Bayer

Forensic samples from crime scenes are important evidence and serve the clarification of the circumstances of an offence. Especially biological material such as tissue and body fluids comprise various information on the presence and involvement of subjects in a given crime and allow to reveal identities of the involved persons. However, conventional assays predominantly based on genotyping exhibit numerous limitations: (I) in case of mixtures (e.g. blood, semen, saliva, vaginal fluid), the exact composition cannot always be determined and assays for vaginal fluid are not commercially available (II) DNA can originate from a variety of body fluids leaving the question about their origin (from which individual) unexploited, (III) sequence variants are difficult to detect and requiring tailored sequencing approaches and (IV) mixtures of multiple individuals' DNA can not be read out. To overcome these problems, we present the development of our LC-MS-based proteomics toolbox allowing for (I) identification of individual body fluids in complex body fluid mixture using specific protein signatures (II) assigning body fluids to specific individuals via identification of subject-specific markers and sequence variants (III) determining the blood type and gender of the subject based on protein and post-translational modification signatures of each body fluid.

In a first step, we screened whole blood, blood plasma, erythrocytes, semen, saliva and vaginal fluid samples for specific protein signatures using sample preparation methods such as FASP and S-Trap (ProtiFi) in conjunction with extensive fractionation, including cryoprecipitation, differential centrifugation, SDS-PAGE, high-pH-RP chromatography, and gas phase fractionation (FAIMS) and state-of-the-art nanoLC-MS (Exploris480 online-coupled to Vanquish Neo, both from Thermo Scientific). We identified ~2000 proteins in whole blood, ~1000 proteins in plasma, ~1200 proteins in salvia, ~1700 proteins in semen and ~1600 proteins in vaginal fluid serving as repositories for determining specific protein and peptide signatures. Accordingly, we were able to design a variety of robust and reproducible targeted assays (via parallel reaction monitoring) for unambiguous identification of all individual body fluids (blood, semen, saliva, vaginal fluid) in body fluid mixtures, which proofed to be highly valuable in multiple round robin tests and can now be applied to real case samples. Further, we were able to identify 27 blood type-specific markers (such as Rhesus, Kell, Diego) in blood samples, which now allow us to assign blood samples to individual subjects (based on their blood-type) using tailored targeted assays. In a next step, we perform a detailed proteomics profiling (including sequence variants) to deciphering additional gender- and blood type-specific signatures to allow for even more explicit assignment of samples to individuals.

In summary, we successfully demonstrated that LC-MS-based proteomics allows to overcome limitations of classical forensic methods and perfectly complements the forensic toolbox. Our robust assays for identifying body fluids in complex mixtures will now be further extended for assignment of body fluids to specific individuals based on gender, blood type and sequence variants. Our results demonstrate the high value of proteomics and will help to pave proteomics' way into forensics for detailed investigations of the circumstances of an offence.

PS-SYMP-6

Time: 5/17/2023 4:30:00 PM - 5/17/2023 4:45:00 PM Location: Goldsaal

Post Symposium "LC-MS" - Erik Niehaves: Mimicking the reactivity of drug metabolites: Protein conjugation of an electrochemically-generated, reactive oxidation product of the antibiotic minocycline

Niehaves, Erik; Karst, Uwe

University of Münster, Germany

Electrochemistry (EC) is a versatile tool for various applications in the field of analytical chemistry. In recent years, it was shown that it can also be used to mimic the metabolism of xenobiotics in the human body. In particular, the online hyphenation of an electrochemical cell to a mass spectrometer (MS) provides a fast and simple method to generate possible metabolites of these xenobiotics. In addition to that, even reactive compounds can be detected due to the short time between generation and detection, which is typically less than a minute. By adding biomolecules like glutathione or different proteins to the electrochemically generated products, the reactivity of these products can also be investigated.

In this work, we used the purely instrumental approach of EC-MS to simulate the oxidative metabolism of the antibiotic minocycline by using an electrochemical cell equipped with a boron-doped diamond electrode. By applying a linear potential ramp to the cell, various oxidation products were formed and online detected by mass spectrometry. One of these products was suspected to contain a reactive quinone substructure. To investigate the reactivity of this oxidation product, the applied potential was first optimized to yield the maximum conversion rate for this particular product. The effluent of the cell was then combined with a second flow, containing glutathione, a common antioxidant in the human organism. This combined solution was allowed to react in a reaction coil and then analyzed online by ESI-MS. The formation of glutathione conjugates with the suspect oxidation product was observed, which indicated a high reactivity of this compound. To further investigate the reactivity of this oxidation product with biomolecules, the developed online method was used for conjugation experiments with Haemoglobin and Lactoglobulin-A. Protein conjugates with one or more minocycline moieties bound to the respective protein were found.

PS-SYMP-7

Time: 5/17/2023 4:45:00 PM - 5/17/2023 5:00:00 PM Location: Goldsaal

Post Symposium "LC-MS" - Manasi Gaikwad: Quantification of proteoforms in commercial protein sample using intact protein mass spectrometry

Gaikwad, Manasi

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Post translation modifications, amino acid substitutions, and truncations of therapeutic proteins (TPs) are very common and responsible for a large heterogeneity, which can alter the functional efficacy of the TPs. Detailed qualitative and quantitative analysis of these multiple protein species - now referred to as “proteoforms” - is critical for the regulatory approval of TPs. Considering the growing demand for TPs and the significance of proteoforms, this study aimed at establishing a fast method for the quantification of proteoforms in TPs. Systematic but fast distinctions and quantification of proteoforms are however challenging tasks for most analytical techniques, because of the highly similar physiochemical properties shared by the proteoforms. The state-of-the-art high-resolution "intact protein mass spectrometry (MS)" was employed and optimized in the present work for achieving proteoform quantification. For circumventing the problem of loss of proteoforms on stationary phases of liquid chromatography columns, flow injection analysis coupled to MS (FIA-MS) was investigated as an alternative method for fast proteoform quantification. Factors contributing towards sensitive proteoform detection in the FIA-MS method were systematically optimized. With an analysis time of only 4 mins per sample and improved specificity of proteoform detection achieved, the eminence of FIA-MS method over the RPLC-MS method was proven. Further on, the FIA-MS based detection of lower abundant proteoforms in the sample could be improved by applying "in-solution supercharging" with sulfolane as an additive supercharging agent. Until the beginning of this study, quantification of full-length proteoforms from isotopically unresolved mass spectral signals was underexplored. Therefore, both- isotopically resolved and isotopically unresolved signals of proteoforms were examined for developing a proteoform quantification strategy. Results of proteoform quantifications by ‘extracted ion flowgram (EIF) strategy' & ‘deconvoluted spectrum-based strategy’ were compared and assessed based on accuracy and precision. Further on, the correlation of quantification results, between multiple deconvolution tools for isotopically unresolved proteoforms was shown for the first time. In conclusion, a novel approach for fast proteoform detection using the FIA-MS method with total analysis time of fewer than 4 mins per sample as well as an accurate proteoform quantification strategy was developed and proven with an Adalimumab sample. Fast FIA-MS-based detection & deconvoluted spectrum-based quantification, can be broadly applied to various TPs for quick proteoform overview.

Postersessions

Poster 1

Time: 5/15/2023 4:45:00 PM - 5/15/2023 6:45:00 PM Location: Panorama Forum + Saal 8

Postersession I

Poster Nr. 1

Investigation of Large Charged ESI Droplets Penetrating the High Vacuum Region of a Commercial ESI-MS under Long-Term Conditions

Heintz, Chris; Braubach, Oliver; Wißdorf, Walter; Schnödewind, Lisa; Benter, Thorsten

University of Wuppertal, Germany

Electrospray ionization (ESI) is among the most important atmospheric pressure ionization (API) techniques due to its ability to ionize large and complex molecules without fragmentation. Basically, an analyte solution is sprayed in a strong electric field generating charged microdroplets. These droplets undergo an evolution of shrinking reactions eventually ending in the release of bare ions. The underlying mechanism is still not fully understood. It is often reported that all droplets evaporate within the ionization chamber. However, calculations and experiments show a lifetime of more than 1 ms, which exceeds the residence time within the chamber significantly.

Current experiments give strong evidence that at least a substantial fraction of the charged droplets penetrates the high vacuum region of common ESI-MS systems. Previous experiments performed on a Bruker micrOTOF allows to directly observe droplet signals by connecting an oscilloscope to an auxiliary ion detector which is located downstream the orthogonal acceleration stage of the TOF. We observed the appearance of very intensive ion signal bursts, which we attribute to droplet signatures. Furthermore, we observed droplet signatures in single mass spectra in terms of infrequently appearing, very intensive spectra. We also reported that crucial parameters for the spray generation (e.g. capillary voltage, liquid flow, nebulizer gas flow and drying gas flow) have a strong impact on the frequency of appearance of droplet signatures observed in the pusher region.

In this work we investigate the long-term performance of a commercial Bruker micrOTOF system in terms of signal stability with respect to the occurrence of droplet signatures under analytical conditions. To estimate the actual amount of individual charges in the observed signal bursts, we installed a fast analog rate meter. The measurements show an interesting effect of a decreasing droplet count within a few hours of measurement. The low droplet occurrence persists in immediately following measurements but recovers during the instruments settle-time. After a couple of hours the droplet occurrence raised significantly. Additional measurements investigate the effect of changing the MS polarity and the inlet capillary. To increase experimental throughput, an automated method is established to systematically change the measurement conditions and to control the used LC pump for direct injection of the used analyte system.

Poster Nr. 2

Molecular Dynamics Based Ion-Neutral Collision Modeling and Application in Ion Mobility Spectrometry

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There are several methods to separate gas phase ions by their ion mobility, one of which is drift tube ion-mobility spectrometry (DT-IMS). Ions are accelerated in a buffer gas filled drift tube by a constant electric field. Thereby the ions exhibit a characteristic ion mobility, allowing to differentiate different species. A second method is differential mobility spectrometry (DMS). DMS builds upon the dependence of the ion mobility on the strength of the applied electric field, utilizing an asymmetric waveform RF voltage with high and low field phases. Only ions with a specific field dependency of the ion mobility can pass a DMS cell.

There are several software packages available (MOBCAL, IMoS), which can estimate ion mobilities at vanishing electric field conditions. However, to be able to accurately simulate ion dynamics and trajectories in IMS and DMS devices, the electric field needs to be variable. The Ion Dynamics Simulation Framework (IDSimF) (https://github.com/IPAMS/IDSimF) is an open-source software written in C++ and allows to simulate ion trajectories for different applications, e.g. ion mobility devices or quadrupole collision cells. The electric fields can thereby be varied freely and ion-neutral collisions are modeled with an previously implemented molecular dynamics (MD) based collision model. This enables more accurate simulations of the collision dynamics compared to more simple descriptions such as hard-sphere models. The MD model is based on a rigid body trajectory method, which solves Newton's equations of motion using an adaptive step-size Runge-Kutta method. The force field, modeling interactions between molecules, contains the 12-6 Lennard-Jones potential and ion induced dipole moment potentials.

First simulation results show a good agreement between IMS simulations of small ions such as Cl– and Li+ in Helium at 20 mbar and 298 K and experimental literature values at different electric field strengths. Additionally, in atmospheric pressure DMS simulations accurate dispersion plot trends could be reproduced, showing validity of the MD model at various pressure regimes. As a further expansion of the force field of the MD model a quadrupole force is introduced to more correctly describe the collision behavior of diatomic nitrogen. Preliminary ion mobilities obtained with the inclusion of a quadrupole force for systems of small ions interacting with N2 show a strong influence of this effect.

Poster Nr. 3

Development and Evaluation of a Novel Accumulation Ion Source for Chemically Instable Compounds

Rieger, Joshua; Benter, Sanna; Langner, Markus; Pengemann, Niklas; Kersten, Hendrik; Benter, Thorsten

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Mass spectrometric detection of molecules and ions at the ultra-trace level requires specifically tailored and optimized sampling and acquisition systems. Analyte accumulation is a common technique used in analytical chemistry but is not used yet for chemically unstable gas phase compounds. The underlying concept behind the novel source is to utilize surface decomposition reactions at selected surfaces. The decomposition products partly remain on the surface, while others desorb back into the gas phase. In a subsequent step, the accumulated decomposition products are desorbed from the surface as ions upon exposure to suitable activation methods and are further guided into a mass analyzer.

The instability of selected compounds on surfaces, as well as the heterogeneous reaction between surface absorbed species and (excited) gas phase species leading to volatile products is well described in the literature. Generally, the decomposition probability depends on the surface material. Pure metals favor the decomposition, whereas (metal) oxides generally inhibit the process. The layer growth rate and thus the accumulation efficiency also depend on the surface and the target compound properties. In the presence of selected gases during the desorption processes, volatile species are formed, which yield a characteristic signal pattern in the mass spectrum. Custom spectrum analysis software allows the identification and relative distribution of the often superimposed fragment species.

Decomposition processes on the sampling tip for validating the ion source performance are monitored via FTIR spectroscopy by measuring characteristic absorption patterns. The exposure chamber is made of glass, minimizing decomposition on the chamber walls and thus almost exclusively on the metal sample tip. The temporal decrease of the gas phase product concentration correlates directly with the amount of decomposed material residing on the sample tip, which allows quantification of the amount of material accumulated.

A linear actuator moves the sampling tip from the exposure environment into a custom ion source, located directly in front of the ion optics of a high resolution TOF mass spectrometer (LTOF, Tofwerk AG, Thun, Switzerland). Ion optical elements guide the subsequently desorbed ions towards the analyzer. In addition to detecting the desorbed ions, the designed ion source also allows for standard EI acquisition, which provides residual gas analysis and calibration of the MS.

For method assessment, relevant samples are synthesized and exposed to the sampling tip in a vacuum chamber. The deposition on the surface is monitored by the time-dependent change of the gas phase concentration via FTIR.

Poster Nr. 4

A Novel Method for the Sampling of Native Ions from a Distant Location

Benter, Sanna; Langner, Markus; Pengemann, Niklas; Kersten, Hendrik; Benter, Thorsten

University of Wuppertal, Germany

An ion optical transfer system is necessary to sample native ions produced e.g. in plasma-reactions. A distance of 150 cm between an EUV-plasma chamber and a time-of-flight mass spectrometer (TOF-MS) was bridged using a segmented hexapole equipped with matching apertures. Ion guiding is mandatory, in addition to gas flow restrictions and a separation stage to decouple the TOF-MS from the plasma chamber. The designs of the hexapole exit aperture and the aperture downstream of a gate valve are critical for an efficient ion transfer, as electric fields between the apertures need to reach into the valve body: In the open position, ions need to travel several cm in an otherwise essentially field free region.

The entire transfer stage design was simulated with SIMION showing promising results with ion transfer rates of up to 90%. Pressure and capacitance measurements were performed using a stainless steel hexapole make-up stage.

The entrance aperture is used for flow restriction from the plasma chamber at 5 Pa and the hexapole held at max. 1E-4 mbar to reduce the number of ion-molecule collisions within the transfer stage. As entrance aperture an Einzel lens is used. The first grounded electrode of the stack, with a diameter of 0.2 mm, is used as flow restrictor. The second electrode is held at about -10 V, to increase the kinetic energy of the ions. Due to geometric restrictions caused by the large and congested plasma chamber enclosure, the ion transfer stage needs to be mounted with in a 150 cm long CF40 stainless steel tube. This reduces the inscribed radius between the rods in the hexapole to 5mm with a rod diameter of 4mm, as the distance of the grounded CF40 tube to the active hexapolar field needs to be sufficiently large. The mandatory gate valve, as per EUV chamber operation rules, mounted at the transfer stage exit, creates a field free region of 4 cm in the open position. Thus, the electric fields of the exit aperture of the hexapole as well as the transfer aperture downstream of the valve have to reach into the clave body to guide the ions through the otherwise field free area. A second Einzel lens is thus mounted at the hexapole exit. Downstream of the valve a DC funnel is used to collect the ions and reduce their kinetic energy for further transfer into the TOF analyzer.

Poster Nr. 5

Observation of charged ESI droplets aspirated into commercial MS high vacuum systems under analytical LC conditions

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Electrospray Ionization (ESI) is an increasingly popular coupling technique between liquid chromatography (LC) and mass spectrometry. LC-ESI-MS systems have become indispensable in today's analytical laboratories, as indicated by the increasing number of papers published and research carried out in this field. Most of these are concerned with the optimization of ion source parameters or the reduction of the detection limit for analytical methods.

In recent years, several experimental observations have shown that the charged droplets initially formed with ESI can be aspirated into the vacuum stages of instruments and can reach even the mass analyzer region which potentially has adverse consequences for analytical performance: Possible effects of the resulting contamination are shortened maintenance periods and complex chemical interactions between analytes and matrix components, which complicate spectrum interpretation. Since initial experiments were limited to short-term measurements by using syringe pumps, the current knowledge about droplet aspiration at actual liquid chromatography (LC) conditions is limited. In contrast, the implementation of an Agilent 1200 series HPLC system allows long-term experiments and thus the assessment of the stability of the system.

The experimental setup consists of a Bruker amaZon ETD with Apollo source and an Agilent 1200 series HPLC-System, which has an integrated binary pump system. Besides the basic stability of the LC-MS system and the general extent of charged droplet aspiration the effects of solvent composition and solvent gradients on aspirated droplets are studied in detail. Variation of the solvent composition obviously affects the internal interactions in the droplets which governs droplet stability. For such experiments, solvent mixtures of ethanol, acetonitrile and water with 0.1% formic acid were prepared, using reserpine (m/z = 609.32) as analyte. Initial results show that the composition of the solvents, as well as transient solvent variations, have strong effects on observed signatures of charged droplets and that a solvent system stabilization time is required to obtain reproducible measurements.

In addition to the investigation of solvent-dependent effects, real analyte injections are planned, whereby the effects of a more complex matrix on the aspirated charged droplets under actual LC conditions are studied.

Poster Nr. 6

Ion Trajectory Simulation in Travelling Wave IMS with an open simulation framework (IDSimF)

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Ion mobility spectrometry is an analytical method that allows for the separation and identification of gas-phase ions based on their mobility in a collision gas. There exists a variety of different analyzer concepts, one among them being traveling wave ion mobility spectrometry (TWIMS). Therein a repeating waveform pattern is applied to a gas-filled RF-only ion guide leading to a sequence of potential waves traveling along a ring-electrode stack. An ion within the TWIMS analyzer can either be swept along by the wave and display what may be seen as "surfing" behaviour or the wave passes underneath the ion in so called roll-over events. The result is a separation of ions according to their electrical mobility similar to regular drift tube IMS, although the process involves more complex molecular dynamics.

The Ion Dynamics Simulation Framework (IDSimF) is an open-source software, written in C++, that contains various models and programs allowing for the simulation of ion trajectories. It provides different simulation applications modeling different experimental setups. In order to gain closer insight on TWIMS processes, a new application was developed that allows for the simulation of ion trajectories and drift times in a TWIMS device. The electrode geometry and electric potentials are defined by SIMION potential array files generated with the fast adjust option. To produce a pattern of potential waves with set distances traversing the analyzer, different waveform profiles in combination with phase shifts can be applied to the electrode stack by modulating the potential across adjacent electrodes.

The influence of various parameters, e.g. wave velocity, amplitude, and shape, on ion-wave interactions and drift times, is investigated. Lower wave speeds lead to a higher likelihood of the ions displaying surfing behaviour; conversely higher wave speeds lead to an increase in roll-over events. The waveform profile influences the ratio of ion drift velocity at the steepest wave slope to the wave speed which in turn influences the motion of ions. Further considerations were made in respect to parameters unrelated to the wave, e.g. the choice of drift gas which was shown to influence ion separation. IDSimF provides access to a sophisticated molecular dynamics based model for the collisions of molecular ions with background gas particles and space-charge simulation capabilities. Using this model detailed and accurate ion trajectories are obtained. We intend to present the newly created IDSimF application and an overview of the simulation results achieved with it.

Poster Nr. 7

Retrieval of time resolved ion mobility from phase dependent modulated compensation voltage in Differential Ion Mobility Spectrometry

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Differential Ion Mobility Spectrometry (DMS) was first conceived in the early 1980's. The conceptual idea was to separate ions in dependence of their differential mobility in an electric field, which is using an alternating asymmetric Separation Voltage (SV) oscillating between a low and high field phase, and a complementary Compensation Voltage (CV) to influence the ion trajectories through the DMS separation cell.

In contrast to the ion mobility separation with a constant electric field, as in classic IMS, the differential mobility is based on the different mobility coefficients of charged species in an electric field with low or high field strengths (referred to as low-/high-field mobility). These differences are substance dependent and can be substantially high and form the basis of this separation method.

Previous simulations have shown that there are several chemical and physical mechanisms which lead to a field dependent differential mobility. With knowledge about the time resolved ion mobility during an SV field oscillation, insights into the field dependent collision and reaction dynamics of the analyzed ions are possible.

This work aims to establish the basis for such an experiment: A phase dependent Compensation Voltage (CV) is simulated in a simplified DMS model. The Compensation Voltage is modulated with a "kernel" function and the effective CV is recorded in dependence of the phase-shift between SV and CV. The resulting phase dependent CV values contain information about the time resolved field dependent oscillating ion mobility, but in a convoluted form. We present results of simulations of a DMS experiment with modulated CV and ways to retrieve the time resolved ion mobility from phase shift experiments.

Poster Nr. 8

Measurement of Distribution of Ion Acceptance (DIA) in APLI ion sources

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Electrospray Ionization (ESI) and Atmospheric Pressure Laser Ionization (APLI) are commonly used ionization methods in mass spectrometry. Since both methods are operated at atmospheric pressure conditions, they are particularly affected by matrix effects. We use the determination of the spatial resolved ion acceptance distribution (DIA) to observe the effects of physical source parameters on the dynamics of analyte ions in the ion source region. For this purpose, analytes located in both, the liquid and gas phases, can be selectively ionized by an actively scanned laser beam to get information about the spatial distribution of observed ion signals. This method can be used to investigate how the ion distribution varies under different chemical conditions and different instrument parameters.

The measurements were performed on a Bruker Amazon Speed ETD quadrupole ion trap (QIT) with an Atmospheric Pressure Laser Ionization (APLI) source. UV laser light (248 nm) was generated with an ATL Atlex-500-I Excimer laser. The laser beam was diverted into the ion source using an automated laser-stage, which allows to scan a wide range (4 x 4 cm) in the source. The laser beam had a width of 1 mm, the resolution of the DIAs and the integration time at individual spatial locations can be varied. Pyrene in methanol (5 µ mol/l), nicotine in acetonitrile (5 µ mol/l), and a mixture of both solutions were used as analytes.

Initial measurements were performed with the same laser stage setup on a Bruker MicrOTOF. In those preliminary measurements, a spatial ion acceptance distribution of the ions in the source was observed. These measurements verify that the basic experimental setup is functionaland that spatial and temporal resolved ion acceptance distributions can be determined in an automated way. Previous measurements with a predecessor setup have already investigated how selected source parameters basically affect the ion distribution. It was shown that the Dry Gas flow and the applied Spray Shield voltage has a strong effect on the distribution of ions. In addition, when studying a mixture of pyrene in methanol and nicotine in acetonitrile, it was shown that the ion distribution differs depending on the analyte considered. In the upcoming series of experiments, DIA measurement series will be performed on the Bruker ion trap. The effects of ion source parameters will be systematically investigated and compared to results from a Bruker microTOF instrument.

Poster Nr. 9

Molecular Dynamics Simulation of charged ESI droplet fragmentation in MS vacuum stages

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Liquid charged droplets generated by Electrospray Ionization (ESI) penetrate deeply into the vacuum systems of commercial mass spectrometers (MS). Recent research indicates that a significant fraction of the ion signal detected in the mass analyzer is suspected to originate from those droplets. The droplet fragmentation and bare ion generation process likely takes, at least in part, place under medium or high vacuum conditions and potentially strong electric fields. Besides the unclear ion generation process, the device is contaminated by comparably large amounts of solvents in the form of aspirated droplets, that potentially have adverse effects for the analytical performance.

Since the dynamics of the droplets under vacuum conditions are largely unknown, molecular dynamics simulations help to rationalize experimental observations and improve the understanding of the complex ion generation process from charged droplets. Classical molecular dynamics (MD) simulations are used to create a microscopic model of the dynamics of collisions between charged droplets and background gas under vacuum conditions. The calculations allow the estimation of internal temperature changes and fragmentation dynamics of liquid droplets at such conditions.

In this approach, various compositions of liquid nanodroplets are set up with moltemplate and heated with a numerical thermostat function with different heating rates in LAMMPS. The simulation shows possible evaporation and fragmentation processes.

The simulations show a clear dependency of the observed fragmentation patterns on droplet temperature and composition. Simplified systems suffice to make preliminary qualitative comparisons of the various chemical systems in droplets. However, the modeling of larger and more complex droplets with LAMMPS proves to be an interesting approach for comparison with experimental mass spectra of droplets. By heating up the same droplet several times with a different random seed, a sufficient statistical set of fragmentation events and thus resulting fragments can be obtained, for example to derive simulated mass spectra. We present the simulation methodology in detail and initial simulation results in our contribution.

Poster Nr. 10

The effects of ion source and transfer parameters on charged droplets formed by ESI ionization

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Electrospray ionization (ESI) is one of the most relevant atmospheric pressure ionization techniques. The ionization method is based on spraying an analyte solution into a strong electric field, resulting in the formation of charged droplets. These droplets undergo a series of shrinkage processes. It is commonly assumed in the community that these processes result in the formation of isolated bare ions within the ion source.

Several experiments and calculations give strong evidence of significantly higher lifetimes of the initially formed charged droplets than previously assumed, which results in their far downstream in the vacuum system of MS instruments. Moreover, recent experiments have shown that a considerable fraction of these highly charged droplets are still detectable in the high-vacuum region of common ESI-MS systems. The analytical performance and maintenance frequency of the instrument can be affected by droplets within the transfer and analyzer region. Thus, the investigation of the physical and chemical dynamics of these droplets is of great value for instrument manufacturers and users.

Droplet signatures can be detected in different ways. Firstly, they become apparent in the distribution of mass spectrum signal intensities. Secondly, droplet signatures can be directly observed by auxiliary ion detectors integrated in specific instruments for monitoring purposes. For this purpose, an oscilloscope was directly attached to a SEV, which is located downstream of the orthogonal acceleration stage at a Bruker micrOTOF. The oscillograms show temporally broad “ion burst” signals with significantly higher intensities than the normal ion current which we attribute to droplets or large droplet fragments.

Using these methods, we observe the effects of source parameters on the droplet generation and frequency of their appearance, respectively. Parameters that have a direct influence on spray generation (e.g. capillary voltage, nebulizer pressure, etc.) have a major impact. Additional experiments investigate the effects of ion transfer stage parameters on the observed droplet signals. It is noticeable that the intensity and shape of the observed ion burst signals are strongly dependent on the RF amplitude of a transfer hexapole. A remarkably clear and reproducible dependence of the signal shape on this RF voltage is observed. We present a summary of the observed ion source and transfer stage effects on the observed droplet signals.

Poster Nr. 11

Fragmentation Dynamics of Aspirated Charged Droplets from ESI in an Ion-Trap Analyzer

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Electrospray ionization (ESI) is one of the most important ionization methods in mass spectrometry (MS). An analyte solution is sprayed into an electric field, resulting in the formation of highly charged droplets containing the analyte. Experimental observations show that a significant portion of the charged droplets generated by the ESI source can pass through the entrance into the vacuum system of the mass spectrometer due to their long lifetime.From experimental observations, it was concluded that the droplets affect the analytical performance and also contaminate the mass spectrometer. This leads to high costs for instrument maintenance. The parameters of the ion source have a significant effect on the electrospray, the formation of the droplets, and thus the ensemble of droplet particles aspirated into the instrument. Direct observation of large charged aggregates, droplets or their fragments, can be performed with ion trap instruments. They allow to isolate droplet fragments and analyze them further by performing MS/MS experiments. Experiments demonstrate that large charged aggregates penetrate even into the mass analyzer region of different ion trap instruments even under actual analytical LC conditions. Systematic fragmentation analysis of the isolated droplet signatures reveal distinct fragmentation patterns, which give indications to the actual structure and stability of the isolated species. Experimental studies are performed with a Bruker Daltonics amaZon ETD Quadrupol Ion Trap (QIT) with an Apollo ion source and an Agilent 1200 series HPLC-System. Due to the high m/z ratios of the droplets, an isolation range between 3500 and 6000 m/z was chosen in the QIT and their CID fragment spectra were systematically analyzed. The analytes used for these experiments are para-substituted benzylpyridinium ions and reserpine in different solvent systems. The solvents used are 1:1 mixtures of ACN, isopropanol or methanol and water with 0.1% formic acid. The operating parameters of the mass analyzer, (accumulation time, and isolation window) have a significant effect on the observed mass spectra of the droplets. In all measurements, a broad, reproducible double peak structure consistently occurred within the isolation window, often with signals having higher m/z values than the primary isolated mass range. This finding is likely due to the fact that the multiply charged droplets are subject to charge loss, resulting in higher m/z values. With increasing storage time, the abundance of analyte ions increased and the double peak signal became broader, which we attribute to fragmentation and evaporation processes of the trapped droplets in the ion trap.

Poster Nr. 12

Elevation of the baseline in Quadrupole Mass Spectrometers: Mechanism and Solution

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Selected quadrupole mass analyzers display an elevation of the baseline, if operated at inlet pressures in the range of a few Pa. The increase of the baseline negatively influences the limit of detection as well as the dynamic range of the device and therefore reduces the data presented in a single mass spectrum. In order to understand and suppress this shift of the baseline a general understanding of the origin for this behavior is necessary. Thorough investigations allowed to form a mechanism that explains the observed phenomenon.

Prior results from our lab motivated the present study: An order of magnitude baseline shift has been observed in an RGA system (QMG 422, Inficon, Bad Ragaz, Switzerland) in filter mode at ion currents reaching the µA range.

Several hypotheses have been constructed to explain this behavior, while experiments indicate that the origin of the shift in the baseline is in the electron ionization source. However, the quadrupole itself exhibits no effect on the baseline which directly translates to non-ionic species as the main culprit, ruling out photons. The aforementioned deflection unit was determined to be the area of ionization, since the influence of electric fields on the baseline differs downstream of this part of the device compared to upstream. Based on these experimental results, a mechanism was formed to explain the transition between a neutral particle and a cation. The ionization mechanism could follow several pathways (photoionization, ions breaking through the analyzer, ionization via gas phase reactions...), however SIMION simulations, theoretical calculations and experiments allowed to narrow the realistic mechanisms down significantly. The proposed mechanism involves neutral species originating from the ion source, which are ionized after traversing the rod system unfiltered. These particles undergo ionization downstream of the rod system but upstream of the detector and might subsequently be collected by the negative electric field of the conversion dynode of the SEM. After deriving the root cause of the shift, a redesign of the detector unit could be realized and tested. The proposed mechanism and the novel detector unit are backed by simulations and experiments alike.

Poster Nr. 13

Detection of plasma ions by coupling a high resolution TOF-MS at minimum distance to EUV-light focus point.

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Extreme ultraviolet (EUV) radiation is continuously applied to various manufacturing processes. To gain a better understanding of the EUV-induced chemistry in the surrounding gas phase consisting usually of hydrogen, a commercially available TOF-MS (LTOF, TOFWERK, Switzerland) is employed, to detect ions generated in the EUV beam path. It is comprised of a time-of-flight mass analyzer (2700 mm flight distance), an ion transfer quadrupole (100 mm rod length), and a custom EI-source, which can transfer the ions from the plasma region to the flight tube, while also providing electron ionization (EI). This modified MS is operated with a compact EUV irradiation system consisting of a discharge-produced plasma EUV source and a beam conditioning system to provide focused radiation at wavelength of 13.5 nm. The MS is coupled through a custom-designed experimental chamber, operated at an adjustable pressure and gas composition. The focus position of the EUV beam is at same height as the MS entrance, at a distance of 70 mm. For the experiment the chamber pressure is set to 5 Pa and the TOF analyzer operates at 1E-7 mbar.

Recent studies from this group have shown that the analysis of EUV plasma generated ions with high-resolution TOF-MS is challenging due to the miniscule number of native ions reaching the analyzer region; generally, the radiation focus is at far distance to the MS entrance. Another main bottleneck is the large pressure difference between the EUV chamber and the TOF analyzer. As an orifice is essential to maintain the MS operating pressure, large ion losses are inevitable. To increase the number of native ions transferred to the analyzer, an optimized coupling stage is placed between the MS and the EUV irradiation system. This stage features a small vacuum chamber, which is pumped separately through a variable leak valve and can be fed with hydrogen background gas up to a pressure of 5 Pa. The minimum pressure in this chamber needs to be < 2E-7 mbar during pump-down to lower the water background, although the pump efficiency is limited to a small diameter (8 mm). The chamber is designed to be as small as possible to a) ensure that the distance of ion formation and the entrance orifice from the TOF is minimized and b) the surface area is minimized. The (unguided) ion travel path is shortened to 70 mm.

The results from this campaign will be presented.

Poster Nr. 14

Fully automated proteomics workflow for liquid biopsies

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Liquid biopsy (LB) is a convenient, non-invasive alternative to surgical biopsies. LB, such as plasma, serum, and cerebrospinal fluid, has been increasingly used in oncology and other clinical applications for genetic and epigenetic studies. Notably, LB analyses with mass spectrometry-based tools could offer a valuable, easily accessible source of biomarkers and evidence for elucidating pathological processes. To unleash this potential, robust sample preparation tools and continuous improvements in LC-MS and data analysis are needed. Here we introduce a proteomics workflow for liquid biopsies integrated into a fully automated sample preparation platform.

Biological fluids from human, mouse, and rat were obtained from BioIVT. Samples (100 µ g total protein per sample) were processed using the iST-BCT kit on the PreON automation platform per PreON software v10.7 instructions. 300ng of peptides were analyzed on EASY nLC 1200 coupled with timsTOF using a 45 minutes gradient. Data were processed using the MaxQuant and Perseus software.

The sample preparation workflow presented here includes biological fluids lysis, reduction, alkylation, digestion, and peptide cleanup automated on the PreON.

Preliminary results showed that over 350 protein groups were identified in undeleted plasma with minimum missed cleavage sites, an increased excellent alkylation rate of cysteines, and minimal artificial modifications.

Samples processed with the PreON platform showed intra-day and inter-day. Hands-on time was reduced to less than 5 minutes, compared to the ~60 minutes required for the manual preparation with the iST-BCT kit. Afterward, the reproducibility of sample preparation was evaluated; the PreON platform showed a 1.8-fold improvement in sample-to-sample variation compared to manual sample preparation. This solution is ideal for low- to mid-throughput analyses, with up to 36 samples potentially processed per working day. It can seamlessly integrate into laboratories working on biomarker discovery in oncology and other relevant clinical fields.

Poster Nr. 15

Streamlined and semi-automated MS-based proteomics pipeline from protein extraction to real-time data analysis by coupling BeatBox, PreON and PaSER platforms

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Introduction

Sample preparation is an often overlooked yet crucial component of successful LC-MS-based proteomics studies. Conventional manual workflows often have various limitations such as low throughput, insufficient reproducibility, and time-consuming protocols. Here, we present a streamlined workflow from sample homogenization to data analysis by combining highly efficient protein extraction on the BeatBox platform with the robust iST sample preparation, fully automated on the PreON platform. Coupling this workflow to the newly developed PaSER technology provides improved and accelerated data processing by making the results available immediately after the run and eliminating the need for separate processing on high-performance computers.

Methods

The described sample preparation workflow was evaluated with human cell lines and various mammalian tissue types. Protein extraction was performed on the BeatBox using sample-specific settings, followed by automated digestion and peptide clean-up on the PreON applying the iST protocol. This combined workflow was compared to traditional sonication and manual iST sample preparation. Data were acquired by a timsTOF mass spectrometer coupled to an Easy nLC 1200 and processed in real-time using PaSER, effectively generating a streamlined workflow from sample preparation to real-time results. To validate the real-time processing, the data were also calculated in MaxQuant.

Preliminary data

From intact sample to finished data analysis (DDA on timsTOF Pro with PaSER data processing, Bruker) in less than one working day and with less than 45 minutes of hands-on time, we processed and analyzed 12 samples in parallel using the described proteomics pipeline. We identified ~4900 protein groups from HeLa cells and ~3800 protein groups from mouse liver tissue using a short 30-minute LC gradient. Compared to sonication, BeatBox homogenization provides an excellent extraction rate and higher flexibility of input material, starting amount, and a number of samples. At the same time, fully automated sample preparation on the PreON platform minimizes technical variability and sample loss and dramatically reduces hands-on time compared to manual processing. The CVs of the technical replicates prepared on the PreON were 1~4% lower for intra-day and 3~4% lower for inter-day repeatability relative to manual processing. PaSER processes the data in real time during the MS acquisition for accelerated data analysis. Comparing the PaSER output with a traditional MaxQuant offline search revealed similar protein IDs but significantly higher peptide IDs, indicating higher sequence coverages.

Conclusion

The presented workflow sets a new standard in speed and robustness in LC-MS-based proteomic sample preparation and data processing.

Poster Nr. 16

Direct Detection and Characterization of Microplastics by Flowing Atmospheric-Pressure Afterglow Mass Spectrometry (FAPA-MS)

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The increasing and rampant consumption of plastic materials has caused countless litter accumulations in remote environments such as Antarctica, mountain peaks, and deep ocean floors,[1] making them ubiquitous materials. Weathering and breakdown of these plastic materials can lead to microplastics (MPs) formation.[2] Flowing atmospheric-pressure afterglow (FAPA) is a plasma-based source that belongs to a large group of novel ambient desorption/ionization (ADI) sources, whose features include less sample preparation, no need for chromatography, and the possibility of direct analysis under ambient conditions.[3] These are attractive features that have rarely been applied to MP analysis.

This work presents a feasibility study to detect MPs directly with a FAPA ionization source coupled to high-resolution mass spectrometry (HR-MS). MPs were custom-made by grinding and sieving to yield particles in the μ m-range from polystyrene (PS), low-density polyethylene (LDPE), polypropylene (PP), and polycarbonate (PC). The influence of FAPA operating parameters on the mass spectral data was investigated and optimized. Plastic materials and MPs were identified based on characteristic molecules from the starting plastics in the m/z 65–500 range with high mass accuracy. With FAPA-MS in positive ion mode, protonated and oxidized species were detected as the major contributing ions. Differentiation of the MPs was done with principal component analysis (PCA).

Furthermore, to explore the influence of thermal desorption on the signal-to-noise ratio, selected materials were submitted to higher temperatures (~450 °C) and directly probed with FAPA-MS. Elevated desorption temperatures were found to induce degradation and enhance fragment ion signals. Lastly, the presence of specific ions in selected materials suggested the presence of plasticizers and additives. Overall, FAPA-MS can potentially become a fast MS-based method for the identification of MP types in the future.

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Poster Nr. 17

Analysis of Polycyclic Aromatic Hydrocarbons with Surface-Assisted Flowing Atmospheric-Pressure Afterglow Mass Spectrometry (SA-FAPA-MS)

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Polycyclic aromatic hydrocarbons (PAHs) have carcinogenic and mutagenic properties and continue to be released into the environment. The analysis of PAHs can be challenging and often requires time-consuming separation steps. In this study, the capabilities of ambient desorption/ionization high-resolution mass spectrometry (ADI-HR-MS) for direct analysis of PAHs were evaluated [1]. Specifically, a flowing atmospheric-pressure afterglow (FAPA) [2,3] desorption/ionization source was used to directly probe analytes from thin-layer chromatography (TLC) surfaces without a preceding separation step. The effect of different surface chemistries on the analyte response was investigated.

A large variety of 30 different PAHs and PAH derivates was deposited onto four different TLC surfaces and analyzed with SA-FAPA-MS. Results indicate a clear trend among the different substrates: Cyano- (CN) and dimethyl- (RP2) modified silica TLCs provided significantly higher analyte ion yields compared to reversed-phase C18 (RP18)-HPTLC and normal-phase (NP)-HPTLC surfaces. PAH solutions with ≤ 1 µ g/mL could be detected on CN-HPTLC and RP2-TLC surfaces. Characteristic mass spectra for all the compounds were obtained in both positive and negative ion mode. Typically, a higher ion yield could be achieved in positive detection mode. Analysis time per dried analyte residue was typically less than 60 seconds. Complete mass spectral imaging of 23 x 23 mm TLC plates could also be performed (60 min). Overall, SA-FAPA-MS is considered a promising rapid screening method for PAHs.

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Poster Nr. 18

Trends in Reductive Elimination from Transition-Organometallate Complexes

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Transition metal catalysts are an indispensable tool in the field of organic synthesis. To further develop and optimize them in a rational process, their mode of action should be understood at a fundamental level. This requires the study of short-lived intermediates that often cannot be characterized sufficiently with traditional bulk techniques. As a fast analytical technique, ESI-MS/MS can probe the innate gas phase reactivity of many such intermediates directly from a reaction mixture. The efficacy of this approach has been demonstrated in multiple works, including studies on cobalt[1] and organoferrate complexes[2]. In the work presented here, instead of focusing on one specific intermediate or reaction, ESI-MS/MS is employed to probe a larger number of late transition metallate complexes for elucidating their trends in reactivity toward reductive elimination and competing reaction channels.

A quadrupole time-of-flight mass spectrometer as well as an ion trap are used in this work to study a range of homoleptic metalate complexes of the type [MRn]– (R = Me, Ph, (trimethylsilyl)methyl (TMSM)). The ions are generated from liquid mixtures of transmetalation reactions. Sample handling requires constant cooling and an inert atmosphere, which can be achieved with pressurized sample injection (PSI) technique. This provides access to a wide range of homoleptic organometallates, in many cases with several oxidation states being observable from one precursor mixture, either already being present in solution or generated in the ESI process. Collision-induced dissociation (CID) allows probing these metalate ions' unimolecular reactivity.

The fragmentation behavior allows some trends to be compiled. Among most examined late 3d and 4d transition metalates, only those with metal centers in higher formal oxidation states of +III show potential for reductive elimination, with oxidation states of +IV and above remaining elusive. Depending on the organyl residue, for Fe(III), Co(III) and Ni(III) either multiple homolytic bond cleavages or reductive elimination can predominate. At lower oxidation states, dissociation preferably takes place through dissociation of organic radicals or fragmentation of the organyl ligand.

Isotopic labelling of the TMSM group reveals differing selectivity between Ni(II) and Fe(II) for the abstraction of a hydrogen atom from one TMSM group by a second organyl group. This hints at close involvement of the metal center in this reaction pathway.

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Poster Nr. 19

Speciation and Unimolecular Reactivity of Tetraalkylaluminate Anions

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Aluminum is the most abundant metal in earth's crust and thus of great interest for practical applications. Since the groundbreaking work of Ziegler in the 1950s, organoaluminum reagents have been widely used. In recent time, anionic organoaluminate complexes are also considered as high-performance electrolytes. To better understand the chemistry of this important class of compounds, we herein investigate their behavior by tandem ESI-mass spectrometry. First, we probe the speciation of different lithium and magnesium tetraalkylaluminates Li+[AlR4]– and MgCl+[AlR4]– in tetrahydrofuran. We then examine the gas-phase fragmentation of the tetraalkylaluminates by MSn experiments in a three-dimensional quadrupole ion trap. The main fragmentation pathway observed corresponds to a β-hydride elimination, which affords [AlR3H]– complexes. These primary fragment ions as well as the resulting ions also undergo β-hydride eliminations, such that ultimately the formation of [AlRH3]– can be observed. As a minor pathway, the loss of dihydrogen occurs as well. Complementary theoretical calculations at the DFT level help to rationalize the experimental findings.

Poster Nr. 20

Using mass spectrometry to determine the degree of deuteration of small molecules

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An exchange of a functional group results in a significant change of a compounds properties but also different isotopes within a molecular structure may cause a different behaviour like the stability of the respective compound. This is the case for molecules with a varying degree of Deuterium used for example in the pharmaceutical industry. There are mainly two questions regarding the characterization of deuterated compounds in contrast to regular materials: the total degree of deuteration as well as positional degree of deuteration to identify positions that are difficult/easy to deuterate with a given method.

Several analytical techniques have been combined and tested to establish a detailed characterization of deuterated compounds to better understand the impact of Deuterium on organic compounds. HPLC-HRMS, GC-MS and NMR as well as isotope ratio MS were compared to find the most reliable technique for the determination of the deuteration degree of an organic compound. The MassWorks™ software was used as a new feature for HRMS data.

The analytical portfolio for the evaluation of deuterated compounds was tested. It was found that all investigated analytical techniques are suitable to determine the degree of deuteration.

Mainly in the case of highly deuterated or bimodally deuterated compounds, HPLC-HRMS using MassWorks™ excels. The deuteration rate of each hydrogen position within the molecule can exclusively be determined using NMR.

Poster Nr. 21

LC with simultaneous IRMS and HRMS detection: A powerful new tool for process investigations

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Compound-specific stable isotope analysis (CSIA) is a unique analytical technique for determining small variations in isotope ratios in individual analytes from complex mixtures. An issue in CSIA by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS) is that any structural information of analytes is lost due to the analysis technique. LC-IRMS was combined for the first time with simultaneous analysis by high-resolution mass spectrometry (HRMS), enabling direct identification of unknown or coeluting species. This is demonstrated for assessing the fate of aminotrimethylphosphonic acid (ATMP) in the environment, which is so far insufficiently understood, primarily due to lack of knowledge about degradation processes.

LC-IRMS+HRMS combines quantitative data on degradation with CSIA while identifying transformation products. In combination with wet chemical analysis of ortho-phosphate (o-PO4), mass balances were established to elucidate and interpret the degradation mechanism. Batch experiments were performed in a photoreactor using a mid-pressure mercury lamp.

ATMP was transformed to iminodimethylphosphonic acid (IDMP), aminomethylphosphonic acid (AMPA), and o-PO4 as main products during photolysis. UV radiation <310 nm was found to be required for ATMP degradation. The degradation of ATMP and its transformation products is slightly affected by temperature and dissolved oxygen concentration. Considering possible reactive oxygen species, such as hydroxyl radicals, it is assumed that the degradation of ATMP occurs to a small extent via a parallel degradation pathway in addition to photolysis. Complementary CSIA revealed an inverse isotopic fractionation of ATMP due to direct photolysis, unaffected by the experimental conditions considered, while no isotopic effect was associated with hydroxyl radical oxidation of ATMP. In UV/H2O2 oxidation of ATMP, four of five additional unknown transformation products, which have not yet been described in literature, could be assigned to postulated structures based on HRMS data. Additional experiments on the influence of pH-dependent speciation of ATMP and its major degradation products IDMP and AMPA on photolysis showed significant differences in reaction rates, formation of transformation products, and isotope effects that occurred.

Poster Nr. 22

(Un)shared features of the reactivity of lithium boronate esters in the gas phase and in solution

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Transmetalation is a key reaction in organometallic chemistry, the understanding of which is helpful to the synthetic chemist to optimize reaction conditions. For this, however, the composition of the short-lived intermediate species involved in this reaction must be known. While (organometallic) reactions can be studied well on a macroscopic scale in solution, the speciation and analysis of reactive intermediates involved in the transmetalation reaction often depends on quantum chemistry to determine their composition, comprehensive kinetic studies or indirect information through crystallization of pre- or post-transmetalation intermediates. The combination of electrospray ionization (ESI) with multi-stage mass spectrometry (MSn) allows for the careful control over speciation, degree of solvation and the analysis of the unimolecular reactivity of ionic species dependent on their total charge. Our work group recently investigated the transmetalation of aryl (Ar) moieties from tetraaryl borates toward Ag and Cu and discerned, which factors govern the preference of transmetalation versus dissociation through a careful analysis of the potential energy surfaces along the reaction coordinates.[1] While Li and Na analogs of these complexes showed no signs of transmetalation, LiX aggregates (X = Cl, Br, I, BF4) of the boronate ester (PhtBuBpin)– (Ph = phenyl, tBu = tertiary butyl, pin = pinacol) unexpectedly undergo transmetalation in ESI-MS-CID experiments performed on [LiX(PhtBuBpin)]– ions. The rate (ratio) of transmetalation versus dissociation of (PhtBuBpin)– is furthermore modulated by X (i.e. BF4 > I > Br > Cl). Quantum chemical calculations verify the preference to undergo transmetalation of Ph– instead of dissociation of (PhtBuBpin)– through an analysis of the potential energy surface along the reaction coordinates. They also show that explicit solvation with one equivalent of THF renders transmetalation kinetically hindered. However, in solution phase experiments, we do not observe transmetalation. The attempt to quench transmetalation intermediates by the addition of H2O to the reaction solution instead leads to hydrolysis of Li(PhtBuBpin) at the ester functionality. Interestingly, the Ph and tBu group remain intact (unprotonated). These albeit simple systems allow for a detailed study on differences in the inherent (intrinsic) reactivity of unimolecular systems and the “extrinsic” reactivity in the solution phase.

[1] (a) T. Auth, K. Koszinowski, R. A. J. O’Hair, Organometallics 2020, 39, 25; (b) F. Kraft, T. Auth, A. Pereverzev, J. Roithová, K. Koszinowski, manuscript in preparation.

Poster Nr. 23

Atmospheric-pressure laser desorption mass spectrometry of safety-relevant low-volatile substances

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The fast and reliable mobile detection of safety-relevant substances is of vital importance for all kinds of security forces, ranging from more civilian settings like police or airport security to military ones. However, the detection of explosives or improvised explosive devices directly from the gas phase is often hindered by the low vapour pressure and volatility of commonly used compounds. Therefore, these substances are usually sampled by wiping a wipe-pad across a potentially contaminated surface, followed by thermal desorption. Unlike in this state-of-the-art method, in the here presented ambient pressure laser desorption method, the analytes are directly ablated from the surface and subsequently sampled with a heated capillary and analysed via mass spectrometry. This procedure brings the additional benefit, that laser desorption is a gentler method that leaves thermolabile analytes more often intact than thermal desorption, which may lead to decomposition.

In this work, the general viability of this concept for the detection of safety-relevant low-volatile substances, in particular of explosives and related precursors are explored. For this purpose, a setup was devised that leads a capillary, heated up to 250°C, in the direct vicinity of a laser desorption spot, to extract the desorbed substance molecules. The transferline was then coupled to a mass spectrometer equipped with an EI, CI and SPI ion source to analyse the desorbed plume. The desorption itself was facilitated by either the second harmonic frequency at 532 nm of a pulsed Nd:YAG laser or a continuous wave CO2 Laser. It was shown that under laboratory conditions low detection limits down to the nanogram scale could be achieved with the Nd:YAG laser for various explosives, such as DNT, TNT, EGDN, Tetryl, PA, AN or TATP. Furthermore, TNT was also detected from real-life samples such as bomb shrapnel or a contaminated briefcase surface. The desorption efficiency is mainly dependent on the laser source, laser fluence, sample surface and analyte substance. This is especially true for using a cw-CO2 laser, which shows a principal thermal desorption mechanism and has a strong correlation between the minimum necessary laser fluence for desorption and used sample surface that spans over an order of magnitude.

In summary, it has been shown that ambient-pressure laser desorption has the general potential to be used in the detection of explosives, although more in-depth research into the influence of possible combinations of substances and surfaces is still necessary and ongoing.

Poster Nr. 24

Comparison of UHPLC-MS Solvents with High-resolution MS

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In the past years, the sensitivity of mass spectrometers increased rapidly. In theory, increasing sensitivity is the key to achieve lower detection limits. However, there are limitations.

One important factor is the solvent purity. Considering the ionization process in the hyphenation of UHPLC and MS, the solvent concentration is clearly higher as the analyte concentration.

In addition, UHPLC-instruments allow higher solvent flow rates than former HPLC-instruments due to their ultra-high pressure stability.

The ratio between solvent and analyte is well known and lead to the development of high purity solvents that are specified for (U)HPLC-MS applications. Today, UHPLC-MS grade solvents are essential to achieve the requirements of a UHPLC-MS system.

Nevertheless, the specifications of different UHPLC-MS grade solvents are not equal, as there are various impurities that influence the ionization process (i.e. trace metals ions, leachables and extractables from packaging, particle size and concentration).

We analyzed state-of-the-art UHPLC-MS grade solvents with a highly sensitive mass spectrometer and compared overall performance by means of background and common impurities.

Poster Nr. 25

In-house packed size-exclusion chromatography columns and native mass spectrometry: a fast and adaptable strategy to study proteins and protein complexes.

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Biological mass spectrometry (MS) plays an essential role in analyzing biomolecules such as proteins, oligonucleotides, lipids, and metabolites. Current challenges in life sciences involve complex networks of interactions among biomolecules moving dynamically around living organisms. This complexity is one of the reasons why MS, especially when working harmonically with high-resolution structural techniques (e.g., nuclear magnetic resonance spectroscopy or X-ray crystallography) is cherished by the scientific community. Mass spectrometry of proteins and peptides has been mainly performed by bottom-up approaches using smaller peptides produced through enzymatic digestion. Despite these methods having been successfully applied in many cases, relevant structural information such as protein-protein and protein-ligand interactions can be lost. Native MS (nMS) is a key technique for these cases as it is capable of retaining noncovalent interactions. This characteristic has made it possible to study intact proteins and their interaction networks with higher confidence than before. Furthermore, the successful combination of native MS with ion mobility (IM) represents another improvement toward high-efficiency structural MS methods. Because IM can separate isobaric species according to their shape and size, IM-nMS can be very useful for investigating the conformational landscape of proteins. A major remaining drawback of these techniques is the sample preparation step. Special attention must be taken in transferring proteins to electrospray MS-compatible conditions without perturbing their higher-order structure. Normally, this is achieved by exchanging the protein solution for a volatile salt-rich one (e.g., ammonium acetate). This step is usually called buffer exchange and helps to keep a native-like environment for the target protein, decrease chances of adduct formation, and improve desolvation efficiency during the transition from the liquid to the gas phase.

Although manual buffer exchange techniques, including the use of spin columns and dialysis perform successfully, they require multiple steps, which negatively impacts the analytical throughput. In recent years, some research groups have started to explore the online coupling of size-exclusion chromatography with nMS (SEC-nMS) to create faster online methods able to perform buffer exchange automatically or to separate and acquire native MS data of several proteins simultaneously. Indeed, this is an attractive idea especially for creating high-throughput methods for native MS. However, conventional SEC operates at flow rates that are rather high for native protein analysis (e.g., 0.250 – 1 mL/min), especially if available sample amounts are limited. To ensure proper desolvation in this case, ESI must be performed under harsh conditions which can induce unfolding events. Moreover, the options for commercially available SEC columns suitable for operating at reduced flow rates are limited. The use of low flow rates with columns of reduced internal diameter (smaller than 2.1 mm ID) facilitates desolvation under soft conditions that favor preservation of a native-like structure. The current work aims to address some of the still-remaining issues around the hyphenation between SEC and native MS. We have developed a procedure for in-house production of SEC columns of different dimensions and stationary phases (i.e., Superdex 75 pg and Tosoh PWxl). The benefits of producing SEC columns in-house include the possibility of adapting them according to the main goal (e.g., online buffer exchange or separation of proteins). This option is more flexible than the use of commercially available SEC columns with pre-defined dimensions, when we need to downscale the procedure as much as possible to favor the coupling with native MS.

Three different columns are reported and tested for online buffer exchange, SEC separation, and nMS analysis of intact proteins. Applications include automated desalting and simultaneous acquisition of native MS spectra from protein mixtures containing ubiquitin, cytochrome c, alpha-synuclein, myoglobin, carbonic anhydrase 2, and bovine serum albumin. Finally, we explore the possibility of doing SEC-native top-down MS of proteins, as well as collision-induced unfolding experiments of protein complexes. With this work, we hope to highlight some of the benefits of using in-house developed SEC columns as the basis for preparing protein samples in non-denaturing conditions just before native MS.

Poster Nr. 26

Investigation of structural features in extremely complex crude oil samples by APPI-FAIMS-FTMS

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While over the last years strong efforts have been undertaken to develop analytical methods for the comprehensive analysis of extremely complex mixtures such as crude oil, the elucidation of structural motifs, let alone of compound structures remains a bottleneck.

In case of heavy crude oils, a comprehensive analysis is hampered on the one hand by the need for sufficient mass resolving power to distinguish isobaric species as close in mass as 3.4 mDa (C3 vs SH4). For structural elucidation by MSn experiments, the additional difficulty to selectively isolate isobaric species also plays an important role. A direct chromatographic separation of species in crude oil, which would remediate both problems, is difficult due to the extreme complexity, the low volatility of numerous compounds in case of gas chromatography or solubility problems that result in the need for less efficient normal phase chromatography in case of liquid chromatography.

As an alternative approach for sample simplification, this study uses High-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS) for the separation of potential isobaric but also isomeric compounds within an otherwise untreated heavy crude oil sample. To this end, the cylindric FAIMS device used has been modified in several ways:

Firstly, the ion source region has been adapted to allow for Atmospheric Pressure Photoionization (APPI) instead of Electrospray ionization, as crude oil is mostly made up of non- to medium polar compounds, many of which contain aromatic moieties. Thus, APPI is the method of choice as a universal ionization method for crude oil analysis.

Secondly, the electrode setup has been modified to reduce the standard electrode gap. Thus, a higher electric field between the electrodes is achieved, which can lead to a better separation of isomeric compounds.

The modified FAIMS device was coupled to a research type Orbitrap Elite mass spectrometer to analyze a heavy crude oil of north American origin. Overview spectra where acquired in full scan mode, while selected mass windows where recorded in SIM mode with a detection window of 30 Da around pre-chosen ions to be fragmented using collision induced dissociation (CID) in the linear ion trap before acquiring the corresponding MS/MS spectra. All spectra where recorded at a mass resolving power of 480k (FWHM at m/z 400) using APPI at 10.0 and 10.6 eV for ionization.

With this setup it was possible to elucidate and to distinguish structural motifs of different types of compounds within a heavy crude oil.

Poster Nr. 27

Is isobar suppression by lasers and a particle accelerator making chemistry redundant?

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For many years, cosmogenic nuclides such as 10Be, 14C, 26Al and 36Cl are routinely determined by accelerator mass spectrometry (AMS) to quantify and date processes in Earth, Environmental and Planetary Sciences. More recently, AMS was successfully performed to measure long-lived radionuclides of (mainly) anthropogenic sources, such as 41Ca, 55Fe, 90Sr, 99Tc, 129I, 135/137Cs, 233/236U, and 237Np. Analytically most challenging are radionuclides (60Fe, 182Hf, 244Pu) with isotopic abundances as low as 10-16. The latter nuclides are the remains from nuclear stellar processes and had been stored in terrestrial archives such as marine deep-sea sediments, manganese iron crusts and nodules.

Applications are very diverse: Starting from cosmochemistry reconstructing irradiation conditions of meteorites and the constancy of the cosmic radiation itself over geomorphology and oceanography to study climate and environmental changes to finally nuclear decommissioning and nuclear waste disposal.

Common to all, however, is the tedious radiochemical separation to deplete matrices and isobars, which was usually a prerequisite for AMS. This prevented fast and affordable analysis and high-sample throughput until recently. Now, the world-wide unique ion-laser interaction mass spectrometry (ILIAMS) system developed at the Vienna Environmental Research Accelerator (VERA)[1] provides isobar suppression by up to fourteen orders of magnitude.

Hence, ILIAMS-assisted AMS enables the direct detection of e.g., 26Al/27Al (~10-10) and 41Ca/40Ca (~10-11) in simply-crushed stony meteorites containing intrinsic ~1% Al and Ca each. The presence of isobars, the natively-abundant elements (15% Mg, 0.1% K), does not cause any analysis problem making any radiochemical separation redundant.

We have also quantified 41Ca/40Ca as low as 10-12 – in the isobaric presence of 0.5% potassium – without any chemical treatment for concrete and simple HCl leaching for heavy concrete (baryte), respectively. Both materials were earlier exposed to thermal neutrons in a nuclear reactor yielding to the specific 41Ca/40Ca, relevant for nuclear decommissioning and legal clearance levels.

Additionally, first 26Al/27Al (~10-11) tests on terrestrial quartz samples from high altitudes used for surface exposure dating look promising to set-up AMS as a pre-screening and sample selection method before starting tedious chemistry for more accurate results, either of 26Al or 10Be. In the near future, when combined with a high-ion current output ion source, high-accuracy results without any chemistry might be within reach.

Acknowledgements: We thank L. Ferrière and D. Heinlein for providing meteorite samples.

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Poster Nr. 28

Mass Discrimination Effects – How Voltage Settings of ESI-IT-MS affect the Analysis of the Substituent Distribution of Hydroxyethyl(methyl)-Cellulose

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The presented work deals with the accurate and precise analysis of pattern profiles in oligosaccharide derivatives by mass spectrometry (ESI-IT-MS, Bruker HCT Ultra ETD II). Sources of potential bias along the MS pathway have been systematically studied.

As additives, cellulose ethers have a wide field of application in food, cosmetic-, pharmacy- as well as textile and building material industry, where they are used, for example, to control viscosity and rheology, adhesive properties, and gelling.

Amongst others, the physicochemical properties notably depend on the chemistry of substituents as well as the degree of substitution and the substituent pattern along and among the cellulose chains.1

The determination of the substituent profiles of cellulose ethers can be done by ESI-MS after partial hydrolysis, following a similar principle to the comparative approach often used in glycomics2. The basic requirement for reliable substitution profiles is the accurate determination of the molar ratios of all possible substitution patterns over a wide mass range – in the case of hydroxyethyl(methyl)-cellulose (HEMC) up to Δ m/z 528. Any kind of discrimination effects along the MS pathway consisting of ionization, ion transport, mass analysis and detection must be excluded.

By a comprehensive study of equimolar mixtures of representative model compounds for HE(M)C, we investigated how the measurement parameters/ voltage settings of the individual components of an ESI-IT-MS affect the quantitative analysis of HE(M)C and how reliable substituent profiles can be obtained.3

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Poster Nr. 29

Microplastic Characterization and Screening by Combining DART and High-Resolution Mass Spectrometry

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Microplastics are small plastic particles (≤ 5mm) that result from both commercial product development and the breakdown of larger plastics. Microplastics are abundant in our environment. Commercial sources include cosmetics, clothing, and other textiles to other pieces of plastics such as water bottles that breakdown with radiation. Currently microplastics have been detected at an alarming level in our marine life and drinking water.

Direct Analysis in Real Time (DART)-high resolution mass spectrometry allows for a rapid fingerprinting of environmental microplastics and the screening of additives. Typical samples of interest are polymers found in the environment like virgin pre-production pellets, microbeads from personal care products, microplastics found in the aquatic environment, and synthetic fibers.

DART was coupled to an impact QTOF (both Bruker, Germany). Traditional GC/MS methods require extensive sample preparation (10-20 minutes) and have long analysis times (> 20 minutes). This new method allows for rapid analysis of polymers (< 5 minutes) with no sample preparation required. DART was coupled to a thermal desorption system (IonRocket, Biochromato Inc., Japan) which delivers temperature gradients from ambient to 600 °C with ramp rates of 150 °C/min. A small sample sliver is cut and placed in a copper pot. When the thermal program is started, the QTOF collects MS spectra at 5Hz for the entire run.

The resulting mass spectra display thousands of discrete peaks. Depending on the temperature, different additives, the polymeric basis as well as degradation products are released and detected at different time points during the run.

Here we compared distinct m/z signals as well as general signal patterns of a microplastic material found in water with standard plastic samples such as a soda bottle, a trash bag and packaging material. Some compounds like at m/z 411 can be found in all samples, referring to a commonly applied additive for these consumer products. While the soda bottle was made of PET, the microplastic clearly had PE as a basis. Since PE is an insoluble polymer, it is difficult to analyze with other MS-based methods like MALDI. The microplastic material showed most similarity with the trash bag, with regard to both certain distinct marker m/z values as well as the general pattern released by the temperature gradient, so it can very likely be traced back to that origin.

The combination of DART with HRMS allows for rapid fingerprinting of environmental microplastics and the identification of unknown compounds with high resolution and high mass accuracy.

Poster Nr. 30

Outstanding performance and low dwell times are not mutually exclusive

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The number of substances to be analyzed using LC-Tandem-Quadrupole-MS is constantly increasing as well as the number of analytes to be measured in one run. Especially in the field of pesticides, regulatory agencies have set maximum residue levels (MRLs) for hundreds of pesticides and their metabolites. Efforts are also being made in other areas to combine more and more analytes or analyte-groups in one method. In most cases, polarity-switching is also necessary to analyze all compounds in one run. Another trend is to significantly decrease the measurement times of LC-MS/MS methods to reduce turnaround times and costs per analysis. For this reason, columns with small particles are used, which leads to very narrow peaks. The combination of all these factors creates a large number of transitions in a very short time causing low dwell times.

The dwell time can only be increased at the expense of the number of data points but with very few data points, reproducibility decreases. If at least 12 data points are available, it ensures that the reproducibility is not negatively influenced by the number of data points. A comprehensive LC-MS/MS workflow for simultaneous quantitation of more than 750 pesticide residues in 20 minutes is used as an example to show that no compromises need to be made because the Agilent Tandem-Quadrupole-MS-systems show very good reproducibility even at dwell times below 1 ms.

Poster Nr. 31

Rapid Analyte-Ion Discriminations through Corona-Discharge-Coupled Ultrasonic Nebulization for Mass-Spectrometric Analyses

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Analyte separation is often a critical step in mass-spectrometric characterizations of complex samples. Conventional chromatographic methods offer stable and high-quality separations of multi-analyte systems, but are often avoided when throughput gains higher priority. However, methods such as ambient desorption/ionization and atmospheric pressure sampling, which offer rapid and high-throughput features, commonly align with the shotgun analysis philosophy, where all analytes are simultaneously detected. The resulting mass spectra are, consequently, as complex as the sample, which can be quite difficult to interpret. In our investigations of an alternative direct sampling method based on ultrasonic nebulization, we discovered distinctive time-domain features for each analyte, which can be used to flag and categorize mass-spectral peaks into different groups. More specifically, without the presence of a separation apparatus, the analyte-specific time-domain features can be used to flag and categorize mass-spectral peaks into different groups, resembling fingerprints that can be used for rapid analyte identification at high certainty.

In this study, we evaluated the potential of ultrasonic nebulization for rapid analyte identification in complex samples using yeast extract as our model sample. The sample was loaded, nebulized, and analyzed by an Orbitrap mass spectrometer. Followed by a workflow based on a modified cross-correlation algorithm that exploits the analyte-specific time-domain signals, hundreds of ion groups were obtained programmatically for direct spectral library search. From sample introduction to analyte assignment, the whole process was within 1 minute, demonstrating the effectiveness of ultrasonic nebulization as a generalized platform for the rapid analysis of complicated samples. Additionally, we isolated the source of the time-domain features in the ion transfer process, which provides insights for further optimization of ultrasonic-nebulization-based platforms. These findings open up possibilities for applying this platform to more accessible instruments, such as ion mobility spectrometry.

Poster Nr. 32

Vacuum Photoionization on a High-Resolution Mass Spectrometric Platform: Prototype and Perspectives for Complex Mixture Analysis

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Gas-phase, pulsed-laser photoionization methods are versatile, soft ionization approaches for mass spectrometry applications. We recently modified an Exactive™ Orbitrap mass spectrometer allowing direct photoionization inside the C-trap (PhotOrbi) [1]. Resonance-enhanced multiphoton ionization (REMPI) allows for a sensitive and selective description of the aromatic profile, whereas the high resolution and high mass accuracy allowed a molecular-level description. The aromatic profile of complex mixtures is of high priority in various research fields due to the environmental and health aspects of polycyclic aromatic hydrocarbons (PAHs).

The PhotOrbi system was developed as a versatile platform easily adapted to the respective research area. Vacuum REMPI was conducted using either a frequency quadrupled Nd:YAG laser (266 nm) or a Krypton-Fluoride excimer laser (248 nm). The system can be easily coupled to different sample introduction techniques, and the specialized data acquisition by Spectroswiss SARL enabled us to record transients with freely adjustable lengths of up to 2s. We explored the capabilities of the PhotOrbi for various complex mixtures and applications areas:

1) Direct gas chromatographic hyphenation, e.g., for the description of bio-oils in the context of alternative fuels and circular economy,

2) Analysis of carbonaceous aerosols, heavy petroleum residues, and asphaltenes by hyphenation of a thermal desorption and pyrolysis approach [2],

3) Direct field usage of the prototype measuring primary emissions of a research ship diesel engine and combustor rig (mimicking aviation emissions) [3].

Remarkably, despite the harsh conditions during the field campaign, the characteristics of the Orbitrap mass spectrometer could be preserved, achieving ppm mass accuracy and >100,000 resolving power across the targeted mass range. Compared to commonly deployed time-of-flight instrumentation, the molecular complexity could be directly tackled, and the mutagenic and carcinogenic PAH profile was accessed, even for highly dynamic processes.

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Poster Nr. 33

2-fluoro-1-methylpyridinium p-toluene sulfonate (FMP-TS) as a new derivatization reagent for vitamin D₃ metabolites using LC-MS/MS

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Vitamin D deficiency has been described as a pandemic health problem worldwide, highlighting the need for accurate and precise measurements of the circulating levels of 25-hydroxyvitamin D (25(OH)D), which is the metabolite used for determining vitamin D status. Recently, there is increasing interest in measuring multiple vitamin D3 metabolites; for example, the C-3 epimer of 25(OH)D3, which has shown significant contribution to total 25(OH)D3; 24,25-dihydroxyvitamin D3 (24,25(OH)2D3), which is the major catabolite; and the active form, which is 1,25-dihydroxyvitamin D3 (1,25(OH)2D3).

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is firmly established today as the gold standard technique for analysis of vitamin D. It provides the opportunity to measure multiple metabolites in a single experiment, thus creating vitamin D metabolite profiles.

Metabolite quantification can be very challenging, especially for low abundant species such as 1,25(OH)2D3. To overcome this problem, chemical derivatization can be employed, which enhances detection sensitivity by increasing ionization efficiency and shifting masses to higher m/z values with less isobaric noise; it also provides specific fragmentation patterns for MS/MS analysis and improves resolving power in the LC separation.

We developed a simple, fast and reliable LC-MS/MS method to measure five different vitamin D3 metabolites in human serum. To our knowledge, this is the first derivatization method using 2-fluoro-1-methylpyridinium p-toluene sulfonate (FMP-TS) for vitamin D3 metabolites. The derivatization reaction was optimized to maximize detection sensitivity. Six parameters were investigated consecutively: 1) presence of moisture in the reaction solvent; 2) added base; 3) base concentration; 4) FMP-TS concentration; 5) incubation temperature; and 6) incubation time. The final, optimized analytical method was validated according to the following parameters: 1) linearity, 2) accuracy, 3) precision, 4) extraction recovery and 5) stability of the derivatization products in the extract.

The method was proven to be accurate, precise, reliable and sensitive; it was also very fast, easy to perform and economical. Another major advantage was the successful separation of the derivatized epimers (3α-25(OH)D3 and 3β-25(OH)D3) and isomers (1,25(OH)2D3 and 24,25(OH)2D3). Finally, our new method was successfully applied to the quantification of the investigated metabolites in human serum samples of healthy individuals.

Poster Nr. 34

A fast and novel urine toxicology screening method using DART-MSMS

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As response to huge number of drug overdose deaths in the 1990's and by 2010, health care providers were testing patients in ever increasing numbers involving immunoassays and LC-MSMS panels with scope to a variety of drug classes. There is a need to develop a quick, fast and inexpensive screening assay to replace long LC-MSMS methods and low specificity immunoassay methods. We investigate DART-MSMS, Direct Analysis in Real Time [1], to provide faster turnaround time and high specificity method. DART has seen unnumerable applications in a variety of analytical areas and has shown to have exceptional versatility [2].

Multi-drugs kits from Pinpoint Testing, LLC. were used, one with drugs in methanol, the other with drugs in urine. Samples were processed according to manufacturer's instructions. Urine samples were extracted and concentrated prior to use. Sample extracts were subsequently spotted on a Bruker HTS96 screen. Analysis was performed with a Bruker DART, ionization temperature 300°C, in helium, in scanning mode.

DART-MSMS data were processed via standard quantitation software and used as traditional LC-MSMS data. All drugs investigated yielded linear regression curves in the range tested, with R2 values of 0.990 or better.

DART-MSMS seems to be a likely technology for urine analysis in forensics and pain management areas. Results obtained thus far are in line with a traditional validation exercise. More work needs to be performed to determine the applicability of this technology in a highly regulated environment.

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Poster Nr. 35

Effects of Feeding and Fasting Conditions on serum Lipidomic Profiles of healthy individuals

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Experiments were conducted to determine the effect of feeding and fasting on the serum lipidome profile of healthy individuals. Serum samples from healthy subjects (biological replicates n=5, technical replicates =3) were collected after fasting (up to overnight) and after feeding, ca after 2 hours. The samples were extracted (using a modified Matyash protocol), and analyzed using a high-throughput LC-MS/MS platform and a targeted approach. Changes in serum levels of 225 lipid species from 13 lipid classes were determined. Higher levels of triacylglyceride (TAG), chol (Cholesterol), and diacylglyceride (DAG) in serum samples of Fed-state samples (compared to fasted-state samples) were found. Several lipid species TAG- 48:0, 50:1, 52:1, 54:1, 54:2, 56:2 ; DAG 34:2; PE- 36:1, 36:2, 38.2,40:4 were significantly changed under studied conditions. Then, we studied the lipidome stability of samples stored at +12 °C in an autosampler for 12 hours and evaluated the reproducibility of measurements for 20 consecutive injections. The lipidomic analysis was found to be stable and reproducible, and no carry-over peaks were detected in the chromatogram of blank samples showing good specificity of the method. Overall, the established method can be used to assess lipid profile alterations of clinical serum samples under different health or disease conditions.

Poster Nr. 36

Identification of disulfide-adducts in human serum albumin resulting from exposure to malodorants

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Malodorants are mixtures containing organic sulfur compounds, that exhibit a pungent smell and have strong, repulsive characteristics [1]. Exposure of humans to these substances causes headache, nauseau, unconsciousness, coma and can lead to fatal respiratory depression [2]. Due to their low olfactory perception threshold, their distribution hardly leads to life-threatening conditions and thus, they may be used as non-lethal agents in military and civilian defence scenarios [1]. Malodorants are utilized in personal protection devices as spray, to control riots, to deny an area or to clear facilities [3-5]. Whenever such a formulation contains synthetic malodorants, it is categorized as riot control agent (RCA). Nevertheless, the use of RCA during war is banned by the Chemical Weapons Convention [1,6]. In order to prove illegal distributions of malodorants, robust bioanalytical methods are needed. Therefore, we present a mass spectrometry (MS)- based in vitro model for the verification of exposure to malodorants. Ethyl mercaptan (SEt) was used for in vitro incubation of human plasma to produce disulfide-adducts with cysteine-residues in human serum albumin (HSA). Resulting modifications were identified after proteolysis by modern micro-liquid chromatography (µ LC) coupled to high-resolution tandem-mass spectrometry (MS/HR MS) as modified dipeptide. SEt was proven to covalently bind to the sidechain of Cys34 in HSA. Such reaction products (adducts) are supposed to be produced with other malodorous compounds as well and thus represent potential biomarkers for forensic analysis to verify exposure.

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Poster Nr. 37

LC-MS/MS based quantification of antibiotics in sepsis therapy

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Sepsis is an acute, life-threatening organ dysfunction that triggers a massive inflammatory response throughout the body. Effective antibiotic therapy is critical to the management of this condition, but individualised dosing is not guaranteed, making it difficult to ensure adequate serum antibiotic concentrations. To overcome this, therapeutic drug monitoring is used to determine the individual antibiotic concentration in the patient's blood. However, this process is laborious and requires a lot of equipment and personnel [1]. Although LC-UV/VIS is currently the most widely used method for antibiotic quantification, it has several limitations: Antibiotics must be determined separately, the separation - and thus accurate identification - of the antibiotics from non-specific impurities is not always reliable, and large plasma volumes are required [2].

To overcome these difficulties an LC-MS/MS based assay was developed for the simultaneous quantification of the antibiotics meropenem, piperacillin and the beta-lactamase inhibitor tazobactam. Initially, the HESI source parameters for all three analytes were optimised as first MS parameters. The second step was the development of the separation method by means of HPLC (Vanquish™ Neo UHPLC). A Hypersil GOLD™ aQ column (100 x 1 mm; 1.9 µ m) was used as the stationary phase and the analytes were separated with a gradient (A: 0.1% FA in water, B: 100% ACN) at a flow rate of 100 µ l/min. In a third step, the linear range of the developed method was tested between 0.01-100 mg/l based on real sample concentrations. We were able to demonstrate that all 3 analytes were detected with the developed method, perfectly separated from each other. Furthermore, the LOQ was determined and a linear correlation was shown over the clinically relevant concentration range of 0.1 - 100 mg/l.

In the next phase of the study, following the development of human blood sample processing, patient samples from the SepsisDataNet cohort (a multi-centre cohort of ~500 patients with up to 4 sample collection time points) will be analysed using this assay. The results will then be used in combination with comprehensive patient data to develop a machine learning model that can predict serum concentrations using artificial intelligence, thereby minimising the need for sample measurements.

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Poster Nr. 38

Mass Spectrometric ITEM-TWO Investigations and Bio-Computational Analyses of Phosphorylation-Dependent Antibody Binding to Cancer-Mutated Linkers of C₂H₂ Zinc Finger Proteins

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Zinc finger proteins are the most abundant class of transcription factors in humans and the subclass of C2H2 zinc finger proteins are encodes by ca. 750 known genes [1]. They play an important role in DNA binding, transcriptional regulation, RNA packaging, regulation of apoptosis, protein assembly, and lipid binding.

Anti-zinc finger protein antibodies have been already applied in proliferation processes and cancer development mechanisms. The monoclonal anti-zinc finger protein antibody that recognizes the specific amino acid sequence motif of the zinc finger linker region HpTGEKP was generated to bind several zinc finger proteins with altered composition.

Interestingly, a point mutation of the zinc finger protein 331 (ZNF331) gene, causing a single amino acid exchanges H®Y of the linker, has been found to be significantly enriched in Uterine Corpus Endometrial Carcinoma (UCEC), Colon and Rectal Adenocarcinomas (COAD/READ), and Skin Cutaneous Melanoma (SKCM) [2]. Expression of ZNF331 has been confirmed in HeLa cells and in follicular thyroid adenomas [3].

To answer the question whether the anti-HpTGEKP antibody was able to detect the in cancer mutated ZNF linker sequence, mass spectrometric ITEM-TWO (Intact Transition Epitope Mapping) analysis in combination with molecular modeling was applied to determine the phosphorylation-dependent molecular recognition motif of the anti-HpTGEKP antibody applying binary and ternary component mixtures consisting of antibody and (phospho-) peptides [3,4].

First, all seven synthetic (phospho-) hexapeptides which went into the study were characterized by experimentally determining their molecular masses. Amino acid sequences of the selected peptides have been chosen to match the antibody's recognition motif or the cancer-related zinc finger mutation plus phosphorylations of threoninyl and tyrosinyl amino acid residues. The purity of the monoclonal anti-HpTGEKP antibody was also checked by offline nanoESI-MS analysis of the intact antibody. To do so, the anti-HpTGEKP antibody was rebuffered into 200 mM ammonium acetate solution, pH 6.7 using centrifugal filters with MWCO 50 kDa.

Then, mixtures of antibody and peptide solutions were prepared to obtain molar ratios of approximately 1:3 or 1:3:3 and incubated at room temperature for at least one hour. ITEM-TWO measurements were performed using a Synapt G2-S mass spectrometer. Before the trap collision energy was step-wise increased, the quadrupole was set to full transmission, and ions were recorded for ca. 2 min, each. The quadrupole analyzer was then applied to block transmission of molecular mass ions below m/z 5200. Immune complex dissociations at increasing collision cell voltage differences were recorded for at least 1.5 min, each. Collision energy was step-wise increased. Upon electrospraying of all the components of the mixtures, that is, hexapeptides, antibody, and intact immune complexes, the produced ions were subjected to mass spectrometric mass filtering. Complex-released peptides were unambiguously identified by their masses with isotope resolution. Molecular modeling studies were

performed using the Schrödinger BioLuminate graphical environment, the OPLS_2005 force field, the Desmond molecular dynamics package, and the "GMX cluster" tool from GROMACS.

From the results of our studies with seven (phospho-) peptides with distinct amino acid sequences, which resembled either the antibody's binding motif or mutations, we conclude that binding of the anti-HpTGEKP antibody to the mutated linker of ZNF331 depends on the following molecular epitope constitution prerequisits: (i) A negatively charged phospho group, located near the peptide's N-terminus is mandatory for antibody binding when placed on the peptide surface at a precise distance to the C-terminally located positively charged epsilon amino group of a lysinyl residue. (ii) A bulky amino acid residue, such as the tyrosinyl residue at the N-terminal position of the (phospho-) threoninyl residue, abolishes antibody binding. (iii) Two closely spaced phospho groups negatively interfere with the surface polarity pattern and abolish antibody binding as well. (iv) Non-phosphorylated peptides are no binding partners of the anti-HpTGEKP antibody.

The testing of peptide-antibody interactions, that is, epitope fine mapping, provides valuable information about binding capabilities of an antibody to its antigen particularly when the actual wild-type or mutated protein had been neither cloned, expressed, and purified nor isolated from a respective tissue/tumor. As shown, subtle molecular differences and their distinctive influences on noncovalent binding behavior between bio-macromolecules and ligands can indeed be probed by mass spectrometric ITEM-TWO analysis.

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Novel Aspect:

Mass spectrometric gas phase dissociation analysis by ITEM-TWO reveals the molecular recognition code of the anti-HTpGEKP phospho-hexapeptide antibody

Poster Nr. 39

Mass spectrometry based virus diagnostic

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Current viral diagnostic methods offer no simple way to simultaneously test for various respiratory viruses, all modern tools are highly specific to individual viruses leading to time-consuming test procedures or a diagnosis purely based on symptoms and experience of the treating doctor. In the case of respiratory infections this circumstance is further aggravated by the fact that many viral respiratory infections present very similar symptoms, especially in the early phases of infections. Shortcomings like these could be addressed using state of the art mass spectrometry (MS) analysis tools.

We aim to develop an easy-to-use MS-based tool to improve modern diagnostics and make early differentiation between dangerous pathogens and “the common cold” easy, fast and cost effective.

Latest results from the first phase of the VirMScan project, focused on identifying suitable peptides for detection of Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2) as well as optimizing reaction settings, and establishing detection limits, will be presented. After the generation of a reliable protocol for the detection of SARS-CoV-2, methods and databases will be gradually extended to detect common respiratory viruses such as influenza viruses, rhinoviruses, human respiratory syncytial viruses or less pathogenic coronaviruses.

Poster Nr. 40

Multiplexed vitamin D fingerprints by one-pot double derivatization using commercial derivatization reagents to reduce measurement time and improve separation and sensitivity

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Accurately determining vitamin D status is important since this prohormone plays crucial roles in many physiological and pathophysiological processes. There are additional metabolites beyond vitamin D3 with clinical importance, namely epimers of 25-hydroxyvitamin D3 (3α-25(OH)D3 and 3β-25(OH)D3), 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) and 24,25-dihydroxyvitamin D3 (24,25(OH)2D3). LC-MS/MS is often used for the determination of these molecules, sometimes combined with derivatization to increase the sensitivity of the method. The most common derivatization reagent is 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) because of its low price and the ease of performing the derivatization reaction. PTAD is a Cookson-type reagent, which attacks the diene moiety of vitamin D metabolites. Even though LC-MS/MS is commonly regarded as the "gold standard" method for vitamin D compounds and pre-column derivatization is still commonly used in clinical studies, which often require analysis of a large number of samples. Unfortunately, no derivatization reagent for multiplexed high-throughput analysis is currently commercially available. There are several suggestions based on attaching alkyl chains of different lengths to the derivatization reagent or using isotope-labelled reagents for multiplexing. However, these derivatization reagents must be synthesized in house, thus making routine analysis in typical clinical labs difficult.

In this study we developed a novel one-pot double derivatization scheme to allow, for the first time, multiplexed LC-MS/MS analysis of vitamin D and its metabolites. The derivatization consisted of both a Cookson-type reagent and a derivatization for hydroxyl groups. The analytes initially reacted with PTAD (0.5 mg/ml), followed by acetylation using acetic anhydride, catalyzed by 4-Dimethylaminopyridine (DMAP) at room temperature. The acetylation was the crucial step as vitamin D3 metabolites vary in the number of hydroxyl groups. The DMAP concentration determined the reactivity and was thus optimized in the range of 1–20 mg/ml. To introduce the multiplexing abilities, we used acetic anhydride und isotope-labelled acetic anhydride. In addition to enable measurement of two samples in one LC-MS/MS run, the acetylation improved the LC separation of the isomers on C-18 columns, which is a major issue in vitamin D analyses. In reversed-phase LC, C-18 stationary phases are most commonly used. Separation of epimers after PTAD derivatization cannot be performed on C-18 columns and usually pentafluorophenylpropyl (PFP) stationary phases are used. However, PFP columns are not as stable as C-18 in long-term use. Acetylation of the C-3 hydroxyl group provided a solution to this problem, as chromatographic selectivity increased and baseline separation of the five metabolites 1,25(OH)2D3, 24,25(OH)2D3, 3α-25(OH)D3, 3β-25(OH)D3 and vitamin D3 was readily achieved on a C-18 column with methanol/water gradient elution.

The derivatization scheme described here for duplexing was tested on a large sample cohort of 240 serum samples of patients with chronic liver diseases. Furthermore, the method was evaluated in terms of dynamic range, intra and inter-day precision, and accuracy using FDA criteria, commercial as well as in house quality control and calibration samples. LC-MS/MS experiments were performed on an Agilent 1290 Infinity II UHPLC system coupled to a Sciex QTRAP 6500+ mass spectrometer, using a Phenomenex Kinetex XB C-18 column.

Our protocol enables the analysis of large samples cohorts, because it minimizes the sample preparation process and reduces the required instrument time by 50%, as injection of two samples at the same time is possible. Also, the two-step derivatization scheme is ideal for separation of multiple vitamin D3 metabolites and baseline separation of 3α-25(OH)D3, 3β-25(OH)D3, 1,25(OH)2D3, 24,25(OH)2D3 and vitamin D3 is achieved using C-18 stationary phase.

Poster Nr. 41

Protoporphyrin IX liquid biopsy for high-grade glioma detection and monitoring

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Introduction:

Protoporphyrin IX (PpIX) is an approved tissue marker during fluorescence-guided resection of glioma. After oral administration of the prodrug 5-aminolevulinic acid (ALA) PpIX accumulates within the brain tumor resulting in an intraoperatively visible fluorescence. Blood PpIX is also explored as diagnostic marker for cancer progression. Most approaches use spectrophotometric or fluorescence detection taking advantage of the strong absorption of PpIX in the region of the Soret band. However, interferences and unspecific sample preparation cause inaccurate results from biological matrices. We used mass spectrometry (MS) in a proof-of-principle study investigating patients with primary glioma (pHGG, 23), recurrent glioma (rHGG, 5) and healthy volunteers (HCTR, 5), and present a workflow for high-throughput analysis of serum PpIX.

Methods:

PpIX was isolated from serum (500 µ l) by liquid-liquid extraction using water/acetonitrile (ACN) followed by anionic-exchange solid phase extraction. Mesoporphyrin (MpIX) was used as internal standard. Both porphyrins eluted with ACN and 2% formic acid. The eluate was dried and reconstituted in dimethyl sulfoxide. RP-HPLC (HP1100, Agilent) with a semi-porous, endcapped column was performed with 10 min run time. For MS detection, a time-segmented method was developed using an ion trap (Esquire3000, Bruker). With this workflow, samples taken pre, during and post surgery were analyzed.

Results:

Quantification of PpIX in serum was performed by MS2 and time-dependent target screening. The singly charged [M+H]+ species was fragmented: cleavages occurred predominantly on the side chains of the tetrapyrrole core structure (59 u (-CH2COOH), 45 u (-COOH), 73 u (-CH2CH2COOH) losses), providing characteristic spectra. PpIX serum levels were significantly elevated in HGG patients within 5.5 to 12 h after ALA administration (Kruskal-Wallis: $p < 0.001$, Dunn-Bonferroni: HCTR vs. pHGG $p < 0.001$, HCTR vs. rHGG $p < 0.001$, pHGG vs. rHGG not significant). Within the 95% confidence interval of the time-point of maximum serum levels, PpIX showed sufficient separation between pHGG and HCTR in receiver operating characteristic analysis (AUC = 0.943, $p < 0.001$, CI95% = 0.884 - 1.000). The cut-off for diagnosing a tumorous patient was 1275 pmol PpIX/ml serum yielding 87% accuracy, 91% specificity, 91% positive and 84% negative predictive value.

Conclusion:

The results recommend the use of PpIX as a blood marker for HGG. Further studies in larger cohorts with an adapted study design are required.

Mass spectrometry related innovations:

Specific PpIX detection in serum, patient proof-of-concept study

Poster Nr. 42

Antimicrobial peptides in pyelonephritis

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Pyelonephritis (PN) is a frequently occurring inflammatory disorder of the renal parenchyma, which is associated with severe morbidity, especially among elderly and immunodeficient patients. The most common treatment is antibiotics, however, multi-drug-resistant strains of uropathogenic bacteria necessitate optimized treatment strategies.

To unravel the molecular and cellular heterogeneity in patients with PN, biopsies from nephrectomized patients with histopathological PN were analyzed by label-free LC-MS/MS-based proteomics and compared with healthy regions of renal cell carcinoma (RCC) resections as a control. The measurements were performed on a quadrupole-ion-trap-orbitrap MS (Orbitrap Fusion, Thermo Fisher) coupled to a nano-UPLC (Dionex Ultimate 3000 UPLC system, Thermo Fisher.

Enrichment analysis using Gene Ontology Biological Process (GOBP) disclosed clear upregulation of biological processes involved in the immune and defense response, including significant upregulation of proteins with antimicrobial function. Among nineteen antimicrobial peptides (AMPs), detected in the kidney tissue, twelve were found to be significantly regulated. Upregulation of AMPs was validated in urine through enzyme-linked immunosorbent assay (ELISA) and complemented by assessing the clinical parameters of the urine and plasma samples of the additional PN cohort. Since AMPs are an integral part of the immune response of the urinary tract with a variety of microbicidal and immunoregulatory functions, our data extend the current understanding of the secretion and distribution of AMPs in the PN.

Poster Nr. 43

Challenge of large-scale proteome studies of red blood cell samples

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The underlying challenge of red blood cell (RBC) proteomics lies in its high hemoglobin content. It marks 95% of the total amount of protein and can cover up other proteins of interest. Therefore, suitable sample preparation and measurement are mandatory.

To establish a specific workflow, multiple methods were approached for isolated RBCs and their White Ghost (WG, only RBC membrane) samples, e.g. SDS-Page followed up by in-gel-digestion, in-solution digestion, single-pot-solid-phase-enhanced sample-preparation (SP3), S-Trap™, and multi-enzyme digestion positive pressure filter-aided-samples-preparation (Med-pp-FASP), which have already been reported in the literature. The prepared samples were measured on the following nLC-MS/MS systems either in DDA mode (LC-Orbitrap-Velos pro, or nLC-Orbitrap-Eclipse) or in DDA-PASEF mode (nLC-timsTOF pro), in order to check for the best preparation method. Remarkably, the speed of chosen mass spectrometer also has impact on the results. The measured samples were analysed using Proteome Discoverer 2.5 and PEAKS online depending on the MS-System.

Preparing only RBC samples in four replicates using MED-pp-FASP and measured in DDA-PASEF mode on nLC-timsTOF pro using a 90 minutes gradient identified in 661 protein groups, which were at least in 3 of 4 replicates of all samples. The repetition of the experiment with RBC and WG samples measured in DDA TopS mode on the nLC-Orbitrap-Eclipse with a 90 min gradient identified in at least 3 of 4 replicates 465 protein groups for the RBC samples and 772 protein groups for the WG samples. Both experiments yielded median coefficients of variation of the protein abundance of under 25% for all groups. Based on the promising reproducibility of the workflow and good impact of the ion mobility device the dynamic range coverage of Eclipse and timsTOF were compared based on the NSAF. The timsTOF showed an enhanced the NSAF range by one order of magnitude.

For further investigation a mix of non-human spike-in proteins is planned to examine the measurable dynamic range and therefore the enhanced detection of non-hemoglobin proteins. The additional aim of this approach is the estimation of respective protein amounts and their variation between different diseases.

Poster Nr. 44

Characterization of the molecular pathomechanism of X-linked hypophosphatemia by mass spectrometric analysis

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X-linked hypophosphatemia (XLH) is a rare disease in which increased renal secretion of phosphate leads to various clinical manifestations such as bone deformities, joint pain or short stature. The hormonally active protein FGF23 plays a central role in the pathogenesis of XLH. The cause of the disease is a mutation in the PHEX gene, which leads to increased FGF23 levels in bone cells and in the blood. Ultimately, this limits phosphate (re-)absorption by suppressing serum calcitriol levels, leading to the various clinical symptoms. Proteolytic regulation of FGF23 occurs in bone cells, but the exact mechanism is not yet known in detail.

Due to the low abundance of proteins involved in phosphate homeostasis in bone cells, proteomic investigation in wild-type cell models or patient samples is limited using currently established methods. Therefore, for the first time, an XLH model was created in the osteosarcoma cell line SaOS-2 by overexpression of FGF23. For this, an additional gene coding for FGF23 was inserted into the genome of SaOS-2 cells by lentiviral transduction, resulting in an increased number of copies of FGF23 in the mutants.

The aim of the study is to gain a closer insight into the intracellular regulation of FGF23 as well as to investigate effects of the increased secretion on bone cells. To this end, the proteome of the FGF23 overexpression model was first quantitatively compared with controls in the undifferentiated as well as differentiated state. After cell lysis, proteins were tryptically digested, measured by mass spectrometry using the data-independent acquisition method optimized for complex sample material, and statistically analyzed. Comparison of the different conditions showed that processes involved in the assembly and regulation of the extracellular matrix were regulated in the FGF23-overexpressing cells. Interestingly, the regulation differed significantly between the states of cell differentiation, suggesting a divergent role of FGF23 in osteoblasts (undifferentiated) and osteocytes (differentiated).

Using the overexpression model, additional studies were performed to determine direct interaction candidates of FGF23 to potentially gain insight into PHEX-based regulation. For this purpose, cells were gently lysed, co-immunoprecipitation was used to isolate FGF23 with potential interaction partners, and mass spectrometry was performed. Several potential interaction partners were determined to play a possible role in the regulation of FGF23.

Poster Nr. 45

dia-PASEF for targeted proteomics: development of large-scale assay in human plasma sample

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dia-PASEF merges the benefits of DIA with the advantages of ion mobility in proteomics experiments making it an advantageous method to be integrated in a platform for large-scale biomarker studies without the need for in-depth method optimization. Here, we use dia-PASEF in combination with the PQ500 kit (Biognosys) to develop a targeted quantitation assay for peptides in human plasma sample. Analysis of the PQ500 kit spiked in non-depleted human plasma using the timsTOF HT allowed all 804 SIS peptides to be analyzed in a 30-minute gradient. The assay was applied to a proof-of-concept study of non-depleted plasma samples from patients diagnosed with lung cancer. In total, 663 peptides and 463 protein groups were identified and quantified, covering around 80% of the PQ500 panel. Of those, 55 proteins were found to be significantly regulated with three of them (Fibronectin, Immunoglobulin lambda-like polypeptide 1, Immunoglobulin lambda-like 1 light chain) detected to be higher abundant in healthy donors. By using a data-independent approach also proteins outside the target panel are measured and can be quantified resulting in a combination of targeted and discovery proteomics. By using discovery-based data processing an additional 26 protein groups were found to be significantly regulated, which were not part of the targeted quantitation assay. Our results show that the applied multiplexed approach has the potential to identify disease biomarkers in non-depleted plasma samples without in-depth expert knowledge using a standard proteomics workflow supported on the timsTOF platform.

Poster Nr. 46

Moving Proteomics towards the clinic: Realtime dda-PASEF and dia-PASEF Plasma Proteomics analysis with the timsTOF platform and PaSER

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Plasma proteomics is one of the main gateway's of entry for proteomics into the clinic, however plasma proteomics presents unique challenges. The high dynamic range of tens of proteins in plasma creates challenges in both identification and the quantification of the lower abundant but more clinically relevant proteins. The timsTOF platform of instruments is well situated to handle such challenges due to the added ion mobility dimension which increases identification while reducing chimeric spectra in both dda-PASEF and dia-PASEF modes. Subsequent to analysis, searching the data generated can be a slow and arduous process that can bottleneck results and be a barrier of entry to the clinic. Here we show that the PaSER platform can, in real time, consistently, confidently, and accurately identify and quantify plasma samples acquired in either dda-PASEF or dia-PASEF mode to a great level of depth.

To demonstrate the plasma proteomic workflow, we analyzed 212 DDA plasma samples with label free quantification enabled. We identified greater than 1200 protein groups and observed a run-to-run correlation average of greater than 0.97 across all quality control runs. In this dataset we were able to quantify a dynamic range of protein intensity spanning 5 orders of magnitude.

We also analyzed 400 different clinical plasma samples by dia-PASEF to investigate if candidate biomarkers could be identified between different clinical conditions. While several hundred proteins were identified in these short runs across all the samples, we clearly identified two proteins that show significant difference between disease and normal state. These two candidates will then be validated as potential biomarkers.

Taken together, DDA-PASEF and dia-PASEF coupled to the PaSER platform can provide deep, accurate plasma results in near real time.

Poster Nr. 47

Protein biomarkers for in-vitro diagnosis of drug allergy

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Introduction:

Drug allergy is very relevant form of adverse drug reactions, as it is hard to diagnose and their symptoms can range from simple rash to life threatening condition. The currently available methods to diagnose drug allergy, which includes combination of medical history, in-vivo skin tests, in-vitro assays, and/or provocation tests show limited success in practice. Thus, there is a great need for a reliable method for routine diagnosis of drug allergy.

The overall aim of the INA project is to investigate - by means of innovative, state-of-the art genomics and proteomics methods – dysregulated genes and proteins which could facilitate the development of an in-vitro diagnostic test suitable for routine diagnosis of drug allergy. Accordingly, we analyzed differential proteins from peripheral mononuclear cells (PMBCs) of drug-allergic patients, after in-vitro stimulation with the suspected drug as compared to those of non-allergic test persons. Using data independent acquisition (DIA) in bottom-up mass spectrometry (MS)-proteomics approach, we identified dysregulated proteins in PBMC samples. We furthermore developed a parallel reaction monitoring (PRM) assay, to verify the dysregulated proteins and checking their capability to differential between healthy control and allergic patient individuals using machine learning.

Results and discussion:

In the biomarker discovery phase, using the MS proteomics data from 14 non-allergic (healthy) and allergic (patient) person's sample treatments (drug-treatment versus no-treatment), we have identified 114 proteins of interest. We further added few proteins and manually refined the list down to the 78 proteins of interest on the basis of their biological significance. We developed and refined a PRM assay, which is more sensitive and accurate method for the verification of these proteins. Using skyline data analysis and export, we have confirmed 4 differential protein signatures found in untargeted (DIA) data using targeted (PRM) data, which are few proteins. Thus, we could confirm differential protein signatures found in untargeted (DIA) data using targeted (PRM) data. Furthermore, Venn diagram analysis suggested that Sulfiredoxin1 (oxidative stress resistance) and GranzymeB (protease activating caspase-independent pyroptosis) are seeming to be general reaction of cultured PBMC cells upon drug treatment/stress. Whereas S100 calcium-binding protein P (known role in immune system), Linker for activation of T-cells family member 1, and Toll-like receptor 8 (endosomal receptor with role in innate and adaptive immunity) are biologically relevant and interesting hits. In Future, we will analyse an independent set of samples using the PRM method to confirm these differential protein signatures in drug allergic reaction.

Poster Nr. 48

Proteome analysis of neutrophils exposed to melanoma cells of various aggressiveness levels

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Introduction

Neutrophils exhibit functional differences inside tumors or inflamed tissues as opposed to those circulating in blood or being resident in the bone marrow. While previous studies focused mainly on the transcriptional heterogeneity of neutrophils in various tissues, MS-based proteomics could provide a real molecular understanding of how neutrophils behave in the presence of tumor cells. However, limited amount neutrophils isolated from murine tumors or inflamed tissues makes proteomic profiling challenging. Therefore, this study is aimed to investigate proteome of few neutrophils in different types of melanoma in an in vitro model.

Methods

Neutrophils derived from murine blood (BL) or bone marrow (BM) were co-cultured with various melanoma cells i.e. cutaneous (CM) and lymph node (LN) for 4 hours. Next, neutrophils were isolated and processed for proteomic analysis using the S-trap micro (Protifi) high-recovery protocol with slight modifications. Tryptic peptides were subjected to LC-MS analysis using a nanoLC coupled to an Orbitrap Tribrid Eclipse (Thermo Scientific) in the DDA mode. MS data were analyzed with Proteome Discoverer 2.5 using the label-free quantification (LFQ) workflow. Gene ontology (GO) analysis was performed with Metascape.

Preliminary data

By performing LFQ proteomics of neutrophils co-cultured with melanoma cells, we could relatively quantify ~2,100 proteins of which 44 and 50 proteins were altered significantly (log2FC ≥ 0.58, adj. p-value ≤ 0.05) in BL and BM neutrophils, respectively. The GO analysis of differentially regulated proteins in BL neutrophils showed a dysregulation of intermediate filament organization as well as phagosome pathway compared to controls. In contrast, BM-derived neutrophils exhibited a change in the neutrophil degranulation pathway, which indicates an early stage of neutrophil activation. We also investigated proteome changes between neutrophils co-cultured with LN or CM cells, which are distinct types of melanoma cells that differ in their aggressiveness and resistance to therapy in vivo. From ~2,000 quantified proteins, 64 and 68 candidates were differentially regulated in BL and BM neutrophils co-cultured with LN compared to CM, respectively. Functional enrichment revealed that pathways associated with the function of both, BL- and BM-derived neutrophils, e.g. degranulation, were differently affected in neutrophils exposed to LN compared to CM melanoma cells.

Conclusion

Our data provide insight into the alteration of immune function and the structural re-organization of neutrophils in the presence of tumor cells. Therefore, tools that enable comprehensive molecular analysis of a small number of immune cells isolated from biologically active tissues are crucial to studying such functional differences.

Poster Nr. 49

Stress granules as a potential origin of abnormal protein aggregation in neurodegenerative diseases

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Nearly one in six suffer from neurological disorders. Despite the heterogeneity in their pathogenesis, the most common neurodegenerative diseases (NDD), Alzheimer´s disease (AD), Parkinson´s disease (PD) and amyotrophic lateral sclerosis (ALS) are characterized by abnormal protein aggregation, which is shown to play a crucial role already in the early stages of said diseases. Until now, it is believed that the abnormal deposition of proteins is mainly caused by an altered protein clearance machinery, however, recent studies identified stress granules (SG) to be involved in aggregate formation. SGs are ribonucleoproteins composed of RNAs and proteins, which reversibly assemble into granules upon cellular stress. Momentarily formed SGs, also called acute SGs are proven to be beneficial to the cell´s survival capacities, whereas long lasting, so called chronic SGs are suspected to develop pathogenic potential over time, as seen in the context of ALS, in which two disease-related proteins, TDP-43 and FUS, were found to aggregate in chronic SGs. Further functional studies could reproduce the SG-associated aggregation of TDP-43 in ALS-fibroblasts and induced pluripotent stem cell-derived motoneurons, reinforcing the connection between SGs and protein aggregation.

Hence in this project we first aimed to verify the abundance of SG markers in brain tissue of NDD patients and healthy controls. For that we carried out an extensive meta-analysis of mass spectrometric data of patients with common NDDs collected from more than 20 datasets and nine studies. With this we were able to create an in-depth comparison of over 700 up- and downregulated SG-associated proteins in chronically stressed brain tissues compared to healthy and unstressed control cohorts.

In a second step, we aim to establish an in-vitro long-term stress model using the most common neuronal cell line (SH-SY5Y). With the application of the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2) and sodium arsenite ($NaAsO_2$) in different concentrations we were able to successfully induce SGs for various time periods. The cytotoxicity of the used ROS was quantified with viability assays (XTT), while immunocytochemistry verified the location and presence of SGs.

Lastly, we aim to characterize the proteomic composition of SGs resulting from different stressors and exposure times to evaluate their similarity to observed SG markers in human brain tissues and to gain a comprehensive view on the relation of SGs and abnormal protein aggregation. With this we want to provide a promising starting point for further statistical and experimental investigations for the role of SGs in NDDs.

Poster Nr. 50

The impact of the expression of different HRAS variants on the proteome profile of human keratinocytes

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HRAS is an early player in different signalling cascades including the MAPK/ERK pathway. The small GTPase HRAS acts as a molecular switch cycling between an active GTP-bound and inactive GDP-bound state. Pathogenic variants in the HRAS gene result in the constitutive activation of HRAS and are common in dermatological malignancies. This includes the germline variant HRASGly12Ser, which results in the developmental disorder Costello syndrome (CS), the post-zygotic variant HRASGly13Arg leading to skin disorders Nevus sebaceous and Schimmelpenning syndrome, and the oncogenic variant HRASGly12Val, which occurs in 7% of all cancers. Up to date, the molecular basis of cutaneous manifestations are unknown. Here, we aim to characterize the impact of different HRAS mutations on the proteome and phosphoproteome in human keratinocytes using Liquid-Chromatography-Coupled Tandem-Mass Spectrometry (LC-MS) to unravel different pathogenesis mechanisms.

Therefore, we generated permanent immortalized human keratinocyte (HaCaT) cells stably expressing the above mentioned HRAS mutations. To obtain single cell clones, cells were transfected with constructs harbouring HA-tagged HRAS variants by electroporation, selected with Geneticin, and single cell sorted by Fluorescence-Activated Cell Sorting (FACS). Single cell clones were further harvested, lysed, and subjected to tryptic digestion following the Single-Pot, Solid-Phase Enhanced Sample Preparation (SP3) protocol (Hughes et al. 2019). For phosphopeptide-enrichment Thermo Scientific's High Select TiO2 Phosphopeptide Enrichment Kit was used. Peptides and phosphopeptides were subjected to bottom-up LC-MS/MS analysis using a C18-Reversed Phase chromatography coupled to an Orbitrap-Quadrupole Hybrid mass spectrometer. Resulting raw spectra were searched by Proteome Discoverer (Version 3.0), including the CHIMERYS algorithm. Data normalization and statistical analysis were carried out in the R software environment and Perseus (Version 2.0.3). Pathway analysis were performed using Ingenuity Pathway analysis (IPA 84978992) and Funrich (Version 3.1.3) respectively.

We found significant differences in the proteome and phosphoproteome of keratinocytes expressing HRASGly13Arg (Nevus sebaceous/Schimmelpenning syndrome). In contrast, Costello Syndrome and cancer-associated variants resulted in more similar proteome profiles, compared to cells stably expressing HRAS wildtype. in particular, proteins involved in the mesenchymal to epithelial transition (MET) pathway were notably affected by the various HRAS mutations. MET plays a role in cellular processes such as metastasis formation, wound healing, metabolic switching, and epigenetic modifications. Next, we aim to investigate the role of MET in the context of different pathogenic HRAS phenotypes in keratinocytes.

Poster Nr. 51

Accurate protein quantification to support comparable viral load calculations

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Human cytomegalovirus (HCMV) is known to cause human diseases. In immunocompetent individuals HCMV infection is typically mild or asymptomatic, whereas in immunocompromised patients, it can cause severe disease. Furthermore, the virus can persist in a latent state and reactivation may be caused by stress factors.

The determination of viral load is important to inform on disease progression and successful medical treatment. However, differences in diagnostic test results may reduce the effectiveness of clinical intervention and disease management.

The ability to determine the number of viral particles present in a biological sample, known as viral load, is essential in our continual quest to reduce the burden of viral infections on society. However, viral load is indirectly measured via the quantification of sequence specific viral nucleic acids, or proteins, in a representative sample. We aim to assess the comparability of viral load measurements using these different approaches to reduce the measurement uncertainty and standardize viral load measurements, thereby improving overall measurement comparability.

Quantitative PCR is the standard analytical method for HCMV diagnoses. Nevertheless, detection of HCMV DNA is a non-specific indicator for active replication of the virus, while viral capsid proteins should only be detectable during HCMV replication. Combining DNA and protein measurements will provide an improved approach for the accurate measurement of infectious viral particles.

In our effort to standardize viral load measurements, HCMV virions were produced in human fibroblasts and purified through a tartrate-glycerol gradient to provide a solution of infectious viral particles. Digital droplet PCR of the HCMV gene UL54 was used to quantify the DNA and calculate the gene copy number within a sample. Additionally, a tryptic digestion-based peptide quantification strategy using mass spectrometry was applied for the quantification of viral capsid proteins. Amino acid analysis, complete digestion, equimolar release of the target peptides and calibration using isotope dilution approaches ensure measurement results are traceable to the SI. Using these methods, we intend to confirm the corelation of DNA copy number and specific target protein amount content with viral load. Furthermore, we estimate the measurement uncertainty according to the guide to the expression of uncertainty in measurement. This enables the evaluation of the major contributing factors to the overall measurement uncertainty. An essential consideration in doing this is to estimate if there are differences in measurement uncertainty between cell culture produced viruses and viruses from patient samples to assess the commutability of the materials used in the study.

Poster Nr. 52

Aging of Metallic 3D-Print Powders Analysed by Secondary Ion Mass Spectrometry

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In contrast to classic processes, 3D printing has advantages in terms of the achievable complexity of the printed parts as well as in terms of their individuality. Typically, 3D printing is considered to have higher material efficiency, which can be accompanied by cost savings.

3D printing is now being applied not only to polymers, but also to metals. For this purpose, spherical metal powders are fused in a targeted manner using either an electron beam or a laser. Powder left over from the process can be separated and reintroduced into the printing process.

However, reuse of the powder in metal 3D printing has not been possible indefinitely to date: Typically, powders show signs of aging after several printing cycles, so that the quality of the 3D print suffers. Therefore, in practice, the powders have to be disposed of after a few printing cycles. This counteracts material efficiency, reduces cost savings, and pollutes the environment.

Therefore, it is important to understand the aging process of the powders. Since the main undesirable effects seem to take place at the surface, it is desirable to detect the aging process by surface analysis.

Secondary Ion Mass Spectrometry (SIMS) represents a valuable method for characterizing surfaces. It is based on bombardment of surfaces with primary ions and mass spectrometric separation and detection of secondary ions desorbing from the surface. SIMS can be used to detect both inorganic and organic sample components in the first 1-5 monolayers.

In the present study, systematically aged metallic 3D printing powders are analyzed by SIMS. Here, the different operating modes of SIMS are brought to bear: spectrometry, imaging and (3D) depth profiling.

First results suggest that especially the thermal energy introduced in the printing process leads to a modification of the sample surface.

Poster Nr. 53

Analysis of Electrode Interphases in Lithium Ion Batteries using Laser Desorption/Ionization-Mass Spectrometry

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Over the past decade, the demand for electronic devices has increased significantly and the need for efficient lithium ion batteries with high energy densities and long lifetimes has grown. The lifetime of a lithium ion battery is largely influenced by the degradation of the electrolyte. Continuous degradation of the electrolyte reduces the lifetime drastically but is counteracted by the formation of a passivating layer of organic and inorganic electrolyte degradation products on the surface of the negative electrode. Consequently, the stability and general properties of this so-called solid electrolyte interphase are crucial for battery lifetime.

In this work, laser desorption/ionization-mass spectrometry (LDI-MS) was used for the first time to analyse the surface of Si/graphite-composite electrodes after cycling in Li-metal coin cell batteries containing the film-forming electrolyte additive 3,4-dimethyloxazolidine-2,5-dione (Ala-N-CA) and other structurally related additives. The combination of LDI-MS with trapped ion mobility spectrometry (TIMS) and tandem mass spectrometry was used for further structural elucidation of interphase components. Additionally, LDI-MS imaging generated mass spectrometric images of interphase components, revealing their lateral distribution on electrode surfaces.

LDI-MS is introduced as a complementary analytical tool for the analysis of electrode interphases to support a targeted design of electrolyte additives. Especially the detection of organic oligo- and polymers is more efficient using LDI-MS, compared to established methods. On the studied electrodes, oligomeric transformation products of Ala-N-CA are detected as part of the solid electrolyte interphase. TIMS-based fragmentation supports and speeds up the structural elucidation of the additive-derived interphase components and enhances the understanding of the interphase-formation process. Similar reactions and transformation products are observed for structurally related electrolyte additives containing the N-carboxyanhydride active moiety. The size of the observed oligo- and polymers, however, varies greatly for the different additives and the obtained information can be employed to choose the most effective additive. Furthermore, LDI-MS imaging depicts the lateral distribution of the detected oligomers. For some additives, the degree of oligomerization varies in different areas of the electrode, which indicates an inhomogeneity of the interphase layer, possibly decreasing battery lifetime, while other additives form a more homogeneous layer. Consequently, the homogeneity of the formed interphase is used as an additional criterion for evaluation during electrolyte additive design. In conclusion, LDI-MS and related techniques enhance the understanding of electrode interphases in lithium ion batteries and, potentially, electrochemical systems in general without the need for extensive sample preparation.

Poster Nr. 54

Analysis of transition metal complexes formed through contact of skin with Euro coins

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It is well-known that some metals/alloys sensitize the skin of susceptible individuals. Trace amounts of metals and chemical compounds containing metal ions can affect human health, i.e., cause allergy or inflammation. However, the details of these metal-induced allergic reactions are not well understood. Complexes of nickel, copper and zinc are known to be easily formed through contact of metal alloys with ubiquitously available organic compounds such as triglycerides, amino acids and fatty acids found in skin secretions. Thus, an analytical technique is required to characterize and identify metal complexes formed on metallic everyday products, ideally offering a chemically specific, laterally resolved view of allergy-causing processes. [1]

We here present an analytical workflow to characterize metal-organic compounds formed on the surface of 1-Euro coins. In this study, we identified different metal complexes having the highest signal intensities in the scanned area of 3 different 1-Euro coins with MS2 experiments using an atmospheric-pressure scanning microprobe MALDI mass spectrometry imaging ion source (AP-SMALDI MSI, TransMIT GmbH, Giessen) coupled to a high-resolution quadrupole/orbital trapping mass spectrometer (Q Exactive HF, Thermo Fisher Scientific, Bremen, Germany). Utilizing autofocusing mode, we also imaged the 3D-surface of coins, mapping Zn+, Cu+ and Ni+ as abundant molecules, particularly in the outer ring, both outer and inner and inner ring, respectively.

Subsequently, laser desorption/ionization (LDI) MSI experiments cleared the corrosion products on the surface of 1-Euro coins in the presence of artificial sweat as Cu(CH_4N_2O). H2O and [C12H3ON4Cu+Na]+. These compounds were identified according to their exact mass by comparison with the theoretical mass list of markers identified in XCalibur.

Acknowledgement

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Conflicts of interest

BS is a consultant of TransMIT GmbH, Giessen, Germany

Literature:

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Poster Nr. 55

AP-SMALDI MSI of Cryptosporidium parvum-infected cells and tissues

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Parasites and resulting diseases pose health and economical threats worldwide. Some parasites of phylum Apicomplexa, i.e. Neospora caninum, Eimeria bovis or Cryptosporidium parvum have not been studied extensively in a biochemical context. The disease caused by C. parvum is known as cryptosporidiosis and is one of the neglected tropical diseases (NTDs), which affect more than 1 billion people worldwide. Efficient treatment options or vaccines are currently not available. Therefore, mass spectrometry (MS) and MS imaging (MSI) were used, combined with high performance liquid chromatography (HPLC) or matrix-assisted laser desorption/ionisation (MALDI), respectively. The aim of the study was to identify molecular biomarkers for parasitic infections of host cells and, if possible, to clarify their function. With MALDI MS(I), infected and non-infected cell pellet samples were investigated in order to detect possible markers. A Q ExactiveTM HF orbital trapping mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) in combination with a AP-SMALDI5 AF imaging ion source (TransMIT GmbH, Giessen, Germany) was used for this purpose (Mass resolution R = 240,000 @ m/z 200; pixel size: ≥ 5 µ m). HCT-8 cells were used as a suitable infection system, which is highly immunoreactive and relate well to the cell types to be infected in vivo. Monolayers of this cell type were suitable for MSI analysis. This allowed for depicting marker compounds in parasite-infected single host cells in comparison to non-infected controls. In addition, C. parvum-infected bovine intestinal biopsy samples were examined by MALDI MSI, thereby mimicking in vivo situation. The software Mirion (TransMIT GmbH) in combination with the Perseus software platform (MPI of Biochemistry, Martinsried, Germany) was used to find potential biomarkers. HPLC-MS/MS (Dionex UltiMate 3000 RSLC-System, Thermo Fisher Scientific, Dreieich, Germany) experiments were employed for structural identification of detected molecular markers. Ions in purified and chromatographically pre-separated fractions of cell pellet extracts were fragmented by HCD. The software LipidMatch (SECIM, Gainesville, USA) was used for preliminary identification of detected molecular markers. It was found that the lipid class of phosphatidylcholines in particular was highly abundant. More than a thousand different signals were found in both positive and negative ion mode, which are significantly increased in infected material. The statistically relevant biomarkers were imaged in monolayers at 5 µ m and host tissue at 10 µ m lateral resolution.

Poster Nr. 56

AP-SMALDI MSI of host-parasite interactions: infection markers significantly change in intensity and spatial distribution

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After infecting hosts, parasites mainly rely on the host regarding nutrient supply. Consequently, parasite infections may result in changes in composition and spatial distribution of metabolites in host tissues. Analyses of these alterations and their link to parasite-host interactions is essential for understanding the processes that occur during infection and might reveal new targets of parasite treatments. Imaging both, parasite and host compartments within the same tissue sample can deliver valuable insights into in vivo processes. We here studied as parasite – host models: a) bovine skin tissue infected with the apicomplexan parasite Besnoitia besnoiti, and b) hamster liver, infected with Schistosoma mansoni blood flukes.

MS images were acquired using an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen, Germany) at 10 µ m pixel size, coupled to a Thermo Scientific Q Exactive HF Orbitrap- based mass spectrometer (Thermo Fisher Scientific (Bremen) GmbH, Germany) at a mass resolution of R = 240,000 at m/z 200. Cryosections of 20 µ m thickness or infected cell layers were coated with matrix using an ultrafine pneumatic sprayer (SMALDIPrep, TransMIT). DHB (2,5-dihydroxybenzoic acid) and DAN (1,5-diaminonaphthalene) were used for positive- and negative ion mode, respectively. In the case of livers from S. mansoni-infected hamsters, LC-MS/MS was also performed using a Dionex Ultimate 3000 UHPLC (Thermo Fisher Scientific) equipped with an ACQUITY UPLC HSS T3 1.8 µ m reversed-phase column (100 X 2.1 mm) (Waters) and coupled to a Q Exactive HF-X system. Lipid extraction was done according to Breitkopf et al.[1] LC-MS/MS data were evaluated using LipidMatch Flow. MSI data of bovine skin were uploaded to METASPACE platform[2] for annotation and quick visualization. Statistical analyses were done by Perseus or MetaboAnalyst.

For both parasite-host systems, infection markers were identified that significantly differed in signal intensity and lateral distribution when comparing infected samples with controls. Additionally, we found accordance with biological structures as identified in optical images of the analyzed tissues.

In B. besnoiti-infected skin, parasite-induced cysts were easily visible to the naked eye. MS images showed both, enrichment and depletion of distinct lipid species inside the cysts in comparison to surrounding tissue. Furthermore, infection markers specific for cyst walls, cyst content and the surrounding unaffected tissue were identified.

In S. mansoni-infected individuals, up to 50% of parasite eggs fail to be excreted by the host but instead are trapped in host tissue. We here investigated hamster livers showing granuloma formation around eggs (egg size = 100-200 µ m)[2] with high lateral resolution, thereby visualizing changes in lipid distribution within granulomatous tissue, granuloma substructures. As an example, ether-linked phosphatidyl-ethanolamines (PE O-) were mainly found in the outer part of the granulomas.

Acknowledgement

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Conflicts of interest

BS is a consultant and SG is a part-time employee of TransMIT GmbH, Giessen, Germany

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Poster Nr. 57

Duo-modal mass spectrometric and emission spectroscopic recordings of 2,5-dihydroxybenzoic acid on cerebellar tissue.

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During the MALDI-process, most molecules that serve as a matrix emit fluorescent light. This light may carry yet unexploited information complementary to MS imaging results. Here we present a method that allows to record emission spectra of matrix and analyte for each individual pixel during MALDI-MS imaging runs using the same laser pulse for both fluorescence excitation as well as ablation/ionization.

Measurements were performed on a modified a tMALDI setup of a dual ion source (spectroglyph) attached to a QExactive plus (Thermo) mass spectrometer. Fluorescent light was collected behind the sample using a microscope objective and focussed into an optical fiber of an emission spectrometer (Avaspec-ULS 2048 CI-EVO-VA-50, Avantes). MSI and spectroscopic data were processed using a Python code and evaluated using SCiLS lab (Bruker). Cryo-sections of mouse brain spray-coated with matrix 2,5-dihydroxobenzoic acid (DHB) served as samples.

We established a bi-modal setup that allows collecting spatially resolved fluorescent spectra under typical MS imaging conditions. Using DHB as a matrix the spectroscopic signal is dominated by fluorescence between 380 nm and 500 nm. This correlates well with the general fluorescence of DHB excited at 349 nm. Because MS- and spectroscopic imaging share the same pixel coordinates, both datasets can be merged into one imzML file using a Python. This way, emission spectra are appended to the corresponding mass spectra as "pseudo-m/z"-values and both spectra can be evaluated simultaneously. As expected from the literature, emission spectra of pure DHB show a maximum fluorescence at f λ ~ 430 nm. Interestingly, a shift of the emission maximum to a longer wavelength range around approx. λ ~ 460 nm is observed for pixels that correlate with tissue regions. Additionally, specific area on the tissue, such as white and grey matter exhibit different fluorescence, allowing to differentiate between tissue regions based on the spectroscopic data alone.

Tissue specific red shifts in the fluorescence spectrum of DHB were observed as compared to pure DHB. In these regions, analyte molecules such as lipids are extracted and incorporated into the matrix layer. We speculate that an interaction between analyte and crystal structure of the matrix may lead to the observed shift of the emission band.

Poster Nr. 58

From Lipids and Drugs to Proteins: Identifying Co-localized Molecules Across Multiple Classes Using MALDI-HiPLEX-IHC

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Introduction

Proteins provide unique challenges for imaging mass spectrometry (IMS), as the low abundance of these molecules makes them both difficult to detect and identify. Recently, Ambergen has developed MALDI-HiPLEX, a new immunohistological staining method that allows the visualization of proteins through photocleavable peptide mass tags attached to protein specific antibodies. The peptides are readily visualized with IMS, revealing the spatial distribution of the protein of interest. Here, we demonstrate the power of MALDI-HiPLEX with our proprietary software, SCiLS Lab, to provide insights into species colocalized with proteins as well the potential to couple the technology to mapping out drug delivery using a JQ1 dosed mouse model.

Methods

Non-perfused whole brain tissues from mice dosed with 10 mg/kg JQ-1 i.v. were provided by St. Jude Children's Research Hospital (Memphis, TN). Coronal tissue sections (10 µ m thickness) were mounted to Intellislides and spray coated with DHAP matrix. Lipid images were acquired on a timsTOF fleX system in positive ion mode using 20 µ m pitch size. The tissues slides were then subjected to the MALDI-HiPLEX method (five antibodies in parallel), coated with DHB through sublimation, and imaged with the timsTOF fleX again to gain the respective protein images via the specific mass tags.

Preliminary data

Each of the five expected peptide mass tags were detected in the imaging experiment. The antibodies Histone H2A.X (HIST) and GLUT-1 showed unique distributions with no spatial overlap and were chosen as targets to co-localize with the lipids. GLUT-1 localized primarily in the corpus callosum, where HIST localized in every other region of the brain. After normalization, JQ1 was found localized in the ventricle. An expected result as the tissue was not perfused prior to collection. Several lipids that colocalize with the drug in the ventricle were found using statistical tools and identified by MetaboScape software according to accurate mass. These results further demonstrate the utility of MALDI-HiPLEX in future drug studies, providing a compendium of the molecular constitution of such heterogeneous tissue while also observing drug-on-target.

Novel Aspects

First multimodal image workflow to co-localize lipid, drug and protein from the same tissue. Acquired by combining two serial imaging experiments including a unique MALDI-HiPLEX sample approach.

Poster Nr. 59

Glycosphingolipids are important for immune response in livers of Schistosoma mansoni-infected hamster liver – Tissue studies using high-resolution AP-SMALDI MSI

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Schistosomiasis is a parasitic infection, classified as a neglected tropical disease (NTD) by WHO. Trematode diecious parasites of the genus Schistosoma are responsible for the disease, with S. mansoni as one of the most important species. Following pairing of male and female S. mansoni, and their settling in the mesenteric veins of the definite host, the female sheds over years up to 300 eggs per day. Most eggs are released in the environment via feces, while a significant part gets trapped in host organs such as spleen or liver. As an immune response of the host, granulomas are formed around these eggs. The formation of granulomas can be divided into different stages.

Among the molecules known to be involved in immune responses are glycosphingolipids (GSLs), however, studies about individual molecular species are rare. To fill this knowledge gap, we developed a workflow combining atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) with nanoflow hydrophilic interaction liquid chromatography tandem mass spectrometry (nano-HILIC-MS/MS). As a model system, we used the liver tissue of hamsters infected with S. mansoni of both sexes (bisex-infected) or with one sex only(singlesex-infected; no eggs can be produced). Non-infected hamsters served as control.

By performing nano-HILIC-MS/MS experiments, we identified 60 GSLs in liver samples of bisex-infected hamsters. Of these 47 were fully characterized concerning their saccharide composition and ceramide backbone moiety. Statistical evaluation revealed significant differences between bisex, single-sex and non-infected groups. Surprisingly, even after single-sex infection (no eggs in the tissue), some GSLs were significantly upregulated compared to non-infected controls.

Based on high-resolution AP-SMALDI MSI data, the upregulated GSLs in the bisex-infected group were primarily localized within granulomas surrounding S. mansoni eggs in the liver. Differences in the spatial distribution of the different GSLs suggest that they can be linked to various cell types, because a granuloma is known to have a highly ordered structure.[1] Moreover, some GSLs exhibited a heterogeneous distribution among the granulomas. This can be explained by changes in the architecture at the different stages of granuloma formation.[2] Additionally, AP-SMALDI MSI experiments with 3 µ m pixel size allowed us to pinpoint egg-specific distributions of GSLs. Located at the egg shell, we found evidence that GSLs are probably important for host-parasite interaction and associated immune responses, which is in line with the literature.[3]

The presented results suggest that the high abundance as well as the accumulation of GSLs in granulomas and trapped S. mansoni eggs are linked to essential processes, probably immune responses, of the parasite-host interaction.

Acknowledgement

Financial support by the LOEWE Centre for Novel Drug Targets against Poverty-Related and Neglected Tropical Infectious Diseases (DRUID) is gratefully acknowledged.

Conflicts of interest

BS is a consultant of TransMIT GmbH, Giessen, Germany.

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Poster Nr. 60

imzML Analyzer - an open source Python application for the rapid MSI data quality evaluation: application to the ecotoxicological model organisms Daphnia magna and Eisenia fetida

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Mass spectrometry imaging (MSI) has emerged as a versatile analytical tool which is extensively employed for the detection, identification and mapping of a variety of molecular compounds present in tissue sections.1, 2 With the rise of the MSI technique from its experimental status, there are also new requirements for ensuring data quality. Here we introduce the imzML Analyzer-an open-source Python tool for the fast assessment of MSI data quality. To demonstrate its potential use in the data analysis pipeline, key features of the tool are applied to high resolution MALDI-MSI measurements of the established ecotoxicological model organisms Daphnia magna and Eisenia fetida.

The imzML Analyzer was written in Python 3.7 and comes as a standalone application with a graphical user interface (GUI). For parsing the imzML file & metadata reading, methods of the imzML Parser3 are employed. The tool will be made available as open source software. D. magna clones were cultivated in an artificial M4 medium and daily fed with green algae. E. fetida worms were held on a substrate recommended by the OECD for ecotoxicological testing. For cryosectioning, worms were snap frozen in LN and subsequently embedded in 3% CMC while daphnids were embedded in 8% gelatine. Measurements were conducted on a Q-Exactive HF (Thermo Scientific, Bremen, Germany) coupled to an AP-SMALDI-AF5 ion source (TransMIT, Gießen, Germany).

In MS the reliable identification of molecules is only possible if both high mass resolution and high mass accuracy are provided. Therefore, an imzML Analyzer key feature is the fast generation of quality reports which display important quality parameters such as mass measurement accuracy plots, ion images & RMSE calculation to evaluate the mass accuracy of each selected peak in a list. In addition, imzML Analyzer offers the possibility to calculate RMSE & extract abundance values of all peaks in a list from either the entire measurement or from a region of interest (ROI). Optionally, auxiliary data (mass errors & abundances) can be also included for each pixel. These features are discussed on the example of MALDI-MSI measurements of lipids showing detailed histological structures of D. magna and E. fetida at 10 and 5 µ m pixel size, respectively.

Reliable statistical analysis of MSI data strongly depends on a sufficient mass accuracy and high-quality ion images. We introduced the imzML Analyzer, an open source Python based tool to quickly evaluate the quality of MSI data in the imzML format.

Poster Nr. 61

Investigation of drug distribution in renal tissue using high resolution MALDI MS Imaging

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The efficacy of therapeutic drugs depends on their ability to reach their site of action and their specific targets, for example proteins. Thus, in situ measurements are necessary to visualize the distribution of drugs in tissue. MALDI mass spectrometry imaging (MALDI MSI) is a versatile tool for visualization of drug compounds due to the label-free detection of a wide range of analytes.

This project focuses on the development of a mass spectrometry imaging workflow to evaluate the distribution of the antidiabetic drug linagliptin in situ in renal tissue. Linagliptin is >75 % bound to its target protein, DPP-4. Since DPP-4 is mainly expressed in the proximal tubules of the nephrons, linagliptin is expected to be present at these sites. All MS imaging experiments were performed using an atmospheric pressure MALDI imaging source (AP-SMALDI5) in combination with an orbital trapping mass spectrometer (Q-Exactive HF) to provide high mass resolution and mass accuracy to reliably identify analytes and also allow high spatial resolution.

For the first time, linagliptin (m/z 473.24080) could be imaged in rat kidney tissue sections with high mass resolution (R>100000 FWHM) and high mass accuracy (RMSE <3 ppm). The detailed distribution in the fine structures of the kidney could be visualized with a spatial resolution of 10 µ m pixel size. The identity of the drug could be further confirmed by comparing tandem MS spectra generated on-tissue under imaging conditions and from the drug standard. The application of this method can contribute to the elucidation of the effectiveness of (potential) therapeutical drugs and to a better understanding of (patho-) physiological processes in general.

Poster Nr. 62

Lipid signatures and inter-cellular heterogeneity of naïve and lipopolysaccharide-stimulated human microglia-like cells

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Microglia are non-neuronal cells residing in the brain and spinal cord and are often referred to as the immune cells of the central nervous system. Their involvement in several neurodegenerative diseases has been implicated recently and multiple morphologically different phenotypes of microglia fulfilling discrete functions have been described. However, morphology is not the sole descriptor of cell status, which is rather based on complex biological processes. Lipids as an example for bioactive molecules can give vital insights into a cell's metabolic status or its response on a changing environment. Here, we characterized, statistically evaluated and localized the lipid signatures in human microglia-like cells and their response upon inflammatory stimulation with a lateral resolution of down to 1.5 µ m.

Microglia-like cells were differentiated from human embryonic stem cells on glass slides and stimulated with lipopolysaccharide (LPS). Atmospheric pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) was employed using a prototype ion-source based on an AP-SMALDI5 AF (TransMIT GmbH, Giessen, Germany) capable of performing at lateral resolutions of 1.5 µ m coupled to a high-resolution orbital trapping mass spectrometer (Q Exactive, Thermo Fisher Scientific, Bremen, Germany). Multiple sample-preparation and matrix-application protocols employing 2,5-dihydroxybenzoic acid or 9-aminoacridine were evaluated and optimized for positive- and negative-ion mode, respectively, employing an ultra-fine pneumatic spraying system (SMALDIPrep, TransMIT). Data was evaluated using Mirion, Perseus or self-written Matlab scripts and lipid annotation was based on database research employing accurate mass measurements using LipidMAPS.

First, sample preparation workflows were optimized for flash-frozen cells and cells fixed with paraformaldehyde (PFA). Flash-frozen cells were best suited for in-depth lipid profiling, while fixation with PFA for one minute preserved the morphology of the cells and was therefore used for high lateral resolution AP-SMALDI MSI experiments. A lateral resolution of down to 1.5 µ m was achieved after optimizing the sample preparation protocol while preserving the morphology of the cells on a micrometer scale as demonstrated by comparison of MSI results to microscopic images of the same sample. Differentiation between cell lines and naïve and LPS-activated conditions of one cell line was achieved based on population-based lipidomics results from AP-SMALDI MSI at 5 µ m lateral resolution using principle component analysis and hierarchical clustering. Especially triglyceride species were found to be increased upon inflammatory stimulation in all investigated cell lines. High lateral resolution AP-SMALDI MSI with sub-cellular resolution down to 1.5 µ m was applied to resolve heterogeneous lipid distributions within cell cultures of naïve and LPS-stimulated cells in positive-ion mode. In these high resolution experiments, increased triglyceride levels were detected for some but not all cells in one cell culture, indicating that a cell specific lipid response manifests in the formation of individual phenotypes within the cell culture. The pro-inflammatory phenotype is characterized by elevated triglyceride levels, most likely a consequence of increased number and/or size of lipid droplets within these cells. While the localization of lipid droplets would have been possible with fluorescence staining techniques and is in line with literature results

for microglia cells, our method enabled charting of the molecular composition of lipids exhibiting elevated triglyceride levels while simultaneously resolving their heterogeneous distribution within the cell culture. Further, phospholipid-based heterogeneity was identified and used to differentiate genetically identical microglia cells into multiple well-resolved phenotypes using a t-SNE based on MS images of selected phospholipids. Especially a reproducibly heterogeneous expression of unsaturated phosphatidylinositol species differing only in the fatty acid composition was observed. Whereas these phosphoinositol expression levels did not depend on stimulation conditions or cell lines, unsaturation of phosphoinositol between genetically identical microglia cells differed between individual cells in negative-ion mode as visualized by AP-SMALDI MSI with down to 2 µ m lateral resolution. In contrast, other phospholipid species with similar fatty-acid compositions did not exhibit cell-specific expression but are homogenously distributed in cells.

Poster Nr. 63

Machine learning and mass spectrometry-based identification of cell- and tissue niche-specific molecular signatures

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Tissues contain a plethora of different niches, where a dynamic cellular crosstalk shapes physiological and pathological processes. Under inflammatory conditions, infiltrating leukocytes obtain a predominant role and significantly alter the tissue microenvironment by a multitude of mechanisms. However, the resulting cellular and molecular adaptations in situ are incompletely understood. Comprehensive analyses of cellular interactions on a molecular level require a multimodal imaging approach. While microscopy visualizes cellular interactions with high resolution, matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) generates high-dimensional molecular information with spatial resolution. Here, we describe a machine-learning and mass spectrometry-based algorithm for correlated microscopy and mass spectrometry data. Using this pipeline, we reveal cell- and tissue niche-specific molecular signatures to decipher molecular adaptation strategies of leukocytes in tissues.

Poster Nr. 64

MALDI mass spectrometry imaging to determine the distribution of key signalling peptides in wild type and mutant Bacillus subtilis colonies

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The emergent functions of bacterial multicellularity in complex biofilms are increasingly recognized and currently studied in many interdisciplinary consortia. One powerful method to investigate intercellular communication in bacterial biofilms is label-free MALDI mass spectrometry imaging (MALDI-MSI). This allowed us to follow the molecular mechanisms of colony growth and cannibalism processes in different Bacillus subtilis strains by recording the distribution of several key signalling peptides, such as sporulation delaying protein (SDP), sporulation-killing factor (SKF) and the recently discovered antimicrobial epipeptide EPE.

B. subtilis strains (3A38 WT, ΔepeAB, ΔepeAB+ΔsrfAA, ΔepeAB+ΔsrfAB, ΔepeAB+ΔcomA, ΔepeAB+ΔcomK and ΔepeAB+ΔcomX and ΔsdpC knockout strains) were incubated in LB medium at 37 °C overnight. An aliquot of the bacterial suspension was transferred to a mixed cellulose ester membrane with 0.45 µ m pore size placed on biofilm-promoting minimal medium (MSgg) agar and was incubated at 25 °C for 1, 2, 4, 6 or 8 days. After a certain amount of time, usually after full colony differentiation, filter membranes were removed from the agar and the colonies fixed with 10% aqueous formaldehyde solution for 30 min. For MALDI-MSI, biofilms were spray-coated with 2,5-Dihydroxyacetophenone matrix. MSI data were recorded in the positive ion mode at a pixel size of 50 µ m. SCiLS Lab software (Bruker Daltonics) was utilized for data visualization.

It could be shown that knockouts of srfAA, srfAB and sdpC were successfully introduced into the bacterial genome because the associated peptides (surfactin and SDP) were not produced. Remarkably, a knockout of comK led to a complete absence of EPE so that a connection to the epeXEPAB operon is assumed. Moreover, it could be shown that the absence of the autoimmunity-encoding genes epeAB affects EPE expression and distribution. In contrast to the wild type strain, EPE in the ΔepeAB mutant is more localized in the centre than on the edge of the colony. In addition, SDP production is enhanced in the ΔepeAB mutant, while SKF was reduced compared to the wild type. The knockout of comA or comX results in an increased or decreased occurrence of surfactins, respectively, relative to the wild type. MALDI mass spectrometry imaging is a powerful tool for deciphering the chemical make-up of Bacillus subtilis communities. Here, we used the methodology in combination with molecular biology to visualize the distribution of crucial bioactive peptides and to study the function of their associated genes.

Poster Nr. 65

MALDI-2 mass spectrometry imaging of uropathogenic Escherichia coli-infected urinary bladders

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An acute infection of the urinary bladder is most often caused by uropathogenic Escherichia coli (UPEC). UPECs invade and damage the urothelium, the first physical defense barrier of the urinary bladder, triggering an innate immune response. Here, we aimed to combine state-of-the-art MALDI mass spectrometry imaging (MALDI-MSI) with immunofluorescence microscopy (IF) to investigate the lipidomic changes in neutrophil-rich environment in a UPEC-infected mouse bladder. For this urinary bladders of female C57BL/6J mice (n=5) were infected by transurethral inoculation with 5*10E8 UPEC strain 536 (O6:K15:H31). Twenty-four hours post infection, bladders were isolated and snap frozen. For MALDI-2-MSI experiments, 8 µ m-thick tissue sections were sublimation-coated with 2,5-dihydroxyacetophenone (DHAP) matrix. MALDI-MSI experiments were conducted on a timsTOF fleX MALDI-2 instrument (Bruker Daltonics) at a pixel size of 5 µ m using the microGRID extension and by use of MALDI-2 post ionization. IF staining was performed on consecutive tissue sections. Sections were incubated with antibodies against epithelial cells, neutrophils and thrombocytes (EpCAM-1, Ly6G and CD42c) in blocking buffer for one hour, each, at RT and nuclei were counterstained with DAPI. The stained tissue sections were imaged on a Zeiss AxioScan.Z1. MALDI-2-MS images of the urinary bladders were characterized by both high spatial resolution and advanced chemical information depth. More than fifty different lipid species were recorded and their differential distribution served to visualize the distinct tissue areas, such as muscle, connective tissue and epithelium. In particular, registration of MSI and microscopy indicated that certain ether phospholipids (plasmalogens) correlated with neutrophils in the different tissue areas. These signals were only present when urinary bladders were infected. The spatial multi-omics algorithm revealed a specific chemical make-up of cells and tissue in bacteria-infected urinary bladders. This approach helps to better understand the molecular mechanisms that regulate neutrophil infiltration in response to a bacterial infection.

Poster Nr. 66

microGRID Technology for Robust High Lateral Resolution Imaging Down to the (Sub)Cellular Level

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Introduction

MALDI imaging is a powerful technique to map biomolecules in tissue. To create a spatially-resolved ion image, most MALDI instruments move the sample using an x-y stage relative to a stationary laser beam, to create a mass spectrum for every pixel. However, approaching spatial resolution of only a few micrometers, over wide travel range, poses a challenge for the mechanical accuracy of most stages.

Here, we introduce microGRID, a new instrument design which combines both stage and laser beam positioning to eliminate imaging artifacts down to about 5 µ m. In combination with MALDI-2, this new technique enables highly sensitive imaging at high spatial resolution without compromising on pixel fidelity.

Methods

Optical encoders with sub-micron resolution were integrated into a regular stepper driven MALDI sample stage and monitor the actual position. Any deviation from the ideal raster is precisely detected by the encoders and sent to the adaptive smartbeamTM 3D laser optics for automatically on-the-fly correction and precisely irradiating the targeted pixel within µ m accuracy.

Images were collected using smartbeamTM 3D systems with ~5 µm laser spot size at raster spacing of 5-20 µm. We used various kinds of samples from single cells to complex tissues with CHCA, DHB and DHAP matrix-coating. Ablation craters were analyzed by high-resolving optical microscopy.

Preliminary Data

The effect of Bruker's new microGRID technology is demonstrated by artifact-free high-resolution imaging of different tissue types. Robust imaging at 5 µ m spatial resolution allows for visualizing small structures not distinguishable at higher pixel sizes such as the Purkinje cell layer in the cerebellum (rat brain) or the glomerular system (rat kidney). The drastically increase in accuracy and spatial resolution also opens the field for the smallest unit of life – single eucaryotic cells. We here present data that allow for the visualization of cellular fine structures and organelles like the

nuclei.

Furthermore, the microGRID laser beam adjustment leads to the reduction of oversampling artifacts like checkerboard patterns and striping and thus increases the validity of high-resolution imaging data. Sensitivity issues due to reduced sample material per pixel are addressed by MALDI-2 post-ionization.

Novel Aspects

microGRID allows for true high-spatial resolution MALDI-MSI down to 5 µm.

Stage movement is compensated by a high resolution positional readout and correction by the laser beam accordingly via smartbeamTM 3D technology.

Sensitivity enhancement with MALDI-2 allows for visualization of numerous molecules in various tissue types and even on the single (sub)cellular level.

Poster Nr. 67

Visualizing spatial distributions of neurotransmitters in cryo-sections of Schistosoma mansoni using high-resolution AP-SMALDI mass spectrometry imaging

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Introduction

The parasitic flatworm Schistosoma mansoni causes schistosomiasis, which affects several hundred million people worldwide and is categorized as a neglected tropical disease by the WHO.[1] In S. mansoni infection, the female worm forms a couple with a male partner and produces around 300 eggs per day, of which many lodge in host tissues causing schistosomiasis pathology. Understanding the development of these parasites and how to prevent it may be key to find new ways of disease intervention.

One unique feature of schistosome development is the dependence of the female of a close and constant pairing contact with the male to reach sexual maturarion. Neurotransmitters are assumed to be involved in this process and are discussed as potential maturation factors relased by the male worm to the developing female. To shed light on the role of neurotransmitters, we investigated their spatial distribution in male and female parasites at different stages of maturation.

Experimental Methods

Mass spectrometry imaging was used to study the distribution of neurotransmitters in mature S. mansoni couples, immature worms, and genetically altered worms. Before measurement, the samples were derivatized with 2,4,6-trimethylpyrylium tetrafluroborate (TMP)[2] to enable the detection of the neurotransmitters. The worms were examined by atmospheric-pressure scanning microprobe MALDI mass spectrometry imaging (AP-SMALDI MSI). Sections were prepared using a cryotome (HM525, Thermo Fisher Scientific). Imaging experiments were performed using an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen), coupled to a high-resolution quadrupole/orbital trapping mass spectrometer (Thermo Scientific Q Exactive HF, Thermo Fisher Scientific (Bremen) GmbH). Data analysis was carried out using the Mirion software package.[3] Lipid annotation was based on the Lipid Maps database.[4]

Preliminary Results

The preparation of the delicate samples was successful, longitudinal sections of the couples but also of immature females were obtained. With these sections, it was possible to distinguish between males and females in optical images, and individual organs, such as the ovary, could also be identified. Furthermore, we were able to section and measure two different couples at the same time to allow direct comparison with the control samples.

As imaging of neurotransmitters is challenging due to poor ionizability and thus interference of strong matrix signals and weak analyte signals in the same m/z range, ionization-enhancing derivatisation is an inevitable approach for MALDI-MSI. We have tested different concentrations of TMP in order to achieve the best possible result for the derivatisation of the investigated neurotransmitters. In addition, other possible derivatisation

reagents were tested, but so far without successful applicability in imaging. In the control worms, various neurotransmitters were detected after TMP-derivatization. Differences in signal intensities were detected within couples, being higher in males than in females. No neurotransmitters were detected in immature females. This supports the idea of a male-dominating factor, potentially involved in female maturation. The results of the measurements of the genetically modified worm couples, in which the neurotransmitter-producing gene was silenced, were inconclusive. Nevertheless, a signal at the m/z value of the downregulated neurotransmitter was unexpectedly found in the genetically modified couple, and there was no significant difference to the control couple measured at the same time. This may indicate the presence of two isomeric forms of the neurotransmitter that cannot be distinguished in MSI analysis without fragmentation. Therefore, we plan to use different derivatisation reagents, which form distinguishable products.

Novel Aspect

On-tissue chemical derivatization combined with high-resolution AP-SMALDI MSI analysis revealed the spatial distribution of various neurotransmitters in Schistosoma mansoni.

Acknowledgement

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Conflicts of interest

BS is a consultant and SG is a part-time employee of TransMIT GmbH, Giessen, Germany

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Poster Nr. 68

Analysis of a Pharmaceutical Formulation using Orbitrap-SIMS

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Pharmaceutical formulations are subject to high quality standards which must be checked at regular intervals. A pharmaceutical review of the composition of the active ingredients is part of the quality assurance of pharmaceutical companies. For this purpose, also mass spectrometric methods are applied.

Orbitrap-SIMS ("3D-Orbi-SIMS") is a comparably new mass spectrometric technique introduced in 2016 [1]. It is a powerful tool to identify organic as well as inorganic components on the surface of a solid sample. Furthermore, it allows the detection of the lateral distribution of these analytes with high mass resolving power. To perform Orbitrap-SIMS on a sample, typically no pre-separation of analytes is necessary.

In Orbitrap-SIMS, a primary ion beam is directed at the sample surface, causing the sample to emit secondary ions. These ions are then mass separated and detected by an Orbitrap mass analyzer. By rastering the surface with the primary ion beam, 2D images reveal the lateral distribution of the molecules.

In this study, the application of Orbitrap-SIMS on selected pharmaceutical samples is tested. The focus is set to the mass spectrometric identification of the active agents as well as on the revealing of their lateral distribution in a cross-sectioned tablet.

One type of sample examined was composed of two active ingredients: Hydrochlorothiazide and Candesartancilexetil. Both active agents belong to the group of antihypertensives: Hydrochlorothiazide is a thiazide diuretic, whereas Candesartancilexetil is an angiotensin receptor blocker [2].

Identification was performed by the acquisition of full mass spectra of the sample followed by data evaluation using Principal Component Analysis (PCA). The detected SIMS-induced fragmentation pattern was in line with the fragmentation behaviour of the active agents determined by tandem mass spectrometry.

At last, mass spectrometric imaging of the sample was performed in order to reveal the lateral distribution of the active components within the sample.

The results give a glimpse into the potential of Orbitrap-SIMS to solve analytical questions in pharmaceutical industry.

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Poster Nr. 69

Capabilities of Surface-Assisted Flowing Atmospheric-Pressure Afterglow Mass Spectrometry (SA-FAPA-MS) for Fast and Semi-Quantitative Drug Screening

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Ambient desorption/ionization mass spectrometry (ADI-MS) can be used for direct molecular mass spectrometric investigations. This makes the method attractive for fast and efficient quality control, preferably including (semi-)quantitative approaches. In the research field of ADI-MS, accurate quantification remains a challenging part [1]. To overcome this hurdle, the method is often combined with additional sample extraction and chromatography which in turn makes it more labor-intensive and expensive.

The presented poster contribution demonstrates the potential to use functionalized surfaces as sample carriers for plasma-based ADI-MS experiments in pharmaceutical analysis. The flowing atmospheric pressure afterglow (FAPA, cf. Hieftje et al. [2,3]) source was used to probe different active pharmaceutical ingredients (APIs) on functionalized surfaces. Specifically, thin-layer chromatography (TLC) plates were used as the sample carrying surfaces, as they show outstanding performance compared to other sample substrates such as glass or metal. A variety of APIs was included in the study to show the capabilities of surface-assisted (SA-) FAPA-MS in this field of application. For this purpose, the influence of different surface modifications (C18, silica, cyano, dimethyl) was investigated. In addition, it was examined how the polarity of the generate analyte ions affects the ADI response. Thus, measurements for selected active ingredients were performed in both positive and negative mode.

As an example for potential clinical applications, untreated saliva samples were directly probed for benzocaine. Samples were taken immediately after consumption of lozenges containing benzocaine and over a period of 120 minutes to semi-quantitatively show the decrease of the API in saliva. The results serve as a proof of principle for SA-FAPA-MS as a direct screening tool not only for screening active compounds in biofluids, but also as a versatile screening tool for other areas of molecular analysis, be it selective reaction control in organic synthesis or studies of contaminant degradation in wastewater treatment.

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Poster Nr. 70

Coupling Capillary Electrophoresis with tims-TOF Mass Spectrometry using the nanoCEasy Interface

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Introduction

Although ion mobility MS has become a well-known technology, it is rarely coupled to capillary electrophoresis (CE). CE and IM separate ions according to their electrophoretic mobility. CE separates solvated ions, whereas IM separates ions in the gas phase. In CE, the separation efficiency and peak capacity are typically much higher than in IM, making the coupling attractive. In recent years, innovative coupling technology of CE to MS has been developed. We recently introduced the nanoCEasy interface, an easy-to-use, robust, and flexible interface with nanoESI sensitivity. The orthogonality of liquid- and gas-phase mobility in a CE-timsTOF MS set-up are studied here.

Methods

An Agilent CE was coupled via the nanoCEasy interface to a timsTOF Pro 2. An acidic background electrolyte (BGE) was applied for the analysis of tryptic Hela-digest (commercial sample) and subunit analysis of proteins (IdeS digest of mAb). A basic BGE was used to separate anionic metabolites from cell culture samples. The interface parameters were established according to Schlecht [1]. The timsTOF Pro 2 was operated in ESI positive (proteins and peptides) or negative mode (metabolites). Calibration of the tims was performed by infusion of a tuning mix directly through the nanoCEasy interface. The nanoCEasy valving option was used for recoating the CE capillary between runs.

Results

Setting up the CE-nanoCEasy-timsTOF was straightforward, leading to sensitive and robust measurements. Different applications have been performed, demonstrating the benefit of the CE-timsTOF MS set-up. To study the orthogonality of CE and IM peptide analysis of tryptic HeLa digest has been performed by the CE-timsTOF set-up using the PASEF approach. Short CE capillaries can be used for this application with fast and efficient separation. A high orthogonality of CE and IM was observed and will be discussed for various peptides differing in size and charge. The analysis of mAb subunits revealed the ability to distinguish between various disulfide bridges. Thus, the combination of CE and on-line timsTOF characterization allows structural information of proteins. Anionic metabolites from cell culture samples were analyzed using a basic BGE. Various sugar phosphates could be separated. Based on migration times of standards and collision cross sections (CCS), the various isobaric sugar derivatives could be unequivocally identified. In summary, the CE-nanoCEasy-timsTOF is a powerful technique with various applications that combine the selectivity and high separation efficiency of CE with the sensitive ESI timsTOF-characterisation.

Poster Nr. 71

Determination of supramolecular complex stabilities using electrospray ionization mass spectrometry

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Soft ionization mass spectrometry is becoming increasingly popular alongside conventional methods like NMR spectroscopy or isothermal titration calorimetry (ITC) for investigating supramolecular complex stabilities by means of titration experiments. Due to the absence of surrounding solvent molecules, it can help to clarify the intrinsic understanding of the interaction of different complex partners. In our work, complexes between native cyclodextrins (α, β, γ-CDs) and five tetracycline derivates as wells as formed from an artificial carbohydrate receptor and β-D-glucopyranosides were the characterized model systems. While the former play an important role in drug delivery applications, the latter are used to study molecular recognition processes. The binding constants (K) of these non-covalent complexes were determined using electrospray ionization (ESI) with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) in both polarity modes. Furthermore, collision-induced dissociation (CID) studies were used to obtain relative gas-phase stabilities in the form of collision energies for 50 % dissociation of the corresponding supramolecular complexes (CE50). These values can be used as semi-quantitative valuation standards for complex stability. The combined results show for the recorded stability constants and CE50 values a positive correlation and a dependence on the ion mode, the charge state and adduct type of the respective complex ion, as well as the hexapole flight time set on the mass spectrometer. The trends of the gas-phase stabilities are largely in line with our theoretical expectations. The investigations provide new thermodynamic comparative values and thus expand the informative value of already existing liquid-phase and theoretical data. From our results, we can also derive that the developed mass spectrometrical methods can assist to rapidly screen active substances in pharmaceutical applications.

Poster Nr. 72

Identification of Hetero-aggregates in Antibody Co-formulations by Multi-dimensional Liquid Chromatography Coupled to Mass Spectrometry

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Antibody combination therapies have become viable therapeutic treatment options for certain severe diseases such as cancer. The co-formulation production approach is intrinsically associated with more complex drug product variant profiles and creates more challenges for analytical control of drug product quality. In addition to various individual quality attributes, those arising from the interactions between the antibodies also potentially emerge through co-formulation. In this study, we describe the development of a widely applicable multi-dimensional liquid chromatography coupled to tandem mass spectrometry method for antibody homo- versus hetero-aggregate characterization. The co-formulation of trastuzumab and pertuzumab was used, a challenging model system, comprising two monoclonal antibodies with very similar physicochemical properties. The data presented demonstrate the high stability of the co-formulation, where only minor aggregate formation is observed upon product storage and accelerated temperature or light-stress conditions. The results also show that the homo- and hetero-aggregates, formed in low and comparable proportions, are only marginally impacted by the formulation and product storage conditions. No preferential formation of hetero-aggregates, in comparison to the already existing pertuzumab and trastuzumab homo-aggregates, was observed.

Poster Nr. 73

Impurity profiling of a newly developed Gd-based contrast agent

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As medicinal products, paramagnetic Gd(III)-based MRI contrast agents (GBCAs) are subject to the European Medicines Agency guidance requiring the identification and quantification of impurities that exceed a content threshold of 0.05 mass%. For this purpose, a new high-performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) method was developed for quantitative impurity profiling of Gd-containing by-products, with significantly improved limits of quantification (LOQ) compared with HPLC-UV analysis. Using this strategy, the impurity profile of a newly developed tetrameric GBCA was investigated by quantification and identification of organic by-products.

Due to the large concentration differences of the main component and the impurities, as well as the very similar chemical properties, with organic by-products often differing from the main component by only one functional group, a powerful separation method for impurity profiling is needed. In addition to the industry conventional reversed-phase (RP) HPLC column with a phenyl-modified stationary phase a mixed-mode RP/ anion-exchange (AX) HPLC column was also used to achieve fast and effective separation of impurities from GBCAs.

With a value of 15 nmol/L, the HPLC-ICP-MS technique achieves a LOQ that is lower by a factor of 10 than the LOQ for HPLC-UV determination. Furthermore, by detecting the 158Gd signal, the elemental analysis allows species independent quantification of Gd-containing compounds via external calibration with a Gd-containing standard substance. Thereby, the detected 158Gd signal intensity of the by-products depends on the number of complexed Gd atoms, which can be derived from the characteristic isotopic patterns of Gd-containing compounds. For this purpose, a complementary HPLC-ESI-HRMS investigation was carried out, which was also used to identify the by-products. Due to the large data volumes of HPLC-ESI-HRMS analyses, the use of automated data processing offers a helpful tool. For this purpose, an additional data filter function was developed for the open-source data analysis programme MZmine3, which allows to check the detected signals for isotope patterns that match the by-products. In summary, this study shows that the HPLC-ICP-MS method is a powerful complement to the routinely used HPLC-UV methodology for the quantitative impurity profiling of GBCAs.

Poster Nr. 74

Mass spectrometric investigations of reaction products of tetrathiomolybdate and Cu ions

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Introduction

Tetrathiomolybdate (TTM) is presently being investigated as a drug for treatment of the Cu storage disorder Wilson's disease. It was found that TTM binds excess Cu ions in the body particularly well and leads to a reduced Cu balance in the bloodstream and hepatocytes. However, the mechanism of action is not fully understood, especially with respect to the way Cu binds to TTM and how it is transported out of the body. To investigate this aspect, this work is the first attempt to characterise the in vitro formed compounds of TTM and Cu ions by electrospray ionisation (ESI) mass spectrometry (MS).

Methods

A solution of ammonium TTM and Cu(II) acetate was prepared. The solution was analysed by a time-of-flight MS featuring an ESI source to identify formed compounds. Stability examinations of the compounds were then conducted by measuring signal intensities as a function of the voltage applied to the ESI source. Further characterisation was carried out by utilising trapped ion mobility spectrometry-MS (TIMS-MS). After addition of glutathione to the analyte solution, the formation of a compound consisting of glutathione, TTM and Cu was investigated by ESI-MS.

Results

By comparing measured isotope patterns to simulated ones, the sum formulae of several TTM-Cu compounds can be determined. The charge states of these ions lead to the conclusion that the Cu ions are singly positively charged due to being reduced by TTM. As the TTM and Cu moieties steadily increase by one and two, respectively, it is suggested that polymeric compounds form in solution. Comparing the ESI voltage-dependent signal intensities of larger TTM-Cu compounds to the smallest, the relative signal intensities of the larger compounds decrease while the relative signal intensity of the smallest compound increases. This can be interpreted as decomposition of the larger compounds to the smallest due to stress during the ionisation process caused by the voltage applied to the ESI source. This corroborates the assumption of the formation of polymeric compounds. Similar behaviour can be seen by comparing the compounds' mobilities with and without induced collisional induced dissociation. These mobility measurements also lead to the finding that the identified TTM-Cu compounds are able to form dimers. ESI-MS analysis of a solution containing glutathione, TTM and Cu showed that glutathione disulfide binds to a TTM-Cu compound but neither glutathione nor glutathione disulfide bind to solely TTM or Cu. This confirms that biomolecule-TTM-Cu compounds can be analysed by molecular MS.

Poster Nr. 75

Pioneering Approach to Investigate Highly Complex Pharmaceuticals Utilizing Ultrahigh-Resolution Mass Spectrometry and Comprehensive Gas Chromatography

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Pharmaceuticals are among the best monitored chemicals and are strictly regulated to secure high production and quality standards. While fully synthesized pharmaceuticals are well investigated during their developing and manufacturing, pharmaceuticals with natural origins are more difficult to characterize and therefore characterize. Ammonium bituminosulfonate (ABS) and sodium bituminosulfonate (SBS) are active pharmaceutical ingredients (API) for numerous pharmaceuticals, which can be tentatively assigned to the class of non-biological complex drugs (NBCDs). These APIs are produced by acid and base treatment of shale oil distillates. ABS and SBS show medicinal effectiveness and, among other, anti-inflammatory and antibacterial properties have been reported. However, these NBCDs are chemically too complex to determine their composition by standard analytical methods described by the European Pharmacopoeia. Its origin as shale oil distillates as well as the sulfonation during manufacturing, makes this API hardly accessible, causing a tremendous isomeric and isobaric complexity. However, further development and understanding of the pharmacological mechanism demand an in-depth chemical description.

Representative for the challenge of NBCDs, ABS, SBS, and their starting material distillates and refined precursor have been analyzed by direct ultrahigh-resolution mass spectrometry (FT-ICR MS) as well as comprehensive two-dimensional gas chromatography coupled to a high-resolution time-of-flight mass spectrometer (GCxGC HR-ToF-MS). Hereby, electron ionization (EI) as hard vacuum ionization technique as well as electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) as soft atmospheric pressure ionization concept were used to cover a wide range of compounds and functionalities. The applicability of the GC×GC methodology for the highly polar API was enabled by online derivatization and verified by FT-ICR MS. On the side of ultrahigh-resolution mass spectrometry, the potential of two platforms, a 7 T Bruker solariX and the 21 T NHMFL user system FT-ICR MS, have been evaluated.

We achieved for the first time an in-depth chemical description of complex APIs at a molecular level via FT-ICR MS and demonstrated the applicability of GC×GC-HR-ToF-MS as a new tool for their analysis. By utilizing ESI and APPI coupled to FT-ICR MS, a broader overview of the ultrahigh isobaric complexity resulting from various chemical functionalities was achieved for each isolated process step. Moreover, the utilization of GCxGC resulted in detailed structural information covering and confirming the high isomeric complexity. The comprehensive analysis of the distillates and the refined precursor verified the effect of the refining process and the applicability of our workflow for the integration into process chemistry. We showed that polar and potentially harmful components like phenols and pyridines were removed. Simultaneously, hydrocarbon and (benzo-)thiophenic core structures are particularly noteworthy as major compounds in the precursor distillates, while their sulfonates were found to be dominant in the resulting APIs. For SBS, the sulfonates were even detected as

sodium adducts via ESI in positive mode. The methodology allowed us to generate a comprehensive chemical fingerprint of the precursors and their resulting APIs. Comparing these fingerprints enabled tracing specific compound classes throughout the process, resulting in first educt-product relation approaches. Furthermore, first conclusions about the influence of aromaticity and degree of alkylation on the sulfonation process became possible.

With this work, we show novel approaches for the in-depth characterization of highly complex drugs like NBCDs and open the possibility for routine process analysis as well as more targeted biopharmaceutical investigations based on marker substances.

Poster Nr. 76

Rapid Screening of Protein-Metallodrug-Interactions using Online Digestion and Trapped Ion Mobility Spectrometry-Mass Spectrometry (TIMS-MS)

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Various metal compounds are applied as metallodrugs. After entering the human organism, many of these xenobiotic species are distributed via a protein network. Therefore, the physiological effects based on the metal-protein-interactions can be used pharmaceutically. Since these interactions are not only relevant for the mechanism of action, but also for potential side effects and toxicity of the drug, research on this topic is of high importance. For this reason, reliable determination of active species and potential binding sites inside the human organism is crucial for understanding the exact mechanism. In this study, we present a method for rapid analysis of protein-metallodrug-interactions via trapped ion mobility spectrometry-mass spectrometry (TIMS-MS) implementing online digestion.

To first determine the active metal species of a metallodrug, a complementary analysis setup comprising LC-ESI-MS and LC-ICP-MS is employed. For the specification of binding sites inside the human organism, enzymatic digestions are frequently used. Usually, the digestions are analysed via a time-consuming LC method. A more time-efficient approach is the implementation of TIMS-MS.

In TIMS-MS, the peptides of digested protein samples form characteristic trajectories when plotting their mobility against their m/z. Metal-binding peptides drop out of these trajectories as they increase in m/z, but maintain mobilities similar to their metal-free counterparts. This behaviour allows for easy identification of binding sites. To further accelerate the analysis of metal-protein-interactions, automation of the enzymatic digestion was applied. Therefore, columns with immobilized proteolytic enzymes were used for online digestion directly prior to mass spectrometric detection. The combination of TIMS-MS and online digestion significantly decreases the total analysis time from multiple hours to several minutes. Furthermore, online digestion enables a more reliable determination of binding sites compared to a conventional enzymatic digestion in solution. Since reaction time after digestion is almost zero due to the hyphenation of LC system and MS, the amount of undesired metal adducts formed with digested peptides is decreased. This allows for a dependable identification of binding partners in intact protein structures. Hence, the found adducts only represent relevant binding sites inside the human organism, completely omitting false-positive results.

To optimize this method, different digestion conditions like temperature, proteolytic enzyme, column length, flow rate and digestion buffers as well as previous reduction of the protein were tested. Digestion yields, signal intensities and carry-over effects were compared. Using the optimized method, binding sites of the gold-containing pharmaceutical Auranofin to human serum albumin (HSA) and haemoglobin (Hb) were determined, and the involvement of free cysteine residues in the metal-binding process could be verified. Furthermore, it was demonstrated that the free cysteine groups of HSA and Hb also act as binding partners for potentially toxic organomercury compounds, such as methylmercury cations.

In summary, the combination of online protein digestion and TIMS-MS enables a fast and reliable determination of metal-binding sites in proteins. This results in a powerful method, which can be used for screening of different metal-protein-interactions to better understand the toxicity and the exact mechanism of action of

metallodrugs. In addition, this method might be useful for toxicity screening of contaminated blood samples to identify the cause of potential poisoning symptoms.

Poster Nr. 77

Sequence Verification and Side Product Identification of Synthetic RNA Oligonucleotides by LC-ESI-MS/MS and OligoQuest Software

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Introduction:

Oligonucleotide characterization by mass spectrometry has gained significant interest recently with the increased use of DNA and RNA as research reagents as well as pharmaceutical drug molecules. Typical DNA primer molecules are in the range of 20mers while single stranded guide RNA (sgRNA) easily involves the analysis of 100mers at 33 kDa molecular weight.

We developed a workflow for the characterization of RNA oligonucleotides in the range 10-100mers using RP-UPLC-ESI-MS/MS and the automatic assignment of the MS/MS fragment ions using the software tool OligoQuestTM.

Methods:

Oligonucleotides were methylated at each nucleotide; UPLC separated and analyzed on a timsTOF Pro 2 (Bruker). In the workflow, multiple spectra are accumulated per charge state and the monoisotopic MS/MS peaklist is calculated using the SNAP algorithm. The OligoQuest software matches the monoisotopic fragment ion list against the theoretical fragment ions calculated from the RNA or DNA sequence including multiple modifications. OligoQuestTM calculates the 5´-, 3´- and internal fragment ions and it provides a visual overview of the match.

Preliminary Data:

The analysis of an RNA 24mer with methylated nucleotides yielded an MS spectrum with an accurate match of the theoretical mass (7969.408 Da), a sequence coverage (SC) of 100% and an intensity coverage of 47%. In addition, 3 early-Rt side products (5-0.5% intensity) were observed with intact masses that indicated a loss of mU (-320 Da), mG (-359 Da) and mC (-319 Da). The simultaneous loss of mG+mU and mC+mG and the addition of mG and mC+mG was also observed in spurious MS peaks. All side products were cleanly separated from the target 24mer at 5.8 min.

The major side product 24mer-mU yielded a fragment spectrum, which confirmed the assumption (100% sequence coverage) that one of the 4 mU residues 11-14 was not properly added to the sequence. The 24mer-mG form matched best to lack of mG-9 with a SC 92%. For the 24mer-mC form 2 positions for lack of mC are possible: mC10 and mC16, none of them could be ruled out at sequence coverages of 90-91%.

The described workflow yielded extensive coverage of the target RNA 24mer fragment ion spectrum and the fragment spectrum quality is well suited to localize sites of missing residues in side products.

The combination of intact mass analysis with the annotated MS/MS spectra in OligoQuest reduces analysis times for targeted oligonucleotide sequence verification as the need for manual investigation of MS/MS spectra is greatly diminished.

Poster Nr. 78

Sulfoxide diazirine (SODA) enables unbiased binding hotspots mapping via LC-MS³

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Photo-affinity labeling (PAL) combined with tandem mass spectrometry (MSn) can reveal noncovalent interactions between small molecule drugs and protein in biological environments. However, the direct detection of the 'hotspots' of the photo-crosslinked binding sites by MS is challenging because of the unknown photo-crosslinking efficiency with the target protein, as well as the unexpected fragmentation of small molecule drugs, especially when these are small peptides. Here, we report on sulfoxide diazirine (SODA) building blocks that can be easily incorporated into peptide-like probes for photoaffinity labeling. The MS-cleavable photoreactive groups allow for a MS2 cleavage event, generating a probe-derived reporter ion and a minimal fragment on the modified peptide. Following a subsequent MS3 fragmentation event, we show that this strategy can be utilized for unbiased identification of the modification sites of PAL probes and mapping the binding hotspots of peptide-like bio-active molecules.

Poster Nr. 79

Comprehensive Characterization of the Beer Metabolome

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Beer has accompanied mankind and the emergence of civilization for thousands of years. Brewing research and the fascination with fermented beverages have produced numerous inventions and findings that have shaped our society today (Pasteur, Enzinger, Linde, Hansen). Rising from and building on this pioneering work, modern analytics allows us to paint a comprehensive picture of beer at the molecular level.

In our work, we examined over 500 beer samples from around the world to reveal the complexity and diversity of molecules in beer. The instrumental range covered the entire analytical space from separation sciences (UPLC) over spectroscopy (2D-NMR) to spectrometry (LC-ToF-MS, DI-FT-ICR-MS). Tens of thousands of molecular signals were detected and characterized. Ultra-high resolution mass spectrometry coming with highest mass accuracy allowed the molecular compositions of each molecule to be determined. Utilizing exact mass signals, the compounds were related by molecular networks (mass difference networks) and specific chemical signatures were found. The brewing process contributes to the complexity of the beer just as decisively as the raw materials themselves. The hopping technology, classic or dry hopping, is reflected in hundreds of bitter acid derivatives and polyphenolic compounds. The basic principles of the Maillard reaction seem to be largely understood; the immense molecular diversity that develops in the brewing process still is almost undescribed ("dark metabolome"). Multivariate chemometric analysis based on metabolomics data made it possible to construct a reaction network comprising more than 2,800 Maillard reaction products. This can be read as an image of the reaction sequences in the malting and brewing process and opens up a comprehensive view of this complex reaction network. Against the background of the German Purity Law, beers were differentiated on the basis of the starch sources used. Molecular networks of the metabolites of barley, wheat, corn, and rice could be detected both at the compositional level (DI-FT-ICR-MS mass difference networks) and at the structural level (LC-ToF-MS2 similarity networks) in the finished beer. From these specific molecular signatures, individual marker molecules were identified that could serve as evidence of the corresponding grain.

Ultimately, the comprehensive non-targeted metabolomics approach succeeded in interpreting the molecular imprint of a historical beer from the German Imperial period (1885). We reconstructed the critical steps of the brewing technology in the late 19th century, when pioneer works by Pasteur, Enzinger, Linde, and Hansen paved the way for modern science and industrial food production.

Poster Nr. 80

Development of protein-based analytical methods and statistical applications for the identification of crustaceans with low-resolution mass spectrometric instrument data

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Seafood, including crustaceans, provides a high-quality animal originated protein source which is a popular food in several human populations and might become even more relevant in the future. Therefore, the identification of marine species is an important issue to prevent commercial fraud and avoid safety risks for human health.

Muscle tissue of selected reference samples from 15 different crustacean species were used to identify species-specific biomarkers. First, an untargeted LC low-resolution MS (LRMS) method was developed and validated to generate peptide profiles. Different modifications of a programmed algorithm were applied to choose marker candidates from peptide profiles with regard to the highest possible specificity and intensity. Then, a targeted multiple reaction monitoring (MRM) method was established to verify the suitability of selected marker candidates as crustacean biomarkers related to the used dataset. The amino acid sequences of the detected biomarkers were determined by de novo sequencing (in silico) with LC-LRMS and additional LC high-resolution MS (HRMS) data using the PEAKS Studio software. Furthermore, a statistical random forest model was created to determine unknown crustacean species based on a LC-LRMS raw data training set using 7 lobster and crab species.

In total, 49 out of the 150 selected peptides were identified as species-specific markers to differentiate 14 crustacean species. To further evaluate specificity, the peptides were tested on additional commercial samples from several crustacean, mussel, insect and fish species. By comparing LC-LRMS und LC-HRMS data, the de novo sequencing (in silico) showed similar results in many cases in terms of the amino acid sequence and for the average local confidence (ALC) value of the remaining biomarkers. The random forest model exemplarily demonstrated the correct identification of a Norway lobster sample in an unseen test data set.

The results show the feasibility to solve authenticity questions, e.g. to identify unknown crustacean species, by applying alternative procedures without using HRMS instruments, requiring a high effort. At the same time, the results prove that certain limitations cannot be overcome using LRMS devices. The evidence of the suggested amino acid sequences from de novo sequencing (in silico) has to be confirmed experimentally after synthesizing selected biomarkers.

Poster Nr. 81

Identification of feed processing marker peptides differentiating blood meals and blood products

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Processing food and feed serves several purposes, which include easier digestibility, increased nutrient availability, and longer shelf life. It does trigger, however, a set of reaction networks (Maillard, oxidation, lipid peroxidation) that result in protein modification or backbone cleavage. According to European Commission Regulation 142/2011 [1], blood-based materials that are intended to be fed to farmed animals may be sold as 'blood products' when being liquid, frozen, or dried or as 'processed animal protein' when being steam-pressure sterilized. Distinguishing these product categories may only be possible based on the effects their different processing, which has not been attempted so far. The latter is, however, of legal relevance for material derived from pig and poultry as feeding rules laid down in European Commission Regulation 999/2001 differ for these product categories.

In order to explore the effects of processing on protein level and to identify markers allowing the distinction between the product categories blood meals (pig: 8, cattle: 2) and blood products (whole blood (6, 7), plasma (7, 6), hemoglobin (7, 3); each liquid and spray-dried) were analyzed. Following samples processing via a standard bottom-up proteomic workflow, peptides were separated and identified using a 2 h gradient on a nanoUHPLC-ESI-Q-Orbitrap-MS. Peptides identified in either of three replicate injections were time-dependently excluded for another three injection replicates. Database search was conducted against the respective Uniprot species proteomes and considering either semitryptic cleavage or 52 dynamic post-translational modifications that were retrieved from food processing literature.

Out of more than 17,000 peptides (> 2,000 proteins) identified in each bovine and porcine dataset, approximately 60% were modified and 10% semi-tryptic (i.e. originating from a protein backbone cleavage. All 52 modifications screened for were identified at least once at peptide level, with deamidation, oxidation, carboxymethlyation, and formylation occurring most frequently.

To identify significant differences in peptide abundance in blood meal compared to the other groups, label-free quantification was performed. Calculated peak area ratios were assessed by applying progressively stricter filters (abundance ratio value, p-value, #proteins, #missed cleavage) to refine the selection of peptides. To further increase confidence in peptide selection, additional abundance ratios were evaluated including peptide/protein ratio to ensure results were not caused by different protein quantities in sample groups. For bovine and porcine material, seven and ten peptides were selected respectivly, which can be utilized to discriminate blood meal from blood products. Peptide identification was verified by synthetic standard peptides.

In conclusion, the presented approach shows that the differentiation of thermally treated feed components from untreated/ less processed materials is possible. Mass spectrometry can complement PCR in official control and thus filling analytical gaps in feed monitoring and allowing to enforce existing regulations.

[1] EU. (2011). Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as

regards certain samples and items exempt from veterinary checks at the border under that Directive Text with EEA relevance. Official Journal of the European Communities, 54, 1–254.

Poster Nr. 82

Novel “Green Chemistry” Analysis for Rapid Determination of Pesticides in Wine

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The global wine consumption is approx. 24.4 billion L/yr. Protection against grape damage by harmful organisms or diseases and guaranteeing product safety for such high volumes is a critical task, also for environmental sustainability. Various fungicides, among other pesticides, are common plant protection products (PPPs) and subject to strict regulations within the EU and other wine-producing regions. GC-MS/MS detection of pesticides requires sample preparation or enrichment to detect residual levels of PPP. Classic techniques, e.g. liquid/liquid extraction, require considerable hands-on lab time and high organic solvent volumes, resulting in hazardous material disposal. Alternatives like (dispersive) solid-phase extraction (SPE/dSPE) offer reproducibility and sensitivity but add costs of specific columns and kits. Presented here is a miniaturized and ultra-sensitive novel method for sample preparation. Benefits comprise speed, low-cost, and compliance with current green chemistry recommendations.

Basis of this technology (Bruker uDrop) is a dispersion of mist of fine microdroplets of an extractant into the aqueous phase, resulting in immediate extraction of analytes. The fine microdroplets become a single microdroplet via centrifugation containing all extracted analytes. High recovery and enrichment factors are achieved. Requiring very little sample (0.4 mL), this workflow offers significant cost and time savings over more traditional methods. 40 samples can be prepared in a total time of 10 min. Further, the volume of hazardous waste is significantly reduced.

Shown here is the determination of 63 common vineyard management pesticides spiked in five types of wine by GC-MS/MS. MRLs (maximum residue level) established by the European Commission are mostly in the range of 10-500 µg/kg (ppb). In order to evaluate the precision and specificity of the method, five sample extractions were made in each of the spiked wines at the 10-ppb calibration level. Applied data evaluation rules met the validation criteria of the SANTE/12682/2019 guidelines for pesticide residue analysis in foods.

The inherent high-sensitivity of this method enables a 100-fold dilution with water prior to extraction. This greatly reduces matrix effects in the different wine samples. A detection limit (MDL) of 0.5 ng/mL (ppb) has been established for all pesticides and in all wine types. The current analytical regulatory criteria were easily met. Multi-residue analyses may be quickly and easily performed at every stage of wine production – from wash water testing to pre-fermentation juices to the finished bottle – with minimal product loss. Further, this method significantly reduces the volume of hazardous waste necessary, supporting modern green chemistry principles.

Poster Nr. 83

Analysis of bio-oils by derivatization gas chromatography resonance-enhanced multiphoton ionization ultra-high-resolution mass spectrometry

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There is no doubt that transitioning from conventional fuels to more sustainable and eco-friendly alternatives is one of the greatest challenges faced by the energy industry. In addition to the emissions of greenhouse gases, the power sector is also responsible for a wide range of other environmental impacts, including the health risks associated with air, water, and soil pollution. Considering the rise of global temperatures, the power supply must undergo a rapid and deep decarbonization process to mitigate its effects and abate the climate change.

As bio-oils are a potential substitute for conventional fuels, great attention is being paid to their different chemical profiles despite their comparable lignocellulosic origin. In order for bio-oils to be industrially upgraded, traditional fossil fuels-based refining processes must be improved and adapted. To achieve that, enlarging structural information at the molecular level is essential and those established analytical procedures will need to be redefined.

Spectroscopic techniques, multidimensional gas and liquid chromatography, and direct infusion high-resolution mass spectrometry have all been used to examine the complex nature of bio-oils. Kösling et al. recently introduced a compact ultra-high-resolution gas-phase laser ionization mass spectrometer platform. It is based on direct laser photoionization in an Orbitrap spectrometer's curved linear ion trap (C-trap). The ion formation is achieved by resonance-enhanced multiphoton ionization (REMPI), which uses a Krypton-Fluoride excimer laser (λ: 248 nm, energy: 192 µJ). REMPI allows for the efficient, selective, and soft ionization of phenols, and monocyclic and polycyclic aromatic hydrocarbons.

This study explores the detection of bio-oil compounds utilizing our photoionization Orbitrap (PhotOrbi), facilitating the silylation of polar constituents. The silyl derivatives present an increase in volatility and thermal stability of polar species, compared with their parent compounds. Furthermore, they reduce the polarity of analytes, enabling GC separation, which is essential to enlarging the chemical space.

The ionizability and behavior of the silylated species are studied for the first time by REMPI. Moreover, fine isotopic fingerprints, including challenging mass splits due to the creation of a CHOxSily-class, are approached. It was possible to observe 13C/12C isotopologues resolved thoroughly presenting low deviations for the intensity distribution. For the isotopologues of a single derivatized compound, it can be secured that a reliable differentiation of Silicon related mass splits, such as 12C/29Si versus 13C/28Si (~3.8 mDa) and 13C12C/28Si29Si versus 13C2/28Si2 (~3.3 mDa) could be achieved with mass accuracy of $\Delta m/z$ 0.1 ppm. A comparison between the silylation derivatization method over standard GC analysis makes it possible to improve the chromatographic analysis, allowing the identification of polar compounds, enlarging the chemical space, and contributing to comprehensive bio-oil sample analysis.

Poster Nr. 84

Direct Analysis of Amino Acids With Surface-Assisted Flowing Atmospheric-Pressure Afterglow Mass Spectrometry (SA-FAPA-MS)

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Ambient desorption/ionization high-resolution mass spectrometry (ADI-HR-MS) enables sample analysis in the native state with little or no sample preparation. Samples can be probed and characterized quickly in less than one-minute analysis time, which is significantly faster compared to classical chromatographic techniques [1].

In this study, thin-layer chromatography (TLC) plates with specific surface chemistries were used as sample support substrates for the direct analysis of proteinogenic amino acids by plasma-based ADI-MS (FAPA, flowing atmospheric-pressure afterglow [2,3]). Analyte ion responses were investigated on different TLC surfaces. Dimethyl (RP-2)- and cyano (CN)-modified silica substrates showed significantly higher intensities for direct amino acids analysis compared to normal-phase (NP)- and reversed-phase-C18(RP-18)-HPTLC surfaces.

Subsequently, the amino acid content was determined by SA-FAPA-MS in both pure-solvent standard solutions and selected real samples (e.g., energy drinks, wine, beer) applied on the aforementioned surfaces. Furthermore, the influence of different dilution solvents on the surface-assisted desorption/ionization process was investigated. The results showed that solvent-surface interactions can affect the signal intensity significantly. Strong diffusion of aqueous sample solutions into the silica-modified surfaces (due to extended evaporation times) resulted in broadened sample spots and reduced signal intensities compared to sample solutions with volatile organic solvents.

[1] J.T. Shelley, S.P. Badal, C. Engelhard, and H. Hayen, Anal. Bioanal. Chem., 2018, 410(17), 4061-4076.

[2] F.J. Andrade, J.T. Shelley, W.C. Wetzel, M.R. Webb, G. Gamez, S.J. Ray, and G.M. Hieftje, Anal. Chem., 2008, 80(8), 2646-2653.

[3] F.J. Andrade, J.T. Shelley, W.C. Wetzel, M.R. Webb, G. Gamez, S.J. Ray, and G.M. Hieftje Anal. Chem., 2008, 80(8), 2654-2663.

Poster Nr. 85

Gas-phase Interaction of Amyloid β and Metals studied by Native MS

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Gas-phase Interaction of Amyloid β and Metals studied by Native MS

Alzheimer's disease (AD) is a neurodegenerative condition that occurs mainly in the elderly. As such, it is a growing worldwide concern due to the aging population. Given the challenges in developing an efficient treatment, further fundamental research is needed. Alzheimer's disease is associated with a specific neuropathological profile of abnormal protein deposits, and is specifically characterized by the formation of plaques composed of different isoforms of the peptide amyloid β (Aβ). High concentrations of metals are found associated with these plaques in AD patients' brains and these metals, particularly iron and copper, are associated with the production of potentially neurotoxic reactive oxygen species (ROS). Previous in vitro studies have linked the human Aβ in presence of Cu to the production of ROS and neurotoxicity, whereas the rodent analogue showed no damaging behaviour. Intriguingly, the rodent peptide differs in only three amino acid residues from the human isoform, indicating that the mechanism of ROS formation is highly sequence-specific.

Several recent literature reports have appeared in which native MS was used to ionise amyloid-metal complexes, after which gas-phase reduction of the metal centre occurred, likely through a radical mechanism. These findings suggest that native MS can potentially be used to study the redox chemistry of these neurotoxic peptides. As in those literature reports, in the current study, we have investigated the interaction between Aβ and transition metals in the gas phase to gain insights into the particular vulnerability of humans to Alzheimer's-linked ROS formation in a controlled environment (i.e., solvent-free, and high purity). The sequence differences between the redox-active human Aβ and the redox-inactive rodent analogue are localized within the N-terminus, which is also the metal-binding-domain. Furthermore, mutations of Aβ within the N-terminal domain (Tottori, English and Taiwanese mutations) have been reported for familial AD, highlighting the involvement of this region in AD pathogenesis.

We started our work with a truncated N-terminal form of human and rodent Aβ (Aβ16) to focus on interactions in the metal-binding domain, and later extended our study to the aggregation-prone full-length (42-residue) Aβ to study the impact of the metal on the entire peptide. The fragmentation of Aβ under metal-free and metal-bound conditions was studied with a Synapt XS ion mobility - mass spectrometer (Waters) equipped with a nano-electrospray ionisation source. Besides the truncated and full-length human and rodent Aβ, customized sequences for more in-depth investigation of the differences between the species were analysed. These custom variants were designed to resemble the human or rodent Aβ16 sequence, with one amino acid being systematically substituted at a time. All Aβ samples were prepared in native-like aqueous solutions. Metal-free and metal-bound precursors were excited by collision-induced dissociation (CID) with varying energies before entry into the ion mobility cell. The gas-phase behaviour of Aβ was evaluated by analysis of the resulting a- and b-ions. Quantifying the fragment ions and studying the reaction energetics allowed us to obtain insight into the formation of the a-ions, in particular whether they were formed through radical or even-electron pathways. Data processing was performed with MassLynx 4.2 and CIUSuite2.

The triply charged metal-bound and metal-free precursors were selected for CID with varying activation energies. Fragment ion yields for the metal-free human and rodent Aβ were very nearly identical, indicating a

generally similar gas-phase behaviour for the two species. Also, the addition of Cu(II) to the rodent Aβ16 and the activation of the metal-bound complex did not alter the b- and a-ion fragment yield. In contrast, the activated human Aβ-Cu(II) complex yielded threefold higher abundance of a14+Cu fragments relative to the rodent analogue and metal-free precursors. Based on a detailed analysis of the fragmentation energetics and reaction products, non-specific radical-directed mechanisms and secondary products of even-electron CID could both be ruled out as the origin of these fragments. Furthermore, the Cu(II) metal centre was reduced to Cu(I) in fragment ions, further indicating radical gas-phase chemistry. Using our custom peptide sequence variants, we were able to determine the essential sequence motif that enables this chemistry, and were able to link this to the known solution-phase coordination chemistry of amyloid β. Ion mobility data of Cu-bound Aβ with and without the key motif further supports the presence of the different coordination spheres of Aβ-Cu in the gas-phase.

Our findings of the reduced metal oxidation state within the human a14+Cu fragment and the fragmentation kinetics have unveiled a previously unreported radical-directed fragmentation mechanism. This mechanism exclusively occurred for the interaction of human Aβ and Cu(II), apparently reflecting the solution-phase redox activity of this complex. Activation of human Aβ in complex with other metals such as Co(III) and Fe(III) did not result in this type of behaviour. Further investigation of this mechanism could improve the understanding of the interaction of copper and Aβ and potentially support drug discovery for Alzheimer's disease, and our results indicate that native MS could play an important role in this type of work.

Poster Nr. 86

Resonance-enhanced multiphoton ionization spectroscopy of surrogates, metabolites and degradation products of explosives and chemical warfare agents

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About 1.6 million tons of conventional munition as well as around 5,000 tons of chemical warfare agents (CWAs) in German seawaters are a result of battles and dumping during and after both world wars. Due to corrosion, the contained substances are continuously being released into the seawater. These chemicals and their degradation products represent a high risk for the health of marine organisms as well as humans. Because of the fact, that in many cases the exact coordinates of the underwater munition (UM) are unknown, it needs to be located for a later removal.

AMMOTRACe is a project, that aims for the detection of UM on seafloors via mass spectrometric systems. For this purpose, munition compounds will be extracted from seawater by a semi-permeable membrane und introduced into a time-of-flight mass spectrometer (TOF-MS). The ionization of the target molecules will be realized by (1+1) resonance-enhanced multiphoton ionization (REMPI). This enables fast and sensitive detection of UM-related target compounds without any sample pretreatment.

In (1+1) REMPI two photons are needed for the ionization of a molecule. The absorption of a first photon leads to excitation into a real molecular electronic state. A second photon being absorbed within the lifetime of the excited state can lead to ionization. Spectroscopic selectivity of the ionization process results from the specific location of the first intermediate state of the compounds. Hence, specific wavelengths need to be discovered for our target analytes.

In our study we are performing REMPI spectroscopic investigations of surrogates, metabolites and degradation products of explosives and CWAs in the wavelength range between 210 to 300 nm. Variable UV-radiation is produced by an optical parametric oscillator, that is used for the ionization in a TOF-MS. A constant mass flow of the measured substances is ensured by a thermogravimetric system, that is coupled to the mass spectrometer.

If nitroaromatic explosives are illuminated with intense UV-laser light, a unique photofragmentation/photo-ionization pathway leads to a selective formation of nitrogen monoxide (NO). The released NO can be selectively ionized at a wavelength of 226 nm by REMPI and the produced characteristic NO+-ions then be detected by a TOF-MS. Our results confirm this pathway and furthermore show the ability of ionization at several other wavelengths with lower intensity. Appropriable wavelengths for the detection of CWAs are only partially known from literature. Current measurements indicate the applicability of REMPI for CWAs with low fragmentation.

Poster Nr. 87

Machine learning-based analysis of mass spectrometry data for targeted detection of viral peptides

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Mass spectrometry has repeatedly been considered a promising approach for the diagnosis of respiratory virus infections. Although viral proteins and virus-induced modulations of biomarkers have been successfully identified and quantified in a variety of human samples, the long time required to perform proteomic measurements and analyze the large amount of data generated has prevented the application of this approach in the clinical setting. Therefore, in addition to optimizing sample preparation, downstream chromatography and mass spectrometry methods, the development of a rapid, reliable, and highly accurate data processing and analysis pipeline is required. Taking advantage of recent advances in machine learning, we are developing a custom neural network that, in addition to a classical feedforward deep neural network, includes a convolutional (CNN) and a recurrent neural network (RNN) specialized for different aspects of mass spectrometry data. Most raw proteomics datasets consist of mass peak tables, which are incompatible with CNNs and RNNs due to their non-uniform structure. As a solution, we are exploring an approach to convert mass peak tables into superimposed sine waves to produce uniform data that meet the requirements of these networks.

Poster 2

Time: 5/16/2023 5:15:00 PM - 5/16/2023 7:15:00 PM Location: Panorama Forum + Saal 8

Postersession II

Poster Nr. 1

An Integrated Cross-Linking Mass Spectrometry Approach to Characterize p53/Sirtuin1 Interactions

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The tumor suppressor p53 is a transcription factor that responds to cellular stress by regulating the expression of a multitude of genes involved in cell cycle arrest and DNA repair. Through these mechanisms, p53 maintains genomic stability and is therefore nicknamed as 'guardian of the genome'. Several post-translational modifications (PTMs) including acetylation, phosphorylation, and ubiquitylation dictate the distinctive p53 response to diverse cellular signals and assist in determining p53's physiological roles. Acetylation in particular has many important effects on p53. It increases protein stability, influences association with other proteins, and augments its sequence-specific DNA binding. Accordingly, deacetylation of p53 down-regulates its transcriptional activity. Sirtuin1 (SIRT1) is a class III histone deacetylase that deacetylates p53 specifically at K382.

Structural characterization of the interaction between full-length, human p53 and SIRT1 is challenging owing to the long intrinsically disordered regions (IDRs) at the N- and C-termini of both proteins. Since the p53 acetylation sites are also mostly located at the C-terminal IDR, the molecular impact of p53 acetylation remains elusive due to the lack of conformational information in these regions. Hence, chemical cross-linking mass spectrometry (XL-MS) comes to the rescue for the structural characterization of the acetylation-dependent interactions between p53 and SIRT1.

For our XL-MS studies, non-acetylated and acetylated forms of human wild-type full-length p53, and human SIRT1 were expressed and purified from a bacterial expression system (E. coli). Acetylation of p53 was achieved by co-expression of p53 acetylase, p300. Acetylation efficiency and specificity of the co-expression system was analyzed via liquid chromatography-mass spectrometry (LC-MS/MS) analysis of tryptically digested, purified acetylated p53 (Ac-p53). The desired lysine at position 382 was found to be acetylated. Both p53 as well as Ac-p53 formed functional tetramers, as evidenced by native mass spectrometry. Thereafter, SDS-PAGE analysis of the SIRT1/p53 cross-linking reaction with the amine-reactive cross-linker disuccinimidyl butyric urea (DSBU) revealed a SIRT1 interaction exclusively with acetylated p53. Furthermore, SIRT1 displayed interactions with monomeric, dimeric, and tetrameric states of acetylated p53, while no interactions were observed for non-acetylated p53. Interestingly, only a single molecule of SIRT1 interacts with Ac-p53, irrespective of p53's

oligomeric state. Our combined XL-MS and native MS results shed light on the molecular details of p53/SIRT1 interaction that cannot be obtained with other techniques.

Poster Nr. 2

Bottom-up and top-down proteomics from single C. elegans nematodes using digital microfluidics and magnetic bead-based sample preparation

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For proteome analysis of samples with limited availability, i.e., in studies with only hundreds of cells down to single cells, or studies targeting single specimens of small invertebrate model organisms, e.g., Caenorhabditis elegans (C. elegans), miniaturization of sample preparation prior to LC-MS analysis is mandatory.

We recently developed a highly sensitive digital microfluidics (DMF)-based sample preparation workflow combining single-pot solid-phase enhanced sample preparation (SP3), high-field asymmetric waveform ion mobility spectrometry (FAIMS), and fast and sensitive ion trap detection on an Orbitrap tribrid MS system [1]. Using this approach for bottom-up (BU) proteomics of single C. elegans specimens, either heat-treated or at default temperature as controls, we consistently identified up to 5000 proteins per nematode, including 45 with a significant abundance difference between the groups.

The method was subsequently modified to also make it suitable for top-down (TD) proteomics [2]. Compared to in-tube sample preparation, the modified workflow yielded an increase in proteoform identifications from single C. elegans by 46% for an average of 673±69 (n=3). Furthermore, label-free quantification using specimens from the same groups mentioned above allowed us to identify abundance changes of proteoforms that would have been masked in BU proteomics.

[1] Steinbach MK, Leipert J, Blurton C, Leippe M & Tholey A (2022). J. Proteome Res. 21, 1986-1996

[2] Leipert J, Kaulich PT, Steinbach MK, Steer B, Winkels K, Blurton C, Leippe M & Tholey A (2023). Submitted.

Poster Nr. 3

Characterization of peptide-protein relationships in protein ambiguity groups via bipartite graphs

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Introduction:

In bottom-up proteomics, proteins are enzymatically digested to peptides before measurement with mass spectrometry (MS), often using trypsin. Because of this, peptides are identified and quantified directly from the MS measurements. The inference and quantification of proteins from this peptide-level data remains a challenge, especially due to the occurrence of shared peptides, which can originate from multiple different protein sequences.

The relationship between proteins and their corresponding peptides can be represented by bipartite graphs. In this data structure, there are two types of nodes (peptides and proteins). Each edge connects a peptide node with a protein node, if and only if the peptide could originate from a tryptic digestion of the protein. The aim of this study (Schork et al, 2022, PLOS ONE) is to characterize and structure the different types of graphs that occur and to compare them between data sets. Furthermore, we illustrate how this knowledge can aid relative protein quantification. Our focus is especially on gaining quantitative information about proteins without unique peptides, as they are neglected by many protein quantification algorithms.

Methods:

We construct bipartite peptide-protein graphs using quantified peptides from three measured data sets (yeast, mouse, and human), as well as all theoretically possible peptides from an in silico digestion of the corresponding protein sequence databases. The graph over the whole data set database can be split up into connected component, where the proteins are not connected via shared peptide nodes. These connected components (called graphs in the following for simplicity) can be handled separately for protein inference and quantification. To reduce the complexity of these graphs, peptide as well as protein nodes with the exact same edges are collapsed. The occurring graphs are grouped into isomorphism classes, sets of graphs with the same structure, which are expected to behave similarly during protein inference and quantification. The structure and characteristics of the occurring graphs are compared between data sets (experimental) as well as between database (theoretical) and quantitative level. Additionally, we developed and applied a method that calculates protein ratios from peptide ratios by making use of the bipartite graph structures.

Results:

We observed a large influence of the allowed minimum peptide length used during the in silico digestion of the database. The inclusion of very small peptides leads to the formation of an extremely large graph where most proteins are connected via chains of shared peptides. When comparing the graphs from the theoretical peptides to the measured ones, two opposing effects can be observed. On the one hand, the graphs based on measured peptides are on average smaller and less complex compared to graphs using all theoretically possible peptides. On the other hand, the proportion of protein nodes with only shared peptides, a complicated case for inference and quantification, is considerably larger for measured data. We also observed that the proportion of graphs containing at least one protein node with only shared peptides rises, when going from database to quantitative level. This effect is slightly increased when protein isoforms are considered.

We show preliminary results of the newly developed relative protein quantification method. By exploiting information from the shared peptide nodes, it is also possible to gain information about protein nodes with only shared peptides.

Conclusion:

Large differences between the structures of bipartite peptide-protein graphs have been observed between database and quantitative level as well as between the three analyzed species. In the three analyzed measured data sets, the proportion of protein nodes with only shared peptides were 6.3 % (yeast), 46.6 %(mouse) and 55.0 % (human), respectively. Especially for these proteins, the usage of information from the bipartite graph structures for relative protein quantification is beneficial.

Poster Nr. 4

Characterizing the Disordered N-Terminus of the Neuropeptide Y₂ Receptor by Cross-linking Mass Spectrometry using Parallel Accumulation-Serial Fragmentation and Data Analysis by Skyline

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Neuropeptide Y (NPY) receptors comprise a family of rhodopsin-like G-protein coupled receptors (GPCRs) that participate in controlling food intake, memory retention, and circadian rhythm, making them highly attractive drug targets. However, the multiligand nature of NPY receptors, such as the NPY receptor type 2 (Y2R), requires a detailed understanding of the receptor's interactions with their natural ligands. Binding assays have so far provided insights into multiple Y2R-NPY conformers with distinct binding affinities that might also be controlled by intracellular Y2R-Gi protein-protein interactions. However, structural biology approaches, such as X-ray crystallography or cryo-electron microscopy, have so far not been able to capture these Y2R-NPY conformers, nor have they been able to resolve the conformational states of Y2R's N-terminus. According to predictions, the N-terminus of Y2R is classified as an intrinsically disordered region. We aim to employ cross-linking mass spectrometry (XL-MS) to characterize the different conformational states and binding modes of Y2R upon binding of its ligand NPY.

For our XL-MS studies, photo-reactive amino acids (photo-leucine and photo-methionine) containing diazirine groups were incorporated at defined positions into Y2R and NPY to undergo cross-linking reactions upon activation with UV-A irradiation. Cell-free expressed Y2R was reconstituted in phospholipid bicelles and mixed with NPY. The mixture was irradiated and enzymatically digested with a surfactant compatible digestion protocol using S-trap columns (ProtiFi). After enzymatic digestion, the peptides were analyzed by LC-MS/MS using a tims-TOF Pro mass spectrometer (Bruker Daltonik) operating in data-dependent acquisition (DDA) approach with parallel accumulation-serial fragmentation (PASEF). Fragment ion mass spectra were processed with MeroX (v 2.0.1.7) to obtain initial cross-link spectral matches (XSMs). Manually validated MeroX XSMs were used to generate a list of target peptides, together with an ion mobility library, for the generation of extracted ion chromatograms in the LC and ion mobility dimensions with Skyline-daily (v 22.2.1.391). Raw DDA-PASEF files were inspected in Skyline, while comparing each cross-link's properties during chromatographic and ion mobility separation with negative control samples, i.e. Y2R-bicelle preparations without photo-amino acid incorporation. These control samples will contain identical peptides, except for the cross-linked peptides.

The combined effects of precursor ion accumulation in the TIMS cell, fragment ion current accumulation through fragmentation events stacking, and the additional ion mobility dimension of peptide separation by DDA-PASEF provided better fragment ion spectral evidence of low-intensity cross-linked peptides. At the same time, spectra generated by co-isolation of high-abundant unmodified peptides were minimized. The comprehensive evaluation of each cross-linked peptide's chromatographic and ion mobility properties, in addition to fragment ion spectra, greatly improved the confidence of filtered peptide identifications. False positive cross-links that passed a lenient false discovery rate (FDR) filter of 10% and were initially manually validated as possible cross-links were

easily discriminated from true positives if a corresponding peptide was also detected in the negative control samples.

For the Y2R-NPY interaction, multiple cross-linking sites were identified between Y2R and NPY, involving the disordered N-terminal region of Y2R. The coexistence of different cross-linking sites between Y2R's N-terminus and different amino acids in NPY point to multiple conformational states of Y2R's N-terminus. With our initial findings we hypothesized the highly disordered N-terminus of Y2R might play a role in NPY recognition and guidance into its final binding site. These results provide first insights on the interactions within NPY and the N-terminal region of Y2R and will serve as basis for developing novel targeted and sensitive MS-based strategies to delineate the molecular mechanisms underlying Y2R-NPY interactions.

Poster Nr. 5

Charge site manipulation to enhance top-down fragmentation efficiency

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In recent years, top-down mass spectrometry has been widely used to study proteoforms, but improving sequence coverage remains an important goal. Electrospray ionisation (ESI) is an important enabling technology for top-down MS, and the presence of multiple charges is particularly beneficial for fragmentation. As a result, methods to increase the overall charge state of protein ions (so-called 'supercharging' approaches) has been extensively studied in the past. However, modification of protonation sites without changing the net charge state is a method that has only recently emerged as having the potential to improve fragment yield in top-down MS. A recent study found that two unique protomer families are formed in ESI of denatured carbonic anhydrase (CA) and that these states produce different fragmentation patterns in collision-induced dissociation (CID). In another report it was shown that the addition of 10% DMSO to the protein solution allows modulation of the protonation sites and consequently the CID fragmentation pattern of the intrinsically disordered protein α-synuclein (αSN). Here, we have extended this work by systematically testing different solution additives with CA as well as αSN. The resulting protein ions were characterised by top-down CID, electron transfer dissociation (ETD), and ion mobility spectrometry.

A Waters Synapt XS ion mobility mass spectrometer was used in the study. To induce the formation of different protomer families, propylene carbonate (PC) and DMSO were added at low concentrations (2% v/v) to either a denatured CA solution (70% methanol, 1% formic acid) or a near-native solution of αSN (50 mM ammonium acetate). Top-down CID experiments were performed in triplicate and changes in fragmentation patterns and fragment intensities were evaluated. In addition, ETD, which in contrast to CID is known to preserve charge positions, was performed to relate protomer formation to the observed CID behaviour.

To rule out conformational effects of the charge modifiers in solution, we aimed to keep the overall charge state change small and also investigated effects on collision cross-section (CCS) values. Triplicate measurements of each of the different ESI solution conditions gave reproducible results for both cleavage sites and observed fragment intensities. Drastic changes in the CID fragmentation patterns of CA and αSN were observed with the addition of both solvent additives, even when the same precursor charge state was selected for fragmentation. Unique fragments were found for each solution condition, resulting in an increase in overall cleavage coverage of up to around 30%. Closer examination of CID fragment intensities also revealed reproducible quantitative effects at specific cleavage sites. Fragments from ETD further supported our hypothesis that proton locations changed as a result of different solution conditions. The results for the 14+ charge state of αSN were in agreement with our previous study and showed a clear charge shift towards the N-terminus of the protein when PC was added to the ESI solution.

The current study used various mass spectrometry techniques to present a novel systematic approach to modifying charge positions in intact protein ions by the use of solvent additives. Even small amounts of these additives can effectively manipulate protonation positions, which in turn alters the behaviour of proteins during collision-induced dissociation and leads to a greater variety of fragments. This approach is advantageous because it results in more fragments that can be used to better characterise unknown proteoforms. The newly developed method allows the commonly available collision-induced dissociation technique to be used for more efficient top-down protein fragmentation. Due to its ease of implementation, this approach not only improves

the quality of top-down mass spectrometry experiments for current practitioners, but also makes top-down protein analysis more accessible to other researchers.

Poster Nr. 6

Community-based development of benchmarks and protocols for top-down mass spectrometry of native proteins and complexes

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The combination of native ionisation with top-down fragmentation allows determination of the stoichiometry of noncovalent complexes, as well as identification of the component proteoforms and co-factors. As both native MS and top-down protein MS are not yet well standardised methods, only a limited number of laboratories routinely use this powerful approach, and it can be difficult for newcomers to enter this field. Furthermore, even for a successful experiment, it can be difficult to identify an appropriate benchmark to assess the quality of the results. Here, we have developed and tested protocols for native MS combined with top-down fragmentation across a range of laboratories, and the resulting data will provide a valuable resource for the community.

A set of protocols and a list of protein standards were issued to nine participating laboratories, who used a total of eleven different instruments in this study, including QTOF, Orbitrap, and FTICR. The set of samples (including carbonic anhydrase, GroEL, and bacteriorhodopsin) contained monomeric proteins as well as complexes, and water-soluble as well as membrane proteins. Proteins were dissolved in aqueous ammonium acetate, with added detergent in the case of membrane proteins. They were then transferred into the gas phase by electrospray ionisation, and subjected to collisional activation, leading (depending on the sample) to removal of detergent and co-factors, monomer ejection, and eventually backbone fragmentation.

Although participants had different levels of experience and used a range of instruments, all were successful in achieving native ionisation of noncovalent complexes. The resulting native spectra were remarkably similar, given the diversity of operators and instruments, although certain instrument types proved more capable of extensive desolvation of large protein complexes, which resulted in higher effective resolving power. Except for very small proteins for which isotopic resolution could be achieved, desolvation efficiency had a far greater impact on the eventual peak width than the inherent resolving power of the mass analyser.

All participants successfully activated at least part of the set of native-like proteins in the gas phase, resulting in monomer ejection and backbone fragmentation. Our set of standard proteins covered a wide mass range up to nearly 1 MDa, and for the largest protein complex, only a few instruments were capable of supplying enough collisional activation to induce monomer ejection. For proteins for which most or even all participants reported fragmentation data, however, results were quite consistent between laboratories. Some fragments were observed by 100% of participants despite differences in exact experimental conditions and data analysis workflows. In many cases, such preferred sites could be rationalised through sequence and/or structural motifs, e.g., aspartic acid-rich sequence regions.

Through surveys, we gained an understanding of which methods participants preferred to use for aspects of the experiment that were not prescribed (including data analysis), as well as which steps were experienced as being the most challenging. Overall, this work provides an entry point for newcomers who wish to combine native with top-down MS. Furthermore, our results serve as a robust benchmark for the expected outcome of such an experiment, and demonstrate that these results are more dependent on inherent properties of the protein or complex than on precise experimental conditions.

Poster Nr. 7

CUBiMed.RUB - a new core facility for bioinformatics in the field of proteomics at the Ruhr-University Bochum

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Introduction: As a newly established central facility at the Medical Faculty of the Ruhr-University Bochum we are succeeding the Service Center "Bioinformatics for Proteomics - BioInfra.Prot“1 of the "German Network for Bioinformatic Infrastructure (de.NBI)“, which is part of ELIXIR. As such we will continue to provide comprehensive portfolios of bioinformatic services in the essential field of proteomics, in cooperation with de.NBI and Forschungszentrum Jülich.

Materials and Methods: We follow a bipedal course – on one hand we provide well-kept tools and workflows for the analysis of mass spectra, peptide identification, protein inference and quantification, as well as further analysis like finding protein variants, also using machine learning. On the other hand, we offer bioinformatic and statistical consultancy and also engage in several training and education activities.

Results: Several bioinformatic tools such as PIA (Protein Inference Algorithm) or MaCPepDB (Mass Centric Peptide Database) have been developed and are maintained. Training courses, such as the beginner course for R have been revitalized and will occur regularly.

Conclusion: With the provisioning of our free and high-quality services, we believe in contributing and becoming a significant part of the bioinformatics infrastructure in proteomics research.

Poster Nr. 8

Deciphering the blood type-specific proteome for crime scene investigations

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Analysis of blood samples or blood stains from crime scenes can provide relevant information on the course of offence. Especially blood types, which are inherited phenotypic characteristics of humans, exhibit a great potential to be employed for identification of involved individuals. According to the International Society of Blood Transfusion there are currently 43 recognised blood type systems which can be accessed by genotyping or serological analyses. However, these assays are often inapplicable in the case of forensic samples from crime scenes, especially due to degradation processes or interference from other biological sample material as in mixed body fluid samples. LC-MS-based proteomics has the potential to determine blood types and assign them to subjects via detection of protein and post translational protein modification (PTM) profiles. Notably, a major challenge with blood samples is the high dynamic range and the complexity of blood which renders tailored sample preparation workflows mandatory for accessing blood type-specific markers.

For setting up such workflows, we perform extensive fractionation of blood samples on multiple levels: (I) Whole blood is fractionated on the cellular level using centrifugation. (II) Proteins are fractionated via SDS-PAGE and cryoprecipitation. (III) After tryptic digestion using S-Trap (according to vendor instructions) and FASP (Manza et al. 2005, Loroch et al. 2022) peptides are fractionated using tip-based fractionation via high-pH-RP or glycopeptide enrichment workflows via ERLIC (Alpert A.J. 2008, Loroch et al. 2015) and further in the gas phase using FAIMS. Samples are analysed by nanoLC-MS in data dependent acquisition-mode using an Exploris480 online coupled to a Vanquish Neo system (both Thermo Scientific) and database search is performed using Proteome Discoverer 3.0 against the Uniprot human reference proteome (102,245 target sequences, including isoforms) with multiple search engines.

Thus far, our approach enabled the detection of ~2000 proteins in whole blood and ~1000 proteins in plasma including the detection of 27 out of the 43 blood-type specific proteins in whole blood but only 7 in plasma (100 % overlap), rendering whole blood as suitable for blood type determination which is most probably attributed to the presence of blood cells, as many markers remain prominently in plasma membranes of cells (especially erythrocytes). Among the most prominent markers were the Von-Willebrand-Factor (VWF), which has been reported to carry ABO blood group antigens, the rhesus system antigens RHD and RHCE carried by transmembrane proteins as well as the Kell blood group glycoprotein. The resulting marker profiles have the potential to identify numerous subjects with a high degree of certainty.

In summary, extensive fractionation in conjunction with state-of-the-art nanoLC-MS allowed us to access most blood type-specific proteins. With the help of these information, we will set up targeted 1D-LC-MS assays for determination of blood types in forensic samples to further extend the toolbox in the field of forensics.

Poster Nr. 9

Detection of allergen traces in food by peptide affinity capture LC-MS/MS

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About 8% of children and 5% of adults are affected by food allergy. [1] Most food-induced allergies are triggered by proteins that mediate a diverse range of symptoms. Beside diarrhoea, atopic dermatitis and shortness of breath, anaphylactic shock can also occur in severe cases. [2] Since a miniscule amount of allergenic protein can cause an allergic reaction, sensitive and specific methods for the detection of allergen traces in food are necessary [3]. The enzyme-linked-immunosorbent-assay is frequently used for the quantitative and qualitative analysis of allergens. However, false positive or incorrect results may be obtained especially for proteins that share high sequence homologies. [4]

One approach to solve this limitation is the affinity capture of allergen specific peptides in combination with detection via high-resolution mass spectrometry. Therefore, a bead-based affinity capture method for the detection of peanut or hazelnut traces in food was developed using peptide specific affinity purified polyclonal antibodies.

In a first step the method was optimized in terms of binding times, temperature and compatible buffers. To ensure that residues of the target-peptide were not present in the antibody serum used due to necessary affinity purification of the antibodies, a crosslinking step was included into the protocol to allow a first elution prior to the final peptide specific affinity capture. This purification step was required since residual peptides were indeed present in the affinity purified antibody sera. Finally, the crosslinked antibodies were incubated with the already digested food sample.

As proof of concept the optimized method was then used for the detection of peanut and hazelnut traces in cookies. It was possible to detect traces of peanut and hazelnut in the low ppm range in the chosen food matrix. Significant increase in LOD was observed for affinity capture.

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Poster Nr. 10

Development of analytical methods to understand emerging environmental contaminants and their health risk

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Mass spectrometry, especially when used with high resolving power, is a powerful analytical technique to answer diverse and complex scientific questions. Concomitantly, technical and methodological requirements on instruments and methods are rising as a result of an increasing awareness of ecotoxicological problems and potential health risks of emerging contaminants. At the same time, ultratrace analyses of compounds in increasingly complex matrices such as environmental and/or bio-related samples become more and more relevant.

This high sample complexity and influence of matrix needs to be lessened prior to the actual mass spectrometric analysis. One example is the contamination of environmental samples such as soil, where the standard soil matrix makes analysis of the contamination extremely complex. In addition to that, understanding health risk and the impact of the contamination on biological systems is not an easy task either. Most often, this is performed by coupling liquid chromatographic separation to MS. Often, the analyte, the matrix or the mobile phase system include non-volatile buffers and/or salts that are problematic for a mass spectrometric analysis, as they lead to extensive clogging and contamination of the mass spectrometers. Additionally, compared to a direct injection analysis, the HPLC coupling leads to a transient analyte signal, thus, limiting potential data depth.

Here, we try to cover the different matrices from environmental contamination to the impact on biological systems by using ultrahigh resolution mass spectrometry coupled with ion mobility spectrometry. By means of a lab-built new FAIMS electrode setup, the separation of salts and buffers from samples, such as peptides and proteins is performed without a chromatography step. Unlike the direct separation in z-direction of standard HPLC setups and the subsequent time limitation for detection, the separation in a FAIMS device happens in a perpendicular direction, thus, allowing continuous measurements without any time constraints. This constitutes a significant gain for the detection of low abundant species such as emerging contaminants or their metabolites within biological systems. In case of intact protein measurements, the sensitivity of the employed Orbitrap Elite instrument could be reasonably increased by accumulating the analytes in the HCD cell instead of classical trapping within the linear ion trap. This allows cleaner signals and higher signal intensities. Currently, PFAS are some of the most important emerging contaminants but there is no common analytical methodology available and their direct health risks are not yet known. Here, we start the search for answers.

Poster Nr. 11

Do you consider "feature-peptides"? Enabling search-engines to search for already annotated feature-information from UniProtKB

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In today's identification workflows, it is common to select FASTA-databases containing mostly canonical and isoform-protein-sequences. The workflows then in-silico digest the database to mimic the digestion-enzyme used in the sample preparation and try to match the resulting in-silico peptides with measured spectra with so-called search engines to generate peptide-spectrum-matches (PSMs). However, in the biological domain, sequences are not as uniform as illustrated in commonly used FASTA-databases, not covering variational sequences in species (except for isoforms) and/or mutations that may occur from diseases or are specific to an individual. Furthermore, biological processes continue which could yield already cleaved signal-/pro-peptides or other peptides, like Abeta 40/42. Although UniProtKB already provides so-called feature-information about molecule processing, variational and further sequence-annotations, the search-engines, on the other hand, cannot identify such spectra, due to missing entries in the FASTA-database. Here, we propose a workflow, which generates FASTA-databases in a sophisticated manner while considering various feature-information provided by the UniProtKB. In this workflow, we first use so-called protein-graphs to represent all possible sequence outcomes. Due to the exponentially growing search space, we further implemented a traversal algorithm as a second step, which takes the MS2-precursors from a dataset and exports only peptides fitting to the MS2-precursor (while considering post-translational-modifications) into a peptide-FASTA-database. The resulting peptide-FASTA-database then can be used instead of the original FASTA-database as a drop-in-replacement in common identification workflows, as long as the search engine is able to search without digestion. We applied this workflow on the dataset PXD007555, containing measured CSF from 12 individuals, and show the applicability of generating such peptide-FASTA-databases. Further, we used Comet and Percolator with the generated FASTA-database to showcase the possibility that search-engines can use such FASTA-databases. We highlight interesting identification results and show that around ~5% of PSMs in PXD007555 originate from peptides resulting from feature-information, demonstrating that such "feature-peptides" are indeed identifiable. Though further investigation is still needed, this approach shows a promising step to further increase the identification-ratio in CSF-Samples.

Poster Nr. 12

Exploring disparate tryptic digestion for bottom-up proteomics: a comparative study

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Serine endopeptidase trypsin is the most commonly used enzyme for protein hydrolysis in proteome analysis. In this work, we present a comprehensive study of a variety of commercially available trypsin from different vendors for comparing cleavage specificity, efficiency and reproducibility. Therefore, an LC-MS/MS shotgun proteomics approach with focus on clinically derived samples (human plasma as well as purified platelets) was conducted. Additionally, to determine the yield of tryptic digestion a targeted analysis using two selected peptides of an antibody was performed. Our investigations demonstrate an equal manner performance of most of the analyzed trypsins, independent of price range. Furthermore, optimal digest time range corresponding to its buffer and temperature conditions were determined for all kinds of quantitative proteome studies.

Poster Nr. 13

Gas-phase fractionation of proteoforms using FAIMS for in-depth top-down proteomics

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Top-down proteomics (TDP) targets the identification and quantification of intact proteoforms, i.e., all molecular forms of a protein, including mutations, RNA splicing variants, truncations, and other posttranslational modifications. Due to the high complexity of proteoforms present in the cell, efficient (multidimensional) separation schemes are essential for in-depth TDP. In addition to liquid-phase separations, gas-phase separation techniques have been shown to increase the depth of TDP analyses. To this end, high-field asymmetric waveform ion mobility spectrometry (FAIMS) has been introduced as an efficient gas-phase fractionation strategy. Recently, FAIMS with external compensation voltage (CV) stepping was investigated using multiple injections with different CVs, resulting in a significant increase in the number of identified proteoforms in a TDP analysis.

Here, we investigated FAIMS with internal CV stepping (multiple CVs within a single run) for proteoforms analysis. For all experiments, a Fusion Lumos Tribrid mass spectrometer equipped with a FAIMS Pro Interface was used in combination with LC-based proteoform separation on a nano C4 column.

Different CVs favor the identification of proteoforms within a certain mass range, e.g., the more positive the CV, the larger the identified proteoforms. For example, at a CV of –60 V, mainly proteoforms in the mass range of 4-8 kDa are identified, but at a CV of –10 V, the mass range of identified proteoforms shifts to 12-20 kDa. An important finding was that this information can be used to optimize MS settings for specific CVs, since, for example, small proteoforms can be identified with lower resolution and number of microscans than larger proteoforms [1]. Thus, we optimized the MS settings for each CV based on the favored mass range and investigated the influence of the number and combination of the different CVs within a single run. Using a combination of four CVs (–60, –50, –40, 0 V), significantly more proteoforms were identified in all mass ranges compared to measurements without FAIMS. In addition, the use of multi-CV FAIMS was beneficial for a wide range of gradient lengths between 60-180 min.

The optimized multi-CV FAIMS method was tested for the analysis of human proteoforms in combination with the most common TDP sample preparation techniques for the enrichment of proteoforms <30 kDa (GELFrEE, molecular weight cut-off filter, and solid-phase extraction) [1]. For all sample preparation methods, the utilization of internal CV stepping almost doubled the number of proteoform identifications and furthermore improved their confidence in terms of residue cleavage, C-score, and p-value compared to measurements without FAIMS. In total, 2,675 human proteoforms from 684 protein groups were identified with FAIMS and only 1,375 proteoforms from 398 protein groups were identified without FAIMS. Note that the acquisition time remains the same for the measurements with and without FAIMS.

Next, we investigated the use of FAIMS for fractionated, i.e., less complex, samples. In a low/low pH separation scheme, 34 fractions were collected and measured via LC-MS without FAIMS and with an optimized multi-CV method [2]. Again, FAIMS doubled the number of identifications, and a total of 5,571 human proteoforms from

1,265 protein groups were identified (without FAIMS: 2,311 proteoforms from 780 protein groups), demonstrating the power of FAIMS also for the analysis of lower complex samples. We also developed a low/low pH concatenation strategy and performed two injections using different multi-CV FAIMS methods to detect the low-molecular-weight and high-molecular-weight regions of the pools, which dramatically increased the number of proteoforms >20 kDa.

As certain CVs target specific mass ranges of proteoforms, we combined our multi-CV FAIMS method with PEPPI fractionation (polyacrylamide gel-based pre-fractionation for the analysis of intact proteoforms) [3]. In the PEPPI protocol, proteins are separated according to their molecular weight on an SDS-PAGE and then eluted from the gel as intact species. MS parameters and CVs were adjusted for the different gel bands according to their mass ranges. For example, for the high-molecular-weight gel bands, more positive CVs were applied and a medium/high acquisition strategy was used, in which MS1 spectra were acquired at low resolution to enhance the identification of larger proteoforms. In total, 8,500 proteoforms from 1,357 protein groups were identified from a human cellular proteome standard using the developed GeLC-FAIMS-MS method. Moreover, the results show that FAIMS is beneficial for analyzing proteoforms from all mass ranges (up to >30 kDa).

Our results clearly demonstrate that FAIMS significantly boosts the number of proteoform and protein identifications in top-down proteomics for a wide range of various applications and holds the potential for further improvements in TDP.

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Poster Nr. 14

HybG and its Role in Diatomic Ligand Biosynthesis of E. coli NiFe-Hydrogenase Studied by Native Mass Spectrometry

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[NiFe]-hydrogenases have a bimetallic active-site cofactor (NiFe(CN)2CO), in which the iron ion carries a CO and two CN- as diatomic ligands (Fe(CN)2CO). While biosynthesis of the CN- ligands from carbamoyl phosphate is clear, biosynthesis of the CO ligand remains unresolved. Six Hyp proteins (HypA-F) are involved in cofactor biosynthesis, but only the FeS-cluster-containing HypD protein is redox-active. HypD is required to transfer the CN- ligands from the HypE-HypF proteins to the iron, and circumstantial evidence also suggests that the CO ligand is generated by HypD from endogenous CO2 already bound to an iron ion on a HypD-HybG (HypC paralogue) sub-complex. The biosynthesis of the Fe(CN)2CO portion of the co-factor occurs on the HybG-HypD complex where HypD acts as an assembly scaffold. The interaction sites for the CN-, Fe, and CO2 within the HybG-HypD complex are unclear. We were able to identify not only the potential binding site for the CN--ligand but also the dependency of CN--binding for CO2 attachment, using native mass spectrometry. We discovered that HybG has two distinct, and apparently CO2-related, covalent modifications that depend on the presence of the N-terminal cysteine residue on the protein, possibly representing intermediates during Fe(CN)2CO group biosynthesis. Impairing this interaction also hinders CO2 binding to HybG. These findings suggest that the HybG chaperone is involved in both biosynthesis and delivery of the Fe(CN)2CO group to its target protein. HybG is thus suggested to shuttle between the assembly complex and the apo-catalytic subunit rather than forming a ternary complex during [NiFe]-hydrogenases maturation.

Poster Nr. 15

Identification of CID fragmented oxidation products of tryptophan with chlorine dioxide solutions as marker of potential cell degradation

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Chlorine dioxide solution is a well known disinfectant for medical applications and treatment of drinking and waste water. In most cases not a pure solution is used but unpurified technical solutions containing basically the whole reaction system of the chlorine dioxide synthesis. The reaction paths are not well understood with the complex technical solution. Therefore, an investigation of the reactions of the pure water chlorine dioxide system appears to be a useful first step towards a more comprehensive understanding. It is proposed that mostly aldehydes are the products of these reactions.

Chlorine dioxide is synthesized with the method established by a.p.f. The gas is stripped out of the solution with wet air. The soluted chlorine dioxide concentration is determined by UV-Vis-Spectroscopy. Tryptophan was prepared with concentrations of 0.05 mmol/l per solution. The chlorine dioxide was applied in different mixing ratios to the amino acid (1:1, 1:2, 1:3, 1:5, 1:10). The reaction time between ClO2 and analyte solution was at least 12 hours. CID mass spectra of the reaction products were measured with a Sciex 6500 Triple-Quad-Mass-Spectrometer.

An initially apparent result is that chlorine dioxide reacts in all cases with the amino acids. Furthermore a strong effect of the ratio of amino acid and chlorine dioxide could be observed. It could be observed that for the oxidation of tryptophan for a full oxidation at least three chlorine dioxide molecules are needed.

As expected, primarily Aldehydes were found as reaction products. In most cases the indole structure opens but the benzene ring remains intact as described in literature.This interpretation is supported by UV-Vis-Spectroscopy. Furthermore no crosslinking between the amino acids was observed.

The second important aspect is the influence of the chlorine dioxide concentration in the solution on the occurrence of each product. There is only a minor effect noticed that needs further investigation.Tryptophan is an amino acid that has an important part in ion channels in cell membranes. Usually the indole part sticks into the ion channel. So any change in that structure can lead to a malfunction of the ion channel. Also tryptophan is an important part of the connection of the protein and the lipid membrane. So the knowledge of oxidized products of tryptophan and the analysis out of CID fragmented mass spectra could be an important marker for the effect of chlorine dioxide on biological systems.

Poster Nr. 16

Identification of Protein-Protein Interactions of Human Mitochondria by In-situ and Blue-Native In-gel Crosslinking Mass Spectrometry

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Many is known about mitochondrial protein functions as well as interactions, especially ones that are involved in energy production and metabolism. Over the last years crosslinking mass spectrometry (XL-MS) revealed not only further insights into mitochondrial protein complex assembly, but also expanded the knowledge of the mitochondrial interactome1;2;3. The use of different types of crosslinker and sample preparation techniques, provides the opportunity to identify interaction partners of proteins, whose functions are so far unknown. Here, after preparation of mitochondria originating from HEK cells overexpressing Small Integral Membrane Protein 26, SIM26, we applied in-situ crosslinking on intact mitochondria followed by digitonin lysis and SDS PAGE. In a second approach, mitochondria were lysed by digitonin, protein complexes separated by blue-native PAGE and subsequently in-gel crosslinked. Mass spectrometry was performed on an UHR-QTOF instrument and Merox as well as MaxQuant software packages were used for identification of crosslinked peptides. With BS3 as crosslinking reagent both approaches resulted in reliable crosslinking hits as we could identify various known interactions. In-situ crosslinking of mitochondria resulted in additional identifications, which shows that both techniques can complement each other. We could identify Voltage-dependent anion-selective channel protein 2, VDAC2, as well as Serine/threonine-protein phosphatase PGAM5 as probable interaction partners for SIM26.

Poster Nr. 17

Infrared laser-based sampling combined with LC-MS/MS enables the three dimensional and spatially resolved proteomic profiling of bladder tissue

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To fully understand cellular processes in organs a spatially and in-depth resolved proteome analysis is required, as different cell layers are associated with different molecular functions and proteomic profiles. Three-dimensional nanosecond infrared lasers (NIRL)-based tissue sampling, a soft tissue ablation technology that generates a homogenized aerosol of tissue molecules, has previously been shown to enable spatially resolved tissue ablation for proteomic profiling. Here, we aim towards a cell layer-resolved proteome analysis of fresh frozen murine bladder to decipher the molecular profiles of different cellular bladder layers, including: urothelium, submucosal layer, detrusor, and adventitia for the first time directly from fresh frozen tissue.

We ablated fresh frozen mouse bladders in layers of about 20 µ m depth from the urothelial side. The aerosols were collected separately and digested with trypsin. Measurement was performed on a nano UPLC (nanoAcquity system, Waters) coupled to an Orbitrap Mass Spectrometer (QExcative, Thermo Fisher) by label-free LC-MS/MS in DDA mode. Data base search was done in Proteome Discoverer with the implemented CHIMERYS algorithm. Consensus clustering was performed to distinguish the bladder layers on the proteomic level. Quantitative protein abundances were compared between different clusters and significantly differential proteins were aligned to the human protein atlas.

Consensus clustering of NIRL-ablation layers revealed different molecular profiles. Identified layers corresponded to the predefined cellular layers of the bladder, as indicated through the abundance distribution of cell type-specific proteins, including uroplakin, elastin, and myosin. This data was further confirmed with histological data from the human protein atlas. Based on our results, a proteomic base profile of cellular bladder layers was generated. The investigation of changes to this profile in future studies can provide new insights into proteome changes during bladder-related diseases, such as cancer (Urothelial carcinoma and squamous cell carcinoma) or urinary tract infections (UTIs).

Poster Nr. 18

Mass spectrometric analysis of amyloid-beta peptides and their interactors for the diagnosis of Alzheimer´s disease

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Objectives: Alzheimer´s disease (AD) is the most common neurodegenerative disorder. Immunohistochemically, AD is characterized by the accumulation of amyloid plaques in the brain of patients. The plaques consist mainly of amyloid-beta (Aβ) peptides, but also of proteins and lipids. Due to the abnormal concentration and the conformation of Aβ peptides in liquid biopsies from AD patients, they are already considered promising biomarkers. In this context, targeting Aβ-interactors is potentially rewarding for the diagnosis and understanding of the disease. Therefore, the aim of this study is the development of a mass spectrometry-based approach for the identification and quantification of Aβ interactors.

Methods: In this work, a co-immunoprecipitation mass spectrometry (Co-IP-MS) approach was developed. It includes Co-IP of Aβ and its interactors from cerebrospinal fluid (CSF), specific MS-compatible sample preparation as well as global and targeted MS methods for the screening and quantification of the interactors. The latter involves testing two MS-based quantitative strategies that use heavy spiking-in peptides: Parallel Reaction Monitoring (PRM) and SureQuant.

Preliminary data: Global proteomic profiling allowed the detection of previously published and several new Aβ-interactor candidates. Two targeted MS-based approaches, namely PRM and SureQuant, have been developed for the quantification of the identified interactors and demonstrate slightly varying sensitivity and accuracy.

Conclusion: The developed Co-IP-MS approach offers high potential to provide added value for uncovering the molecular network of Aβ at the protein and peptide level and improving AD diagnosis in the future.

Poster Nr. 19

Mass spectrometric identification of autocrine factors from CHO cell secretome - adapted extraction, purification and MS strategies

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More than half of today's biopharmaceuticals are produced using mammalian cells, such as Chinese Hamster Ovary (CHO), with typical products like monoclonal antibodies (mAbs), vaccines or hormones. Secreted host cell proteins (HCP) play a major role in biopharmaceutical production and their characterization and identification in culture supernatant of production cells is beneficial for two reasons. First, the identification of challenging HCP impurities enables downstream processes (product purification) to be improved through cell engineering (e.g. gene knock-outs) or bioprocess adaption. Second, the identification of autocrine factors allows to optimize serum-free and chemically defined media for an improvement of growth and cell productivity.

Here we present a workflow for extraction of autocrine factors in culture supernatant of the antibody-producing CHO cell line XL99 G4, followed by nanoLC-Orbitrap-MS analysis. For this purpose, the comparison to the parental cell line CHO XL99 was carried out with a SILAC quantitation approach (stable isotope labeling with amino acids in cell culture) of HCPs. The workflow for obtaining the secretome samples includes depletion of extracellular vesicles to enable the sole analysis of soluble proteins in the culture supernatant. Also, a large part of the antibody product was removed by means of affinity chromatography with protein A (or protein L) yielding a purified HCP fraction that was analyzed by MS.

With this improved sample preparation and an optimized MS acquisition a large part (43 %) of the Top300 protein IDs is clearly designated as extracellular according to determined Gene Ontology terms. In sum, our optimized strategy based on SILAC quantification revealed regulated production cell specific autocrine factors that are associated with effects on extracellular matrix remodeling or important signal transduction pathways, but also factors (such as proteases) that can have an impact on product quality.

Poster Nr. 20

Matrix development for the detection of phosphorylated Amyloid-β peptides by MALDI-TOF-MS

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Amyloid-β (Aβ) peptides, including post-translationally modified variants thereof, are believed to play a key role in the onset and progression of Alzheimer's disease. Suggested modified Aβ species with potential disease relevance include Aβ peptides phosphorylated at serine in position eight (pSer8-Aβ) or 26 (pSer26-Aβ). However, the published studies on these Aβ variants essentially relied on antibody-based approaches and the available mass spectrometric evidence for the occurrence of endogenous pSer8-Aβ and pSer26-Aβ in biological samples is ambiguous at best. Technical challenges of detecting phosphorylated Aβ peptides by MALDI-TOF-MS include the commonly known limited 'flyability' of Aβ peptides, the susceptibility for methionine oxidation during sample preparation, and the typical phosphate loss under mass spectrometric conditions. To address these issues, we developed a customized matrix formulation, referred to as TOPAC, that allows for the detection of synthetic pSer8-Aβ(1-40) and pSer26-Aβ(1-40) with high signal intensities, while minimizing methionine oxidation and phosphate loss (Liepold et al., J Am Soc Mass Spectrom, 10.1021/jasms.2c00270). The TOPAC matrix is based on trihydroxyacetophenone/diammonium citrate (THAP/DAC), a matrix preparation widely used for the analysis of phosphorylated peptides in positive ion MALDI-TOF-MS, and includes as additives the non-ionic detergent n-octyl-β-D-glucopyranoside (OGP), phosphoric acid (PA), and the commonly used peptide matrix compound α-cyano-4-hydroxycinnamic acid (CHCA). TOPAC not only improved the analysis of intact pSer8-Aβ(1-40) and pSer26-Aβ(1-40), but also the detection and sequencing of the proteolytic cleavage products pSer8-Aβ(1-16) and pSer26-Aβ(17-28). We expect that TOPAC will facilitate future efforts to detect and characterize endogenous phosphorylated Aβ species in biological samples, and that it may also find its use in phospho-proteomic approaches apart from applications in the Aβ field.

Poster Nr. 21

Minimizing Identification and Quantification Errors in Proteomics by DIA

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Label-free protein quantification (LFQ) by mass spectrometry (MS) is progressing rapidly with a large number of published tools and software, especially in the field of data independent acquisition (DIA). However, the performance of the new technologies is often unknown.

While proteome coverage and quantitative precision can be characterised by ID numbers and standard deviations, quantitative accuracy and validity are hardly accessible. While benchmarks offer the possibility to investigate these quality attributes, they often remain uncharacterised. We note a lack of suitable benchmark analysis software, which we aim to address in the presented work. The aim is to extend current benchmark procedures1,2 with analysis software to investigate previously undescribed sources of error.

We used the "classical" benchmark setup (cited above) consisting of human, yeast, and E. coli mixtures with expected log2 changes of 0, +1, and -2, respectively. Four replicates of DIA measurements were performed using 90-min gradients from a Q Exactive HF mass spectrometer. The remaining raw data were obtained from PXD028735 3. The DIA data were processed using DIA-NN v1.8 and analysed using our in-house benchmark analysis software. A variety of quality attributes and sources of error are described using data visualisations and summary statistics. A key process within the performance evaluation is the use of differential expression analysis with Limma5 to produce summary statistics in style of a confusion matrix.

The output of the benchmark analysis software is excellent for detecting and describing errors arising from incorrect normalisation, identification errors, dispersion, and skewness. Based on the newly gained knowledge, we have simultaneously optimised proteome coverage and quantitative accuracy. The resulting overall performance of our DIA - Q Exactive HF workflow surpasses that of a diaPASEF dataset, among others. In particular, our results show little bias or skewness in the form of ratio compression, which is readily observable in the diaPASEF data. It also reduces all kinds of quantification errors, including the rate of peptide misidentifications.

In total, we have developed a strategy to achieve highly valid and accurate protein quantification while maintaining a sensitivity comparable to benchmark datasets obtained with modern mass spectrometers.

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Poster Nr. 22

NAM-MS: Application of Proteomics to Accelerate Development of New Approach Methodologies for Investigating the Skin's Microbiome Relevance for Melanoma Progression

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Humans are permanently exposed to external influences of different sources such as UV radiation and xenobiotics. Due to its barrier function and sophisticated cellular repair mechanisms is the skin generally an efficient protection shield against these external factors. Failure of the skin's protective mechanisms can lead to fatal tissue damages, which may culminate in melanoma formation. In Germany, melanomas account for approximately 5 % of all newly diagnosed cancer cases. Recently, the commensal microbiome of healthy skin was reported to contribute to human pathogen protection, wound healing and immune modulation. Notably, melanoma formation likewise is accompanied by perturbed balance of the microbiome. Yet the interplay of melanoma progression and microbiome dysbiosis as well as underlying mechanisms remain elusive.

This prompted us to establish a co-culture system of 3D-skin melanoma models colonized with a skin swab of a healthy volunteer. The skin model is based on the MelanomaFT™ (MatTek). Preliminary results from histology and immunoassays indicate decelerated melanoma progression. For example, elevated secretion of the cytokines IL-1α, CD40 ligand and GM-CSF into the culture supernatant was detected which associates with good survival in cancer patients. Furthermore, increased levels of the melanoma marker S100B were detected. S100B is expressed by melanocytes and its release indicates reduced cell integrity and cell death.

We will now apply a proteomic approach to melanoma models that are non-colonized or colonized with either a skin swab, a defined bacterial community (DBC) or a DBC including melanoma-associated pathogens to assess global proteome changes in melanoma cells. This will enable identification of proteins and pathways that are involved in modulating melanoma growth in colonized skin. In a second step, a metaproteomic approach will be applied in order to create a comprehensive proteome database of the skin's microbiome which will then be used to identify microbes with beneficial effects on melanoma repression.

Poster Nr. 23

New CID cleavable cross-linkers suitable for IMAC enrichment and structure elucidation of proteins via MS

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Chemical cross-linking in combination with mass spectrometry (XL-MS) has developed into an important method for characterizing protein conformations and protein-protein interactions.[1] Especially the development of CID cleavable cross-linkers enhanced the effective detection of derivatized peptides and opened the avenue for automated MS data set analysis.[2] Nevertheless, it remains a challenging analytical task to differentiate cross-linked peptides from unmodified peptides in complex mixtures resulting from protein digestion.[3]

To further improve the selective analysis of the cross-linking process tri-functional reagents for chemical cross-linking have been developed in the last years. These type of cross-linking reagents contain an additional moiety designed for affinity chromatography enrichment. We aim to expand the currently available group of trifunctional linkers by designing reagents that allow an enrichment of cross-linked species via immobilized metal affinity chromatography (IMAC). The symmetrical linkers for chemical cross-linking have the CID urea moiety in common, making them sensitive upon CID.[4] The projected trifunctional cross-linker containing a CID cleavable group in addition to an IMAC-enrichable function contains two Tyr moieties in the new linker reagent. The phenole side-chains should allow the characteristic complex formation with immobilized iron aimed to selectively retard cross-linked peptides via IMAC. This approach was inspired by the catechol based siderophores in terrestrial bacteria resulting in the idea of using the oxygen iron interaction for an IMAC purification.[5] This enrichment strategy has been established for a phosphonic acid containing cross-linker that allows the enrichment of cross-links based on IMAC.[6] The synthesis and initial proof-of-principle results with tandem MS of a NHS-Tyr-Urea-Tyr-NHS linker reagent are presented. We obtained the cross-linker from commercially available tyrosine over six steps with an overall yield of 20 %. Furthermore, we were able to react the cross-linker with thymopentin to prove its effectiveness as an amine reactive CID cleavable cross-linker.

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Poster Nr. 24

Phosphoproteomics with dia-PASEF and short gradients

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Protein phosphorylation is one of the most studied post-translational modifications due to its prominent role in cellular signaling and many diseases. Mass spectrometry-based phosphoproteomics studies have collectively identified well over 100,000 phosphorylation sites in the human proteome. To disentangle the underlying phosphorylation networks and further extend applications to primary clinical samples, improvements in throughput and sensitivity are highly desirable. Here, we demonstrate how key features of dia-PASEF translate to phosphoproteomics. Phosphorylated peptides enriched from HeLa full proteome digests were separated within 7- and 21-minute ACN gradients. The timsTOF Pro instrument was operated in dia-PASEF mode with an optimized acquisition scheme for phosphopeptides using the py_diAID software (Skowronek et al., 2022). At low sample amounts equivalent to ~20 ug protein digest per analysis, we quantified over 12,500 phosphopeptides including ~9,200 class I phosphosites in 21-min gradients without a spectral library. Decreasing the gradient time to 7-min, we still quantified about 80% of the class I sites, while maintaining a virtually complete data matrix and quantitative accuracy with a median CV <10% in four replicates. We found examples of positional isomers of nearby phosphosites that were separated by ion mobility but remained unresolved by fast chromatography. Increasing the throughput and sensitivity of phosphoproteomics is highly desirable. Here we show the application of dia-PASEF for short-gradient phosphoproteomics from low sample amounts without the need for a spectral library, enabling rapid and accurate quantification of major cellular signaling pathways.

Poster Nr. 25

Proteomic characterization of neuromelanin granules in health and disease

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Neuromelanin granules (NMGs) are organelle-like structures present in the human substantia nigra (SN) pars compacta. Besides neuromelanin, NMGs further contain proteins, lipids and metals. NMGs are of high clinical relevance, since especially neurons with a high NMG content are more vulnerable in the context of Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Therefore it is believed, that NMGs play a role in neurodegeneration. However, this role is not completely understood yet and needs further investigation. Furthermore, the synthesis of neuromelanin and the mechanism behind the formation of NMGs remain largely unknown. Since NMGs are absent in the brain of most common laboratory animals (e.g. mice, rats), researchers are dependent on human post-mortem brain tissue, which is highly requested and therefore limited.

To overcome this limitation, we established a protocol for the proteomic characterization of NMGs in comparison to the surrounding SN (SNSurr.) tissue, based on the use of laser microdissection and bottom-up proteomics. First, we isolated NMGs and then subsequently collected SNSurr. tissue from the identical tissue slice. Both sample types were afterwards prepared for mass spectrometric analyses by lysing the isolated tissue followed by tryptic in-solution digestion. We performed data-dependent acquisition (DDA) experiments on an Orbitrap Fusion Lumos mass spectrometer to detect significantly regulated proteins between NMG and SNSurr. tissue, as well as parallel-reaction-monitoring (PRM) experiments, the latter as a validation for DDA-derived results.

In a next step, we applied our established workflow, which was published in 2021 (Wulf et al., 2021), on samples from a cohort consisting of five donors diagnosed as DLB cases and five donors classified as control (CTRL) cases after neuropathological examination. In all of our samples, we were able to identify a total of 3,090 proteins. Comparison of differential proteins between SNSurr. and NMGs of CTRL cases resulted in the identification of various RNA-binding and stress granule-related proteins to be significantly higher abundant in NMGs. In addition, we could show that proteins involved in dopamine synthesis are highly abundant in NMGs, which could also be verified by PRM for tyrosine hydroxylase (Wulf et al., 2022a). When we compared the proteomic profiles of NMGs of CTRL and DLB cases, we found alpha-synuclein and protein S100A9 to be significantly higher abundant in NMGs of DLB cases (Wulf et al., 2022b).

In summary, these studies add up to the knowledge on the complex biology of neuromelanin and NMGs. Our results point towards a mechanism for NM synthesis, including tyrosine hydroxylase and the autooxidation of dopamine, which is one of the most frequently discussed hypotheses. In addition, the results of our studies indicate stress granules to be related to NMGs, either as their precursors or as aggregates forming in close proximity to them, thus adding new perspectives to the research topic of the role of NMGs under physiological conditions. The high abundance of alpha-synuclein and protein S100A9 inside of NMGs of DLB cases raises the question of the presence of potentially neurotoxic alpha-synuclein fibrils inside the NMGs, since S100A9 was

reported to modulate the fibrilization behavior of alpha-synuclein. The presence of such fibrils may explain the vulnerability of especially those dopaminergic neurons, which contain the highest numbers of NMGs, in the context of DLB and PD. To test this, we are currently performing a follow up study, including tissue from ten CTRL and eight PD cases.

Poster Nr. 26

Proteomics of the Aspergillus fumigatus host-pathogen interaction

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Aspergillus fumigatus is an important human pathogenic fungus, which can cause life-threatening invasive pulmonary mycosis of immunocompromised hosts and serious allergic reactions. Recently, A. fumigatus was classified by the WHO as a fungal pathogen with the highest priority for taking countermeasures, due to rapid infection dynamics as well as difficulties in diagnosis and treatment, including resistances to antifungal drugs. Proteome analyses massively contribute to elucidating host-pathogen interactions. In order to decipher the underlying molecular processes of the pathogenicity of the fungus and the human immune response, we investigate the interactions from both sides and on every conceivable level.

Surface proteins and cell wall components of asexual spores (conidia) are crucial for infection as they mediate the first contact between pathogen and host. We analyzed the fungal surface proteome during all developmental stages (resting and swollen conidia, germlings and hyphae) by both surface biotin-labeling and trypsin shaving approaches. We found that the abundant conidial cell wall protein A (CcpA) belongs to the immune evasion proteins that reduce the immune recognition of fungal spores (stealth effect). Deletion of the CcpA gene in A. fumigatus resulted in a strain with attenuated virulence.

Alveolar macrophages, neutrophils and lung epithelial cells have important functions for the elimination of inhaled fungal conidia. A. fumigatus evolved strategies to resist phagocytosis and intracellular digestion. We could show that the fungal cell wall protective pigment layer consisting of DHN (1,8-dihydroxynaphthalene) melanin interferes with the acidification of the phagolysosomes by affecting the vacuolar ATPase.

If A. fumigatus conidia germinate and form hyphae, these can no longer be easily eliminated by phagocytic cells and extracellular defense mechanisms such as the production of extracellular vesicles (EVs) are used. The secretion of extracellular vesicles is a widespread mechanism for both intercellular and microbial communication. Their release from human epithelial and immune cells is of particular biological importance because it has been shown that EVs from neutrophils effectively inhibit fungal growth.

The mass spectrometry-based identification of proteins at the host-pathogen interface poses analytical challenges to researchers as different biological systems are studied simultaneously, increasing the complexity of proteomic profiles and expanding the dynamic range of protein abundances. We discuss challenges and strategies to analyze host-pathogen interactions using mass spectrometry-based proteomics.

Poster Nr. 27

Quantitative proteomics of signalling proteins in human spermatozoa by parallel reaction monitoring (PRM)

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Fertilization in humans is a complex process, during which the sperm cell undergoes several changes. After ejaculation, sperm become 'capacitated' in the female reproductive tract, a process by which sperm acquire the capacity to fertilize an oocyte. A multitude of proteins have been suggested to play a role in signal transduction during fertilization. Many of these proteins were identified in mammalian model organisms such as mice or rats; however, whether these findings also apply to humans is unclear. We used different mass spectrometry (MS) techniques to identify and quantify proteins that are present in human sperm and play a role during capacitation. Additionally, we show that many sperm proteins that have been implied in fertilization are present at very low copy numbers, if expressed at all.

Data-dependent acquisition (DDA)-MS with prior offline basic reversed-phase fractionation was used to assemble the proteome of human sperm. In three biological replicates, we identified ~6,000 different proteins. As an indicator for the sensitivity of our analysis, we note that we could identify all 10 subunits of the cation channel of sperm (CatSper). We searched our results for proteins that were previously implied in human sperm physiology and signalling. These proteins were assembled in a 'positive' list entailing 39 proteins. Another 'positive' list comprises 22 proteins that are unrelated to the capacitation-associated signalling but that serve obvious sperm-specific functions such as acrosomal exocytosis, gamete recognition, or axonemal structure. By contrast, proteins that had been commonly implied in sperm signalling but were not detected by DDA-MS are assembled in a 'negative' list. For selected proteins from these lists, using parallel reaction monitoring (PRM)-MS, we determined either their copy numbers (number of molecules/cell) or for those, which we could not detect, their limit-of-detection (LOD) values. For absolute quantification, we used the proteome dataset to identify suitable peptide sequences for generating standard peptides labelled with stable isotopes. If proteins were not identified in our DDA analysis, sequences for standard peptides were chosen from the peptide atlas (www.peptideatlas.org) and these standard peptides were used to determine the LOD value for the corresponding proteins. Proteins extracted from human sperm were digested with trypsin according to a protein aggregation capture (PAC) approach. Defined amounts of standard peptides were added to the endogenous peptide mixture, which was derived from a known number of human sperm cells. Heavy standard peptides have the same physicochemical properties as the respective endogenous peptides, which leads to the same behaviour during LC-MS/MS analysis. This allows for direct comparison of signal intensities of the standard and endogenous peptides and, thereby, for quantification. LOD values for endogenous peptides were titrated by loading 1 attomole to 10 femtomoles of the standard peptides on the LC-MS column along with endogenous peptides derived from a known number of sperm cells.

Four proteins that reportedly are key to transport of bicarbonate, which plays an important role during human sperm capacitation, were not identified by DDA-MS: SLC26A6 (a solute carrier), SLC4A4 (electrogenic sodium/bicarbonate cotransporter 1), CFTR (cystic fibrosis transmembrane conductance regulator, a Cl- channel), and SCNN1A (epithelial sodium channel 1 subunit alpha). The LOD values for these proteins vary between measurements and also between different peptides owing to their different intrinsic properties. Of note, the minimum number of molecules/cell detected is calculated from the lowest LOD value of a standard peptide

obtained from the most sensitive MS measurement. For different standard peptides, the lowest LOD values ranged from 1 attomol to 50 attomol, equivalent to 7 and 335 molecules/cell. Importantly, in similar PRM experiments performed with HEK293 cells, SLC26A6 and SLC4A4 could be readily detected, which rules out the possibility that these peptides escaped detection in human sperm for technical reasons or due to their membrane-associated nature. By contrast, other proteins involved in bicarbonate regulation feature a high copy number in human sperm such as the carbonic anhydrase (CA2) with 154,100 ± 67,000 (mean ± s.d., n = 4) molecules/cell and chloride-anion exchanger (SLC26A3) with 23,100 ± 15,400 (n = 4) molecules/cell. The large dynamic range between the very low LOD values and the high copy numbers of detectable proteins suggests that SLC26A6, SLC4A4, CFTR, and SCNN1A are either absent or physiologically irrelevant in human sperm. Additionally, we determined the copy number of five subunits of the CatSper channel and the Slo3 potassium channel: 2,500 ± 1,800 (n = 4) molecules/cell for CatSper 1; 7,100 ± 2,500 (n = 4) molecules/cell for CatSper 2; 5,000 ± 2,600 (n = 4) molecules/cell for CatSper 3; 2,700 ± 1,300 (n = 4) molecules/cell for CatSper 4; 5,400 ± 1,000 (n = 4) molecules/cell for CatSper B; and 5,300 ± 2,500 (n = 4) molecules/cell for Slo3. These copy numbers are of the same order as in sea urchin sperm. In summary, PRM measurements with spiked-in standard peptides reveals either the absence or the copy numbers of signalling proteins or proteins of other kind. These results provide a basis for systematic and quantitative analysis of human sperm signalling.

Poster Nr. 28

Relative peptide quantification to determine the extent of species substitution in seafood by targeted LC-MS/MS

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Adulteration affecting foods of animal origin has been raised consumers' awareness due to recent incidences. Especially substitution of meat and seafood ingredients with other inferior species is commonly done in order to increase profit. Effective and sensitive analytical methods for species identification are needed to ensure the correct labelling of food products. In addition, quantification methods are required to determine the extent of substitution in order to distinguish intentional adulteration from contamination.

Mass spectrometry has become a promising alternative to routinely used protein- and DNA based methods in the field of authenticity control. Emphasis has been placed on bottom-up proteomics and the identification of species-specific marker peptides which have been successfully applied to highly processed food products [1].

In order to determine the extent of species substitution using LC-MS/MS Watson et al. developed an MRM based approach named corresponding peptides from corresponding proteins (CPCP) [2, 3]. Species-specific marker peptides showing small variations in their sequence and deriving from the same protein are considered as corresponding peptides. Ratios of MRM transition peak areas of corresponding peptides were used to calculate the amount of each species in binary species mixtures. 1% (w/w) of one meat species added to another was successfully determined.

Seafood mislabelling has been increasingly documented over the past decade. Litopenaeus vannamei and Penaeus monodon are two of the most commercially relevant penaeid shrimp species. Their phenotypic similarities especially after removing the external carapace makes them potential targets for species substitution. Based on the CPCP approach, a workflow was developed for the relative quantification of shrimp species mixtures. Corresponding peptides were identified based on aligned protein sequences followed by experimental verification using pseudo-MRM MS. Binary mixtures were analysed to verify the linear correlation of peak area ratios and species composition. Reproducibility and sensitivity of the method was investigated using raw and processed species replicates.

[1] C. von Bargen et al. J. Agric. Food Chem. 2014, 62, 39, [2] A. Watson et al., J. Agric. Food Chem. 2015, 87, 20, [3] Y. Gunning et al., Food Control 2019, 101.

Poster Nr. 29

Structural and functional relationships of plant allergenic proteins during gastrointestinal metabolism

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Food proteins frequently result in severe allergic reactions after gastrointestinal digestion and absorption by the intestinal mucosa. Little is known about global properties of plant-based food allergens and their resistance against digestion on a molecular level so far.

Human gastrointestinal digestion was simulated with a standardized in-vitro digestion model (COST Infogest). Time dependent degradation of allergens was analyzed by SDS-PAGE, while identification of the highly complex set of degradation products was performed by LC-HRMS/MS on a Q-TOF instrument in a software assisted proteomics approach. Degradation rates were estimated by label-free quantification.

Digestion of pland-based foods results in a highly complex mixture of low-molecular weight products. Based on these identified peptides a multistage data processing approach was developed in Python to provide the basis for a set of data visualizations to further interpret the behaviour of proteins during gastrointestinal digestion. Protein digestion seems to follow a multi-step mechanism in repetitive patterns regarding time dependent formation and degradation kinetics of peptides with different length. Investigation of digestion kinetics by label-free quantification verifies that some proteins are degraded into larger peptides at an early digestion state, whereas others are degraded as late as in the end of gastric phase.

Regions in the amino acid sequence of degradation products overlapping with previously identified epitopes could be determined, suggesting that these peptides can trigger allergic reactions after intestinal absorption. As a result, the N-termini of the soy allergen Gly m 6 alpha-subunit and wheat allergen and celiac disease related protein alpha-gliadin were shown to exhibit unusually high stability, cover known epitopes and are characterized by high degrees of disorder.

The development of a multistage analysis and data processing approach in Python allows to characterize plant-based food allergens during gastrointestinal digestion, providing insight into highly stable regions in context with secondary structure features.

Poster Nr. 30

Structural Assessment of the Full-Length Wild-Type Tumor Suppressor Protein p53 by Mass Spectrometry-Guided Computational Modeling

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The tetrameric tumor suppressor p53 represents a great challenge for 3D-structural analysis due to its high degree of intrinsic disorder (ca. 40%). We aim to shed light on the structural and functional roles of p53's C-terminal region in full-length, wild-type human p53 tetramer and their importance for DNA binding. To investigate the structural properties of p53, with a special focus on the understudied disordered C-terminal region, we adopted an integrative structural biology approach. Specifically, we investigate the topology of p53 in the absence and presence of DNA in solution. In contrast to the majority of structural data published for p53 where the intrinsically disordered regions (IDRs) are missing completely or where IDR-derived peptides are used as substitutes, we employ full-length, wild-type human p53. Our approach combines complementary structural MS methods, AI-based structure prediction with AlphaFold2, data-driven protein modeling, and in-depth scoring and validation of derived models. By this, we gained novel insights into the C-terminal IDR of p53 and evaluate potential structural changes thereof upon DNA binding. Our results show no major conformational differences in p53 between DNA-bound and DNA-free states, but reveal a substantial compaction of p53's C-terminal region. This supports the proposed mechanism of unspecific DNA binding to the C-terminal region of p53 prior to transcription initiation by specific DNA binding to the core domain of p53. The synergies between complementary structural MS techniques and computational modeling as pursued in our integrative approach is envisioned to serve as general strategy for studying intrinsically disordered proteins (IDPs) and intrinsically disordered regions.

Poster Nr. 31

Targeted 3D tissue sampling with a nanosecond infrared laser for nanoliter-scale spatial quantitative proteomics

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Introduction

In the last years, ultrashort pulsed infrared (IR) lasers, operating at a high peak in the absorption spectrum of water, were shown to be used for sample homogenization and subsequent mass spectrometric (MS) omics analysis. In our latest publication we utilized 3D tissue sampling with a nanosecond infrared laser (NIRL) in combination with differential quantitative proteomics, revealing spatial proteomics of intestinal epithelium as well as the layerwise proteome analysis of mouse intestine. In this study, we demonstrate targeted 3D NIRL-based sampling of tissue with sample volume in the lower nL scale for spatial proteomics with even higher resolution.

Methods

Murine tissue samples were ablated directly from the sample with a wavelength of 2940 nm by a nanosecond infrared laser (NIRL). The emerging sample aerosol was collected on a glass slide. Afterwards, bottom-up proteome analysis with liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed. The proteins were identified with a database search and statistically analysed with Perseus. Based on the quantitative data with spatial information, region specific proteins of tissue samples should be identified and further confirmed by IHC staining using the human protein atlas.

Results

We reduced surface adsorption losses by utilizing microscopic slides with small wells for aerosol collection. Furthermore, we avoided all sample transfer steps by using a one-pot sample preparation protocol for tryptic digestion. By this, we improved the number of identified proteins with high reproducibility in a reduced sample volume compared to our previous study.

We reduced the ablation volume from 700 nL down to 2 nL, determined by volume measurements with optical coherence tomography (OCT), which corresponds to 3000 and 5 laser pulses, respectively. Almost 2000 proteins across the samples could be identified with quantitative information using bottom-up proteomics. This study confirms us a more than three hundred times higher spatial resolution for our targeted 3D tissue sampling approach with NIRL-based ablation directly from the sample.

Poster Nr. 32

Top-down fragmentation of the native tetrameric 102 kDa Concanavalin A complex using ECD on a QTOF system

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Electron capture dissociation (ECD) is well known to be effective for middle- and topdown

fragmentation of proteins. The combination of ECD with native MS enables the investigation of non-covalent complexes to study differences in the behaviour upon binding of ligands or complex partners or structural changes.

Here we present data from the analysis of the tetrameric Concanavalin A (Con A) complex. Con A is a commercially available 25.5 kDa lectin from Canavalia ensiformis which forms a tetrameric complex known to bind carbohydates with a preference to internal mannosyl and glucosyl residues. It is widely used in glycoprotein analysis as well as for glycoprotein purification.

The ECD was performed using a Waters Synapt G2-S QTOF system equipped with an e-MSion Inc. ECD cell being located between the ion mobility and the transfer region of the TriWave module. Ion generation was performed using static nanospray with the Waters offline nanoESI source. The Stepwave ion guide was additionally modified with a device changing the pressure in the Stepwave region to improve desolvation and transfer of big non-covalent complexes (MS Vision).

While fragmentation of native Con A using CID gives no indication four bound metal ions, ECD fragmentation of the native tetrameric complex revealed binding of Calcium and Manganese to the fragments, allowing to localize these metal ion ligands in agreement with the known Xray structures in the N-terminal part of the Con A sequence. ECD of the native complex with glycans did so far not result in the direct observation of the glycan being bound to the fragment peptides, yet significant differences in the ECD fragmentation pattern upon glycan binding could be observed resulting in a shift of the oberved fragments which indicates some sort of shielding of the known glycan binding region from ECD-based fragmentation.

The results show that ECD in combination with native MS is a valuable tool to investigate ligand binding and can help to localize ligand binding sites in the protein structure, in particular when techniques like cryoEM fail e.g. because of mixture of free and ligand-containing protein. Yet the conditions under which bound ligands can be observed still have to be further evaluated.

Poster Nr. 33

Triclosan alternatives modulate the immune response in human macrophages with distinct modes of action as revealed by proteomics

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Triclosan (TCS) is a broad-spectrum antimicrobial agent, widely used in various personal care products, e.g., toothpaste, mouthwash or hospital soaps. Due to its versatile effects on human health, TCS has been banned from European biocide products of the product cateogory 1 in 2016 and the FDA in antiseptic wash drug products, rendering substitution products more relevant.

Macrophages, which belong to the innate immune system, form the first barrier against pathogens and harmful substances. There is growing concern about potential adverse effects of TCS and its alternatives on macrophages and recently it was shown, that TCS alters the immunometabolism in human macrophages, but effects at the molecular level are not entirely understood and knowledge on substitution products is lacking.

To gain insights into the mode of action, endotoxin-stimulated THP-1 macrophages were treated with TCS or the substitutes, including Benzalkonium chloride (BAM), Benzethonium chloride (BEC), Chloroxylenol (CEO), Chlorhexidine (CHX) and Cetylpyridinium chloride (CPC). Effects on the cytokine release were determined by ELISA, and molecular changes were revealed via untargeted LC-MS/MS-based proteomics using tandem mass tags (TMT). Effects on the pathway level were determined using Ingenuity Pathway Analysis®, and Weighted Gene Correlation Network Analysis (WGCNA) was applied to identify key drivers.

First, cytokine release was analysed, with TCS showing a high release of TNF after 16h and 24h as well as higher IL-1b release than the control, suggesting the assembly of the NLRP3-inflammasome. In contrast, the alternative CHX completely abolished cytokine release. Proteomics revealed, that TCS and CHX elicited the strongest response on the protein and pathway level compared to the other alternatives studied. TCS inhibited oxidative phosphorylation, glycolysis and TCA cycle and upregulated NRF2-mediated oxidative stress response, indicating mitochondrial dysfunction possibly due to oxidative stress. In CHX-treated cells, glycolysis, TCA cycle and oxidative phosphorylation were down regulated, with the exception of ATP synthesis. In addition, significant inhibition of signalling pathways related to translation was observed, e.g., EIF2 signalling and ribosome. Surprisingly, the network analysis revealed key drives only for the treatment with CHX, e.g. COX5B and COX6C, ITGAX and ITGAM, TF, EIF2S1, many ribosomal proteins, e.g. RPL10, but not for TCS.

Taken together, the results of this study will help to elucidate the molecular mechanisms of TCS and its novel alternatives, thereby aiding the development of strategies to minimise adverse effects.

Poster Nr. 34

Unraveling the Complexity of Short ORF-Encoded Peptides in Blautia producta: A Combined Top-Down and Bottom-Up Proteomics Approach

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The human gut microbiome (HGM) is a complex and dynamic community that plays a crucial role in human health and disease. While short open reading frame (sORF)-encoding peptides (SEPs) have been identified in the HGM, their biological function remains elusive. To shed light on the role of SEPs in the HGM, we conducted a study utilizing bottom-up and top-down proteomics, aiming to identify SEPs in Blautia producta, a member of the HGM, under various growth conditions and stress exposure.

We identified 45 SEPs, including those previously reported only to be produced in the microbiome community, indicating that their production is not exclusively dependent on interactions or communication within the microbiome. Our findings suggest that specific SEPs are influenced by growth conditions or pH-stress, highlighting their potential role as stress response proteins in B. producta. Top-down proteomics provided comprehensive insights into different proteoforms of the SEPs, including N- and C-terminal truncations and other co- and post-translational modifications. Our investigation revealed truncated proteoforms that contained methionine as the initial amino acid residue, indicating the possibility of alternative initiation as a common feature in B. producta.

In summary, these findings emphasize that SEPs represent an understudied subgroup of non-annotated polypeptides within the HGM that warrant further investigation. Further, our study demonstrates the benefits of top-down proteomics for the identification of proteoforms, but also the value to perform bottom-up experiments in parallel.

Poster Nr. 35

Validation of immunoaffinity-based mass spectrometry approaches for the detection of ruminant-specific peptides in animal feed

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To prevent transmission of bovine spongiform encephalopathy, strict feeding restrictions for animal by-products were enacted by the European Commission (Regulation (EG) 999/2001). Increasing reauthorizations imply analytical challenges for official control as established PCR- and microscopy-based systems are insufficient to discriminate authorized (e.g. milk) and from non-authorized materials (e.g. meat-and-bone meals). We thus previously developed two analytical methods for the detection of illegal protein additives using antibody-based immunoaffinity enrichment coupled with mass spectrometry for the detection of ruminant-specific proteins by surrogate peptides in animal feed.

One method detects ruminant hemoglobin using commercially available antibodies and materials. Hemoglobin is enriched at the protein level using magnetic beads and a manual magnetic separator. Tryptic peptides are analyzed by LC-MS/MS.

The second method comprises a sophisticated two-tier multiplex assay for the detection of tissue-specific ruminant proteins. Meat and cartilage are determined using myosin-7 and matrilin-1. Complement factor 9 and α-2-macroglobulin are used for the quantification of plasma proteins. Blood and meat meal incorporation to feed can furthermore be quantified using hemoglobin subunit α. Bone meals can be distinguished from milk based on the relative amount of osteopontin in relation to the aforementioned proteins.

Both methods rely on tryptic digestion of the proteins, magnetic bead-based immunoaffinity enrichment of protein surrogate peptides and isotope-labelled peptide analogues (or the protein itself), and analysis by nano-liquid-chromatography coupled to high-resolution mass spectrometry (parallel reaction monitoring). Ruminant- and tissue-specific peptides are quantified using internal standards and an external calibration curve.

For method validation, five blood products and nine meat-and-bone meals of ruminant origin were mixed into vegetal feed matrices. Accuracy, linearity, parallelism, precision, recovery and sensitivity of the method were determined and analyte stability was determined for multiple storage conditions. Twelve different animal feeds served as feed matrices to demonstrate that a wide variety of feeds neither produce false positive signals nor interfere with the detection of the surrogate peptides. In order to exclude suppression of the analytes by other proteins present in the feed matrix, interference of the analytical method with milk powder additives up to 60 % (w/w) was assessed. Our validation results for the analytical methods demonstrate that adulterants with ruminant protein of 0.1% (w/w) in the feed matrix can be detected qualitatively and 1% (w/w) quantitatively in laboratory routine operation.

Poster Nr. 36

4D-TIMS quantitative tissue lipidomics for lipid therapy response monitoring

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Lipids play essential roles in many physiological processes and pathological conditions, and emerge as important candidates for therapeutic agents, biomarker discovery, treatment response, and follow-up monitoring in clinical research. However, continuous efforts are required to expedite clinical research and profiling applicability. 4D PASEF lipidomics enables analysis of the lipidome using 4 different dimensions, i.e., retention time, CCS, m/z, and MS/MS spectra, at high speed and mass accuracy and thus emerges as a valuable tool to increase lipidome coverage owing to excellent sensitivity and molecular resolution.

Here, we investigate lipidome changes in heart and brain tissues, as well as in plasma, in response to lipid-modifying therapies. For this, we first developed a platform based on 4D PASEF-MS for reproducible tissue lipidomics profiling and quantification, encompassing parallelized tissue extraction, confident lipid annotation, and reproducible quantification. Efficient chromatographic separation and IMS-MS separation and detection within 20 minutes analysis time were obtained using UHPLC coupled to tims-TOF-MS in PASEF acquisition mode. Using in-house generated tissue-source specific 4D libraries, the annotation confidence significantly increased due to controlled and consistent spectral purity, matching, matrix effects, and background in the PASEF data. This method was applied to investigate the lipidome changes in brain and heart tissues of a mouse model of epilepsy in response to sub-chronically pre-treatment with PEA.

By applying the developed high throughput 4D-PASEF-based quantification workflow we were able to identify and quantify more than 300 lipid species in heart and brain tissues obtained from a mouse model of epilepsy in response to peripheral PEA administration. 4D-PASEF lipidomics using the timsTOFpro MS (Bruker Daltonics, Germany) allows deep coverage of the tissue lipidome including cis/trans, SN1/SN2 isomer identification, and isomers originating from distinct fatty acyl chain composition, thus a comprehensive lipidomic tissue pheno-mapping. This coverage is shown here as instrumental to assess and elucidate the response of the lipidome to therapies relevant to neurological diseases. Preliminary data reveal strong metabolomic changes in the heart lipidome due to PEA administration depending on the degree of unsaturation of the fatty acid chains.

We here demonstrate the suitability of this method to unravel the impact of subchronic PEA treatment on seizure-induced changes of numerous structurally heterogeneous lipids extracted from minute amounts of heart and hippocampus tissue, respectively, and reveal new aspects of lipid metabolism in heart and brain following therapies in animal models.

Poster Nr. 37

Analysis of tuberculostearic acid-containing phosphatidylinositols in sputum from patients during antibiotic therapy for infection with mycobacterium tuberculosis

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis, one of the most prevalent and most fatal infectious diseases worldwide. Due to the increasing drug resistance, treatment of tuberculosis is continuing to become more difficult. On this basis, analysis of specific molecules, that indicate infection status and therapy response are highly necessary to individualize the patients' treatment. Mycobacteria are equipped with a complex cell envelope, containing high amounts of carbohydrates and lipids. The latter include unique structural features that are not present in eukaryotic lipidomes, such as species containing the fatty acid 19:0 (tuberculostearic-acid (TSA)). Previously, it was shown that phosphatidylinositol (PI), of which quantification is well established in lipidomics, is the most abundant phospholipid class in various Mtb-lineages. More specifically, PI 35:0 (16:0_19:0) is the major phosphoglycerolipid in the cell envelope complex (1).

Here, we investigate whether TSA-containing PI are detectable in sputum samples derived from patients infected with tuberculosis, using shotgun lipidomics performed by nanoESI-MS/MS (Q Exactive™ Plus Hybrid Quadrupol-Orbitrap™). Lipid identification and quantification is carried out with the software LipidXplorer (2). The patients all started standard antibiotic treatment (isoniazid, rifampicin, ethambutol, pyrazinamide), as their infection was caused by drug-responsive Mtb. Sputum was sampled before therapy, after two weeks and after two months, so the course of the PI is depicted. Quantitative analysis of the antibiotics is carried out by LC-MS/MS (Xevo-TQ-MS) in the sputum and compared to the measurements from plasma that was sampled at the same time points as the sputum. At the side of sampling smear microscopy was carried out with the sputum samples, so correlation between the targeted PI and microscopic results is evaluated.

TSA-containing PI could be detected in the sputum samples. The concentration of PI 16:0_19:0 decreased during the two months of antibiotic treatment. In the sputum samples in which smear microscopy did not reveal any acid fast bacilli, like mtb, concentration of PI 16:0_19:0 was lower than in the samples with positive microscopy results.

The results of these analyses provide further data for the discussion about the possible application of TSA-containing PI in clinical settings.

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Poster Nr. 38

Charge inversion of phosphate-containing lipids by dimetal complex formation for desorption electrospray ionization mass spectrometry imaging

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Introduction

The research field of lipidomics includes structural elucidation of lipid species as well as investigations regarding their interactions with cells and other metabolites. Phospholipids represent one of the most abundant subclasses of lipids in plants and animals. Some phospholipids, such as phosphatidic acids (PAs) and phosphatidiylinositols (PIs), play a vital role in lipid biosynthesis or signal transduction processes. Challenges regarding the research of these lipids include low ionization efficiencies in positive-ion mode due to their acidic phosphate head group and low abundances in cells. One approach to overcome these challenges is to employ charge inversion chemistry, for example using biomimeting dimetal complexes that selectively bind to phosphate groups. Moreover, applying desorption -electrospray -ionization (DESI), extensive sample preparation can be avoided and spatial lipid distributions in tissue can be elucidated using mass-spectrometry imaging (MSI).

Experimental methods

Lipid standards (Avanti Polar Lipids, Inc.) were prepared with a final concentration of 1 mM in methanol or chloroform (Merck, LiChrosolv HPLC grade). Mouse kidney tissue samples were sectioned (20 µm thickness) using a cryotome (Thermo Fisher Scientific GmbH, Dreieich, Germany). Dimetal complexes were dissolved in a mixture of methanol/water (95:5; v:v) or acetone/water (1:1; v:v). Spray coating was done with a home-built nebulizing sprayer. All DESI-MS(I) experiments were performed with a home-built DESI stage coupled to a LTQ FT Ultra or Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany). Images were created using the Mirion software package and lipid assignment was done using the LIPIDMAPS database.

Preliminary results

Metalloenzyme-derived dimetal complexes [M2(bpbp)(R)]n+ (M = Ga3+, Zn2+, In3+) were synthesized from their metalperchlorate salts and triflate salts, respectively, with the heptadentate ligand 2,6-bis(bis(2-pyridylmethyl)aminomethyl)-4-methyl-phenol (Hbpbp). Reaction of the complexes with different lipid standards of phosphatidic acids in solution and after spray coating on glass slides show characteristic complex - lipid adduct signals in positive-ion mode within one hour of reaction time. Different adducts were identified depending on lipid species, the dimetal complex and the counterion of the complex. Complex - lipid adducts were characterized by MS/MS experiments. Spray-coated lipid standards and tissue samples showed the same complex - lipid adducts as in solution, enabling the application of the method in MSI experiments. A spatial resolution of 100 µm was established for imaging experiments.

Novel aspect

Combining the use of DESI-MS(I) with charge-inversion capabilities of biomimeting dimetal complexes for enhanced ionization efficiencies in positive-ion mode and spatially resolved lipid analysis.

Poster Nr. 39

Deep lipidomic profiling reveals sex dimorphism of lipid metabolism in calcific aortic valve disease

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Calcific aortic valve disease is the most common valvular heart disease in the aging population. Valve replacement or implantation remain the only treatment options, as a pharmacological therapy does not exist. CAVD is characterized by chronic inflammation and lipid accumulation, and is driven by differentiation of valve cell subpopulations, promoting tissue fibrosis and consecutive calcification. However, the underlying biochemical mechanisms are not well understood. In particular, remodelling processes of the aortic valve lipidome have never been studied at the molecular level, even though lipid dysmetabolism was proposed to play a significant role in disease progression.

Poster Nr. 40

Development of a LC 4D-PASEF-MS method for analysis of acidic glycosphingolipids in biological matrices.

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Glycosphingolipids (GSLs) are essential components of the cellular membrane. Their functions include stability maintenance of the cell membrane, promotion and regulators of cell-cell interaction, as well as serving as recognition motifs for viruses and pathogens to enter the cell. Hence, it is crucial to perform qualitative and quantitative measurements of GSLs. Given the high level of structural isomers, the deep structural characterization and isomer separation is a prerequisite to individual functional isomer identification and quantification. Current understanding of GSLs is far from complete because analysis of GSLs faces many analytical challenges. They have high isomerism, structural diversity and exhibit a dual nature: hydrophilic and hydrophobic, thus requiring class and/or sub-class tailored extraction and analytical approaches. Elucidating the structure-function relationship of GSLs in many biological processes and diseases has been traditionally achieved by thin layer chromatography with immunostaining. However, this method is targeted with no large multiplexing capabilities and resolution, and has low throughput. The majority of the achievements that have been made in GSL analysis have been accelerated by mass spectrometry (MS), which has a higher throughput and sensitivity than conventional methods1. Unfortunately, the untargeted MS-lipidomics currently in use lack the sensitivity needed to either detect low abundant GSLs or to elucidate the delicate structural characteristics in a total lipidome extract, which could be used to help the discovery of the precise biological activities of GSLs. It is impossible to comprehensively determine the relationship and interaction between structure and biological function of the membrane and/or signaling lipids because GSLs and other lipid classes are typically studied in isolation from one another.

Consequently, it is essential to develop a method that can analyze the membrane lipidome in its entirety. Therefore, we devised and present here a strategy to: i) translate typical untargeted LC-MS lipidomics protocols to include GSL analysis; ii) determine the separation performance of GSL using LC-MS coupled with TIMS and infer criteria for TIMS based conformation separation; iii) develop a PASEF-MS method for structural elucidation of GSLs and optimize MS/MS spectra for database addition; iv) generate subclass and class specific spectral library and analyte list containing collision cross section (CCS), retention time (RT), precursor m/z, ion type and fragmentation spectra for 4D-PASEF MS identification of GSLs; and v) profile GSLs in customized mixture and biological sample using the database library.

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Poster Nr. 41

Dual-omics cellular mapping: Development of a dual-omic approach in low cell number profiling towards molecular pathways determination

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Recent advances in cell isolation and mass spectrometric technologies have allowed single-cell multiple omics investigation for exhaustive unraveling of cellular function and/or profiling of different cellular functional stages. However, most of the high-cell resolution omics studies are currently restricted to dual RNA and protein analysis from the same cell population. Inclusion of lipid profiling from limited number of cells is, however, imperative to determine the complete molecular mechanisms and pathways underlying cell function as well as the lipid metabolic change of cells in their different functional stages.

In this context, a cell phenotyping approach using a novel dual-omic analysis including, transcriptomics and lipidomics from low number of cells was developed to advance single-cell resolution. For this purpose, multi-extraction of lipids and RNA was developed to allow comprehensive dual-omic profiling from the lowest possible number of cells following the procedure by1,2. Untargeted 4-dimensional lipidomics for cellular phenotyping was developed using 4D-TIMS; for low abundant lipids of interest LC/MRM was used, while the transcriptomic study was achieved by RNAseq and/or qPCR. Reference cells models were used to assess the lowest number of cells from which such multi-omics can be performed.

The dual-omics cellular data were processed with software-based tools for pathway analysis to determine the molecular pathways underlying a specific cell phenotype.

This higher cellular resolution dual-omics will prospectively enable early-stage identification of cellular phenotype changes in pathological and physiological conditions with subsequent determination of the molecular pathways underlying disease mechanisms but also of molecular targets for therapeutic intervention.

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Poster Nr. 42

Elucidation of Cardiolipin Alterations by HPLC-MS/MS

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Phospholipids are characterised in subclasses, according to their polar head group. The subclass of cardiolipins (CL), exclusively located in the inner mitochondrial membrane, is thereby of increasing interest. Current research results indicate a connection between widespread disease patterns, such as cardiovascular diseases, diabetes or neurodegenerative diseases, and the functionality of mitochondria as power plants of the cell. A particular focus is thereby set on alterations in the natural CL distribution, potentially leading to mitochondrial dysfunction. High contents of polyunsaturated, long-chain fatty acyl residues make these lipids sensitive to oxidative stress. The elucidation of the natural occurring CL distribution is therefore essential to the understanding of pathogenesis on a molecular level.

The analysis of CL species comes with challenges in terms of low concentrations and high structural diversity, demanding a powerful analytical setup. Hyphenating reversed phase liquid chromatography (RP-HPLC) to electrospray ionization high resolution mass spectrometry (ESI-HRMS), the CL distribution in total lipid extracts of isolated mitochondria from HeLa cells was ascertained. Furthermore, a special focus was set on the identification of modified CL species such as monolysocardiolipins (MLCL) and oxidation products, originating from oxidative stress in mitochondria induced by raised levels of reactive oxygen species (ROS).

Sufficient chromatographic separation of homologues CL species based on the fatty acyl residues was achieved while HRMS detection allowed identification of CL species by their accurate masses, confirmed by additionally performed data-dependent MS/MS experiments. Based on characteristic fragments, bound fatty acyl residues were identified for most CL species. For visualization of the data set acquired, Kendrick mass plots were utilized, normalized on the CH2 Kendrick mass defect (KMD). Taking into account the above mentioned detection and data processing a reliable identification of CL species according to carbon chain length and degree of saturation in the fatty acyl residues despite complex sample matrices is achieved.

Poster Nr. 43

Highly sensitive and rapid quantification of Dolichyl phosphates using reverse phase liquid chromatography-mass spectrometry after TMSD methylation

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Dolichyl monophosphates (DolPs) are important regulatory lipids in highly conserved glycosylation pathways across almost all major domains of life. DolP analysis by LC-MS has remained challenging due to their very low abundance, and a wide span of high lipophilicity. In the present work, we describe a novel method for the determination of intracellular levels of DolPs based on derivatization with Trimethylsilyldiazomethane (TMSD) prior to RPLC-MS analysis. Derivatized DolPs were found to have enhanced ionization efficiency and detection sensitivity. Almost complete derivatization of individual DolP species was accomplished in 60 min. The RPLC-MS method was optimized and validated for the simultaneous determination of various DolP species in a single 40-minute run. The highly sensitive method enabled quantification of the least abundant DolP species in a very low sample size of ~1×106 cell number. The strategy was applied to selectively detect and quantify 9 key DolPs from cellular lipid extracts of Hela, fibroblast, and Saccharomyces cerevisiae cells using full scan MS analysis. Moreover, results demonstrated that the applicability of the fast and sensitive determination of intracellular DolP levels could expedite further research in Glycosylation studies.

Poster Nr. 44

Hyphenation of supercritical fluid chromatography with high-resolution MS and MS/MS for the identification and structural elucidation of polar cereal lipids

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The focus of this work was the identification of polar glyco- and phospholipids of different cereals (oat, barley, rye, emmer, spelt, einkorn, wheat). In prior publications, lipid profile comparisons of different cereals were limited because only certain cereal varieties and marker lipids were of interest or because of the techniques used [1, 2]. For this reason, the polar lipid profile was comprehensively analyzed, including less studied lipid classes, such as N-acyl-phosphatidylethanolamines (NAPE), N-acyl-lysophosphatidylethanolamines (NALPE) and acyl-monogalactosyldiacylglycerols (acyl-MGDG). These lipid classes are of particular interest due to their structure, as they are all additionally acylated in their polar head group, and still very little is known about their biological function.

Supercritical fluid chromatography (SFC) combined with high-resolution mass spectrometry (HRMS) has proven to be a useful tool for various applications in the past, such as lipidomic studies. [3] In our study, SFC-HRMS is used for the analysis of cereal lipids for the first time. The SFC offers a high sample throughput due to short chromatographic run times and is therefore well suited for the screening of the large number of investigated grain samples. Online coupling with high-resolution electrospray-MS and data-dependent MS/MS experiments in positive and negative ionization modes were used to determine lipid species of different lipid classes for the study of lipid profiles in different cereals. MS/MS fragmentation experiments in the negative ionization mode allowed the identification of lipid species based on the length and degree of saturation of bound fatty acid residues, whereas MS/MS in the positive ionization mode provided complementary information. In particular, acylated galactose head groups in acyl-MGDG and N-acylated head groups of NAPE could be identified by fragmentation experiments in the positive ionization mode. Moreover, the two lipid classes NALPE and PE can be distinguished from each other based on their different fragmentation behavior in the positive ionization mode. Therefore, the complex lipid profile consisting of various lipid species belonging to all major polar lipid classes could be unambiguous identified by the use of SFC-MS and SFC-MS/MS in the investigated seven plant species.

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Poster Nr. 45

Identifying epilepsy lipid markers in mouse models by multiplex LC/MRM and high end 3- and 4 Dimensional-trapped ion mobility mass spectrometry based assays

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Over the past decade electrospray ionization (ESI)/ liquid chromatography/multiple reaction monitoring (ESI/LC/MRM) mass spectrometry (MS) were so tremendously improved regarding sensitivity and precision that serve today as the golden standard analytical tool for clinical applications, such as the characterization of disease protein-, peptide, glycan, metabolite, or lipid-markers.

More recently, the dimension of ion mobility (IM) was coupled to high-resolution mass spectrometry (HR-MS) and has been investigated as an alternative method for lipid analysis. The significant advantage of IM-HR-MS is its ability to separate isobaric and isomeric analytes by ion neutral collision cross sections. LC-IM-HR-MS generated 4D-lipidomics render lipid and in general molecular identification based on the following dimensions: 1) mass/charge 2) chromatographic retention time 3) ion mobility, and 4) fragmentation pattern. Moreover, MALDI coupled to IM-HR-MS allows improved sensitivity and molecular resolution of small and large molecules compared to the traditional MALDI approaches, MALDI-IM-HR-MS generates dispersions of analyte signals in the mobility space, which allows the detection of low molecular weight metabolites ($m/z < 1500$). Owing to high tolerance to speed of analysis, salts, minimum amount of sample volume and good ionizability of lipids from crystalized matrices, MALDI IMS has enabled high spatial resolution lipidomics in tissue slices and cells.

We here valorized the added dimension of ion mobility to the MALDI-IM-HRMS to develop a very (3-5 seconds per sample). 3D Lipidomics platform for cellular and biological fluid analysis. For faster identification and annotation of lipid profiles we establish a 3D-lipid database for perspective lipid marker discovery, as well as ISTDs-based quantitative lipidomic strategy applicable to minute amounts of tissue or biological fluid volumes.

Poster Nr. 46

Improved selectivity and sensitivity in LC-MS/MS-based sphingolipidomics by two-dimensional multiple heart-cut liquid chromatography

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Sphingolipids gained increasing attention concerning their involvement in bioactive cell signalling and their association with neurodegenerative and cardiovascular diseases. In this contribution, we present a two-dimensional multiple heart-cut HPLC method with subsequent high resolution-mass spectrometric detection for the analysis of sphingolipids. To demonstrate the applicability, a complex sample of the nematode Caenorhabditis elegans (C. elegans) was investigated. The latter is an established model organism in biological studies or studies concerning neurodegenerative diseases and drug discovery.

Alkaline depletion is a commonly employed sample preparation step in sphingolipid analysis to deplete more abundant and interfering glycerophospholipids. We avoided this labor-intensive measure by 2D-HPLC, i.e. separating the sphingolipids in the first dimension from the more abundant glycerophospholipids (like phosphatidylcholines) via hydrophilic interaction liquid chromatography (HILIC). The fractions were cut out, stored in a sample loop and transferred onto the second dimension reversed phase liquid chromatography (RP-LC). In addition, high resolution MS and MS/MS experiments were conducted to further elucidate the chemical structure of the sphingolipids.

The 2D-separation, in contrast to the 1D-LC, allowed for a confident identification of lower abundant species, e.g. dihydro-sphingolipids (DhSLs) due to the chromatographic separation from the glycerophospholipids and reduced matrix effects. A total of 45 sphingolipids (ceramides, hexosylceramides and sphingomyelins) were detected in the lipid extract of the model organism C. elegans.

Poster Nr. 47

Lipid Composition of Human Lymphoma Cells Revealed by LC-MS Utilizing Parallel Accumulation Serial Fragmentation (PASEF) Acquisition

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Lymphoma as one of the most common cancer types today is still challenging to treat and even classify caused by its molecular diversity. A connection between lipid peroxidation and ferroptosis, an iron mediated cell death, is well documented and even considered a hallmark of ferroptosis. [1] Understanding the underlying mechanisms of lipid peroxidation and thereby ferroptosis is mandatory to help treating lymphoma. Double bonds of unsaturated fatty acids are most susceptible to lipid peroxidation. Fatty acids are primarily contained in more complex lipids like phospholipids (PL) and can be classified into mono-/ poly-/-unsaturated (MUFAS/PUFAS) and saturated fatty acids defining their oxidative susceptibility. In human cells PL are the most abundant lipid class, each subclass found to be responsible for different biological functions. This is why PL species with their respective fatty acid chains are of particular interest in the context of lipid peroxidation and hence ferroptosis. Due to the highly complex composition of PL species in human lymphoma cell lines, powerful hyphenated mass spectrometric techniques are necessary. For lipid extraction, an established protocol by MATYASH et al. was conducted whilst an internal standard was used to monitor extraction efficiencies. A newly developed reversed phase (RP) liquid chromatographic (LC) method was used to separate PL species. Prior to mass spectrometric measurements with a Quadrupole-Time of Flight MS (Q-TOF-MS), a trapped ion mobility spectrometer (TIMS) was utilized to gain further information and improve separation power. Additional information generated by TIMS allows another dimension to be considered for signal identification resulting in an overall higher confidence. The PASEF (Parallel Accumulation Serial Fragmentation) mode of the timsTOF instrument was used to gain diagnostic MS/MS data along with the respective ion mobility. Hereby generated data was automatically processed and annotated by MetaboScape software using a 4D approach considering ion mobilities, MS/MS spectra, exact mass to charge ratios (m/z) and retention times. The data was revised manually utilizing a 4D Kendrick Mass Plot for each PL class and respective scores calculated by MetaboScape software.

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Poster Nr. 48

Lipid-class specific internal standard normalization of HILIC-MS/MS data embedded into untargeted data processing and interactive exploration

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The complexity of the lipidome calls for appropriate data normalization procedures for LC-MS-based lipidomics workflows. Data normalization must address differences in experimental conditions, e.g., sample preparation, instrument-specific variations, and differences in analytical targetability between individual lipid classes. In the rapidly growing field of lipdomics, incorporation of streamlined data analysis workflows into commercial software packages is key to improving data comparability cross experiments, instrumental platforms, and laboratories.

In this contribution, we present a new software workflow that allows for accurate and precise normalization of lipid class concentrations using stable isotope labeled internal standards (SIL-IS) representative for each lipid class.

The workflow, which is fully integrated into the MetaboScape software platform, is based on lipid class separation by hydrophilic interaction liquid chromatography (HILIC). In HILIC, each SIL-IS coelutes with the corresponding phospholipid class, thus enabling class-specific normalization of LC-MS/MS data. Post-ionization trapped ion mobility spectrometry (TIMS) separation is orthogonal to HILIC and facilitates separation of co-eluting isobars based on gas phase mobility in an electric field. Hyphenation to high-resolution mass spectrometry, and fragmentation experiments by parallel accumulation - serial fragmentation (PASEF) allowed unambiguous assignment of lipids to classes and species based on retention time, mobility, accurate mass, and fragmentation behavior.

Prior to untargeted feature finding, data for the configured SIL-ISs are extracted using a target list. SIL-ISs may be assigned to a lipid category, lipid class or lipid subclass, according to the LIPID MAPS hierarchy [1]. After preprocessing, rule-based lipid annotation is applied, which also assigns annotated features into the appropriate class and level of the hierarchy. Class-specific intensity normalization is then performed on all features covered by a SIL-IS according to their classification.

The workflow allows for further data processing and exploration, for example with basic statistics or Kendrick mass defect plots.

We applied this workflow to NIST (SRM 1950) plasma sample. The SIL-IS normalization could eliminate systematic errors in lipid concentrations so that the relative standard deviation among the covered lipids could be improved from 11.2 % to 6.6 %. As a result, more accurate values are obtained for comparing lipid distribution and abundance in different samples.

[1] https://www.lipidmaps.org/shorthand_nomenclature/rules/hierarchy

Poster Nr. 49

Lipidomics workflow to analyze regulated lipid metabolic pathways in response to 3-week treadmill training

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Efficient energy metabolism during physical activity depends on the breakdown of glucose and lipids from muscle and peripheral tissue. To study lipid pathways activated or silenced in highly performing muscle, a marathon mouse model paternally selected for high treadmill performance (specially selected endurance mouse line: Dummerstorf marathon mouse) and an unselected control line were compared under sedentary conditions or after three weeks of treadmill training (both males). At the age of 70 days, total muscular RNA was isolated and sequenced. The differential expressed genes between the mouse lines and treatment groups were determined before performing KEGG pathway analysis.

In parallel, serum lipids were measured by a metabolomics approach using an ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) system (Vanquish UHPLC-System with heated electrospray ionization (HESI) QExactive plus Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA)) in the positive and negative ionization mode. MS data were acquired over a scan range of 100–1200 m/z with a full MS resolution of 70,000 and a data-dependent MS2 resolution of 17,500. Chromatographic separation of the lipids was performed on a reversed-phase column (Accucore Polar Premium 100 × 2.1 mm (2.6 µ m) with guard column: Accucore Polar Premium 10 × 2.1 mm (2.6 µ m); Thermo Scientific). Compound Discoverer 3.1 and Lipid Search Software (Thermo Scientific) conducted annotation and relative quantification of individual lipid metabolic species.

The most differentially-expressed genes (DEGs) were found in the comparison group trained marathon vs. trained control mice, of which again most could be assigned to metabolic KEGG pathways. Transcripts involved in fatty acid degradation were increased in marathon compared to control mice, independent of training. Sedentary marathon mice exhibited downregulated triacylglycerols (TAGs) and upregulated anti-inflammatory lipid mediators compared to sedentary controls and increased serum levels of Cholesterylester as storage lipids & oxylipin when compared to trained littermates. In the control line, instead, training did not lead to changes in lipid degradation and serum metabolites. Comparison between trained mouse lines revealed downregulated mediators of inflammation in marathon mice.

In summary, marathon mice displayed increased muscle lipid degradation accompanied by downregulated inflammatory response during training than control mice.

Poster Nr. 50

Lipotypes of the honeybee Apis mellifera: from in-hive duties to risky tasks

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Honeybees that belong to the same colony display a near-homogeneous genomic background together with a high level of behavioral and physiological plasticity and, thus, are an ideal model for functional lipidomics studies. Due to their nutritional specialization, the only external source of lipids is pollen. However, the diet of bee queens differs from that of workers. Queens are fed glandular secretions provided by the workers. Theses secrestions mainly contain saturated and monounsaturated fatty acids (FA). The comparison of the FA composition of queen and worker bee tissues revealed that FA of queens are mainly monounsaturated, whereas unsaturation increases in workers with pollen consumption. The high amount of saturated FA could in part explain the extended longevity of honeybee queens, in accordance with the “oxidative stress” or “free radical”-theory of ageing.

In the worker caste, there are different adaptations to physiological specializations, which follow a loose chronological sequence (“chronological polyethism”). Newly-emerged worker bees usually start as cell-cleaners, before feeding brood. Leaving the hive for foraging is typically the last step of temporal polyethism. At the transition from in-hive duties to the risky task of foraging, worker bees strongly decrease in weight and storage proteins, whereas the masses of their flight muscles increase. There are also subgroups of similar age but differing specializations, such as cell heaters using the flight muscles to produce heat and non-heaters. The flight muscle of honeybees has one of the highest maximum rates of energy turnover of any animal tissue studied so far. Therefore, cell heaters offer an interesting model for functional lipidomic research, because they can be expected to have a high energy turnover and can be compared to same-aged, non-heating individuals. Thus, the present study aimed to elucidate the lipid composition of flight muscles from differentially specialized bees. Our results show that differences in the lipid class of cardiolipins reflect the difference in energy turnover between heating and non-heating hive bees. A near-comprehensive set of A. mellifera lipotypes is presented.

Poster Nr. 51

Method development for the analysis of short chain fatty acids by direct infusion mass spectrometry

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Objectives: Short chain fatty acids (SCFAs) are metabolites produced by the fermentation of complex carbohydrates by the gut microbiota. They play a pivotal role in people with multiple sclerosis (pwMS) and other neuroimmunologic diseases. Recent studies have shown that the serum and feces of pwMS contain significantly reduced amounts of propionate compared to controls. In this context, quantitative analysis of the SCFAs has the potential to improve our understanding of the mechanisms of the disease development and to aid in targeted studies on SCFAs supplementation. In this work, we present a simple, rapid, semi-automated, direct infusion mass spectrometry (DI-MS) approach for the quantitative analysis of SCFAs.

Methods: A new workflow has been developed for the analysis of SCFAs (acetate, propionate and butyrate) in serum, plasma and stool. It combines a specific protocol for extraction and preparation for MS analysis and a targeted DI-MS method for SCFAs. The latter includes determination of the lower limits of detection and linearity range for the three SCFAs. The developed method was further used for quantitative analysis of propionate in serum of healthy subjects before and after its oral supplementation.

Results: The semi-automated DI-MS approach substantially simplifies the SCFA quantification and allows a reduction in MS analysis time to be reduced to 2 minutes. The linear detection range of the DI-MS method is from 1 to 100 µ g/mL for acetate (R2=0.973), from 0.01 to 10 µ g/mL for propionate (R2=0.953), and 0.05 to 50 µ g/mL for butyrate (R2=0.993). Subsequent measurements of SCFAs in blood plasma, serum, and stool samples showed that the measured levels were within the expected range.

Conclusions: Our newly developed mass spectrometry-based workflow provides a significantly improved method for quantifying SCFAs. Due to its simplicity and shorter analysis time, it is particularly advantageous for use in clinical studies with many samples.

Poster Nr. 52

Parallel reaction monitoring-PASEF-based targeted acquisition strategy for lipid biomarker monitoring in metabolic pathways

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Multi-dimensional mass spectrometry methods have recently been adapted to enhance the structural annotation and profiling of lipidome from biological samples. Although several selected reaction monitoring strategies have been used for clinical profiling of known targets [1], novel targeted acquisition strategies such as parallel reaction monitoring (prm) possible on high-resolution mass spectrometers allow high acquisition speed sensitivity and higher selectivity [2] owing to superior m/z accuracy of the precursor/fragment ions.

The work here demonstrates the advantages and performance of a prm-PASEF-based targeted acquisition strategy for lipid identification and quantification in NIST Human plasma SRM. PASEF acquisition mode allows for higher sensitivity than untargeted profiling, especially benefiting the detection of low abundant molecules in biological samples due to the 100% duty cycle offered by relatively novel HRMS such as timsTOF.

For prm-PASEF data processing, e.g., quantification, open-source, vendor-neutral software Skyline was used for the peak extraction from the four-dimensional 4D- (trapped ion mobility mass spectrometry) TIMS lipidomic data. The extracted peak areas were used to quantify using an external calibration curve multiple selected lipid targets belonging to specific lipid pathways. The quantification was cross-validated by multiple reaction monitoring (MRM) and data-dependent acquisition (DDA) [1] TIMS strategies.

The value of such an analytical strategy was demonstrated by its use for targeted acquisition and quantification of potential lipidomic biomarkers [3] for several physiological and metabolic pathways [4,5].

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Poster Nr. 53

Tandem mass spectrometry of doubly-charged lipid-metal complexes

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Lipids are essential constituents of cell membranes and play important roles in signaling pathways. Changes in the lipidome are associated with a range of medical conditions such as diabetes, cancer and cardiovascular diseases. The diversity of lipid structures complicate full structural analysis of lipids, i.e. lipid class, C=C position and geometry, and sn-isomerism. Mass spectrometry based techniques are on the forefront in the field of lipidomics. Here, we present novel tandem-MS methods for the analysis of several lipid classes, focusing on glycerophospholipids, as doubly-charged lipid-metal ion complexes with the goal to confidently assign lipid structures on the molecular level.

All lipids were analyzed as doubly-charged lipid-metal ion complexes in electrospray ionization (ESI) experiments. Spray solutions consisted of synthetic lipid standards or an E. Coli PE extract (Avanti Polar Lipids, Alabaster, Alabama, USA) with 100 mol% metal salts added in water free methanol. Measurements were performed on high-resolution orbital trapping mass spectrometers (Q Exactive HF-X, Orbitrap Elite, Thermo Fisher Scientific, Bremen, Germany). Data analysis was performed manually using Xcalibur QualBrowser and Origin.

Optimization of the spray solution composition allowed investigation of previously not detected doubly-charged lipid-metal ion complexes [L+M]2+. Metal screening experiments were performed to determine the influence of the metal-ion identity on complex formation efficiency and fragmentation behavior. Comparing tandem mass spectra of [L+M]2+ with M2+ being alkaline earth or transition metals, showed significant differences. Tandem spectra of [POPC+Co]2+, [POPE+Co]2+, [POPS+Co]2+, and [POPG+Co]2+ contain fragment ions corresponding to the cobalt(II) adduct of oleic acid. Tandem MS of POPG and POPS show additional fragment ions which can be attributed to loss of FAs as [FA-OH]+ as well as headgroup loss. Furthermore, we detected previously unreported fragment ions attributed to fragmentation of the FA alkyl chain for HCD-MS^2 of [PE+M]2+. This fragmentation occurs only if at least one unsaturated FA is present. Further experiments with deuterated lipid standards indicate that the fragmentation occurs exclusively at the unsaturated fatty acids and involves radical processes. As [FA+Co]+ complexes are generated for all investigated lipid classes, their analytical value was further explored in MS^3 experiments. Resulting CID/HCD-MS^3 of the fatty acid metal complexes allowed the localization of branching, cyclopropane modifications as well as indications on C=C positions based on characteristic fragment ions and mass differences. Subsequently, the method was employed to characterize structural features of PEs in a commercially available E. Coli extract.

Poster Nr. 54

HPLC-MS Based Quantification of Short Chain Fatty Acids in Mouse Feces – Study of a Sepsis Mouse Model

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The clinical picture of sepsis is a common cause of death in intensive care units, although 30% of the intensive care unit budget is used to take care of sepsis patients in hospitals.[1] This indicates the relevance of studying the course of sepsis in more detail to find possible treatment methods. Fecal microbiota transplantation (FMT) presents an already approved therapeutic method for curing gut-derived sepsis in mice models. Due to FMT, non-pathogenic microbes can be reintroduced into the infected gut by bringing in healthy donor feces.[2] In this context, it is already known that short chain fatty acid (SCFA) concentrations decrease due to sepsis and therefore their increase could be key of the curative effect. Furthermore, it is suspected that transplanting stool from septic mice can train the immune system of healthy mice for the consecutive stimulus of sepsis and thus increase chances of survival.

To investigate this hypothesis, a sepsis mouse model was used. In a first test group, SCFA contents of healthy mice were compared to those in mice that have been treated with cecal ligation and puncture (CLP), an well-established method to induce sepsis in rodents. In a second group, mice feces was tested before and after performing FMT on healthy mice using feces of sepsis-effected mice. The last group examined the influence of preventatively conducted FMT with sepsis-infected feces before treatment with CLP.

SCFAs from mouse feces have been extracted using an aqueous extraction procedure. The separation of SCFAs was performed using ion exclusion-chromatography. With gradient elution using H20 + 0,005 % formic acid and acetonitrile, it was possible to separate all SCFAs from each other, as well as their respective iso-isomers. Evaluation of the aqueous eluent indicated that the addition of more than 0.005 % formic acid led to high ion suppression especially for acetate and propionate. To additionally enhance the ionisation efficiency, a post-column flow of 0.05 % ammonia in methanol was added, enabling four- to sixfold lower limits of detection. For increased selectivity, high-resolution mass spectrometry has been used in negative ionisation mode for detection. Quantification of SCFAs was conducted via stable isotope-labelled internal standards.

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Poster Nr. 55

Comprehensive workflow for targeted cell metabolomics using automated sample preparation, HILIC chromatography, LC/TQ, and a statistical analysis software suite

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Researchers aiming to understand the biological processes commonly use targeted LC/MS methods to quantify and profile the metabolome in large scale studies. For these metabolomic studies it is advantageous to have the most analytically-sensitive analysis and the most comprehensive list of metabolites to increase biological insights from the samples tested. Furthermore, the most effective LC/MS metabolomics workflows are simple to implement, reliable, reproducible, and provide deep biological insights with ease. Herein, is a new workflow for cellular metabolomics aimed at users with various LC/MS backgrounds by utilizes a Bravo automated sample prep and LC/MS for sensitive and reproducible studies.

Polar metabolites were effectively extracted from K562 cells using a robust Agilent Bravo-automated protocol.

A comprehensive dMRM method can be built using the custom database of ion transitions and retention times for over 500 analytes.

The Poroshell 120 HILIC-Z column was selected for retaining polar analytes and ionization in both positive and negative mode. It was paired with an Agilent 1290 Infinity II Bio LC.

The 6495C LC/TQ provided needed speed and analytical sensitivity to detect analytes with <5ms dwell times. Limits of quantitation vary but are in the fmol range in matrix for the isotopically-labeled standards.

Data analysis in MPP can help researchers find trends in the data quickly with or without quantification.

Jump-start biological research projects with a comprehensive workflow that includes sample prep, transferable chromatography and extensive dMRM database.

Poster Nr. 56

Evaluation of Data Dependent MS/MS Acquisition Parameters for Non-targeted Metabolomics and Molecular Networking of Environmental Samples

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Analyzing dissolved organic matter (DOM) is a challenging task since it contains a diverse and complex mixture of molecules from both biotic and abiotic origins. Non-targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a widely used tool for the detection and annotation of small molecules in complex environmental samples. Data Dependent Acquisition (DDA) of product ion spectra is thereby currently one of the most frequently applied data acquisition strategies in mass spectrometry. The optimization of DDA parameters is central to ensuring high spectral quality and coverage to boost the number of compound annotations within complex samples. In this study, we optimized 10 central DDA parameters of the Q Exactive mass spectrometer platform for analyzing natural organic matter samples from ocean, river, and soil environments. Open-source mass-spectrometry data processing tools such as MZmine3, GNPS were used for creating classical and Feature-Based Molecular networking. Network metrics, MS/MS placement, and MS/MS coverage were used to define the best settings. The results revealed that Dynamic Exclusion, Automatic Gain Control, Mass Resolution, and Microscans are the most critical parameters, while Isolation Width, TopN, and Collision Energy have moderate effects, and Apex Trigger, Monotonic Selection, and Isotope Exclusion have minor effects. The here-presented insights into the data acquisition ergonomics of the Orbitrap platform provide guidance for LC-MS/MS method parameters, some of which may also be transferable to other sample types and MS platforms.

Poster Nr. 57

Identification and quantification of multiple phytohormones by targeted metabolomics in different plant species

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Sustainable food security for a growing population faced with progressive climate changes is nowadays the driving force behind the efforts to improve crop plant yield. Secondary plant metabolites, also called specialized metabolites, play an important role in crop adaptation, architecture and grain yield. Within the Cluster of Excellence on Plant Sciences (CEPLAS) we investigate the complex network of plant hormone signaling molecules - phytohormones - that influence the plant performance as a function of environmental and developmental stimuli.

Phytohormones are divided into five classical types of hormones: auxins, cytokinins, gibberelins, abscisic acids and ethylene. The list of hormone classes is meanwhile expanded, and brassinosteroids, jasmonates, salicylates, and strigolactones are also considered as major phytohormones. These compounds range from being highly polar (acidic/basic) to non-polar (neutral), and provide activity at very low concentrations. Within each class of hormone, all members of the same class have similar physiological effects but their chemical structures can vary extremely.

From the analytical point of view, the many different chemical properties make an adaptation of all analytical techniques indispensable. At first, extraction solvent and protocol must be properly selected while considering the different solubility of the phytohormones. Regarding the different polarities of the phytohormones, an appropriate column must be chosen for LC-separation. Thus, for the accurate determination of phytohormones using an MS-based approach it is essential to optimize metabolite extraction and LC separation in advance.

Within CEPLAS, we currently focus on the quantification of cytokinins, auxins, gibberellins, salicylates, abscisates, jasmonates and related derivates in rice and barley extracts. To simultaneously profile their hormone variation at different growth stages in diverse organs or tissues we have developed a highly efficient liquid-liquid solvent extraction method. The protocol enables the enrichment of the phytohormones from complex plant matrices like shoot and root, and tissues like meristem or leaves, across different scales ranging from solely 5-20 mg fresh weight.

Interestingly, LC-MS experiments revealed a high occurrence of isomers for several phytohormone classes. To give an example, the cytokinin Zeatin can be present in six different glucoside isomers (cis-Zeatin-O-Glc, cis-Zeatin-7-Glc, cis-Zeatin-9-Glc, trans-Zeatin-O-Glc, trans-Zeatin-7-Glc and trans-Zeatin-9-Glc) in barley. Or with respect to jasmonic acid, we found several isomeric features in rice. One of the greatest challenges is now to unambiguously identify and quantify these isomeric metabolites, which often fulfil different biological functions.

Poster Nr. 58

Investigation of the Phase I Metabolism of a New 20-Keto-Steroid S42 by C-6 Oxidation and GC-MS Analysis

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Introduction

Selective Androgen Receptor Modulators (SARMs) are relevant as illicit drugs for performance enhancement in the sports doping context due to their anabolic properties combined with less side effects based on their tissue selectivity.[1] In 2009, a new synthetic 20-keto-steroid S42 was introduced as a new SARM candidate.[2,3] Since S42 showed anabolic and anti-catabolic effects on cultured myotubes, it was proposed to be applied as a cure for muscle-related diseases,[4] triggering also use in sports doping.[5] However, fundamental understanding of the GC-MS behavior of S42 and its metabolic, is indispensable for reliable qualitative and quantitative analysis of this pharmaceutical and of its metabolites in body fluid matrices.

Preliminary Data

S42-mono-OH, S42-bis-OH and S42-tris-OH were found by GC-MS analysis after S42 phase I metabolism. The structure of S42-mono-OH was closely analyzed, and we assign the hydroxylation position to be in ring A or B based on 3 experimental findings. Firstly, the mass difference of TMS-S42 isomers and TMS-S42-d6 isomers decreased from 6 Da to 5 Da after phase I metabolism. This implies that the possible hydroxylation position is at C-1 to C-3, C-6 or C-7. Secondly, the dominating signals in the GC-Mass spectrum of TMS derivatized S42-mono-OH are found at m/z 219.1200, at m/z 245.1359 and at m/z 297.1671, suggesting that the TMSO functional group is attached to either rings A+B or A+B+C. This assumption rests on the significantly mass shifted fragment ions of the respective TMS-S42 plus 88 Da ([M-H+TMSO]) molecular ion. Furthermore, [TMSOH]▪+ and [TMSOD]▪+ peaks were found of the ions noted above, indication the TMS moiety in tandem MS experiments. However, the TMSO group may not be at the A ring since this elimination reaction is unfavorable at an aromatic ring. Thirdly, an indicator ion at m/z 143.0887 which originated from the underivatized D-ring of TMS-S42, was observed. Therefore, a hydroxylation at the D-ring is ruled out. In addition, a S42-C6-ketone was synthesized and the TMS derivatized substance showed similar fragment ion formation with a mass difference of 2 Da to TMS-S42-mono-OH. These observations indicate that the B-ring hydroxylation in the positions C-6 or C-7.

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Poster Nr. 59

Large scale quantification of metabolites in plasma samples utilizing high-resolution mass spectrometry and Skyline data analysis workflow

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Metabolite quantification from a variety of biological samples is required for clinical and biomedical translation of metabolomics research. One of the ongoing challenges in biomedical metabolomics studies is large-scale quantification of targeted metabolites due to the complexity of biological sample matrices. Further, the response of compounds in liquid chromatography mass spectrometry (LC-MS) analysis is influenced by the properties of compounds, chromatography, eluent composition, sample preparations, type of MS ionization and analyzer used. To facilitate these metabolite quantification, we proposed a complete analytical and computational workflow using novel quantitative LC-MS library combined with Skyline data processing platform, which allows large-scale quantification and sharing of results. The quantitative assay involves high-resolution mass spectrometry with Orbitrap Exploris240 and calibration curves using commercially available standards. As a spectral library, the validated 96 compounds both in positive and negative mode are reported with their accuracy and reproducibility. Our protocol with Skyline allows for the quantification of these compounds in plasma samples without the need for further reanalysis, and this approach can be useful as an important building block for the targeted data analysis of other biomedical metabolomic applications.

Poster Nr. 60

LC-MS quantification of low- and no-calorie sweeteners (LNCS) in 24h-urine to assess consumption and associated food sources

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Non-nutritive or low/no caloric sweeteners (LNCS) are approved in the EU for long time, and their use is widespread. With respect to emerging discussions about sugar reduction, sugar substitution gains even more importance. In contrast, there are still open questions regarding exposure, especially with respect to mixed LNCS consumption. While LNCS intake is often inferred from market data, biomonitoring data are scarce. To gain insight into LNCS use in south-west Germany, we established a targeted RP-LC-ESI(-)-MRM method covering several common, structurally diverse LNCS in urine. A previous method was extended by several analytes and adapted to UPLC-MS/MS conditions, resulting in a 7-minute duty cycle on a BEHC18 column. Samples were prepared just by dilution, in combination with matrix-matched, internal calibration. The method was successfully validated, and applied to 301 24h-urine samples from a previous Metabolomics study (KarMeN, Karlsruhe Metabolomics and Nutrition). 24h urinary LNCS excretion was normalized to osmolality, whereby concentrations up to 294 mmol/L per mOsm/kg urine were found. Quantitative results were used to calculate associations with dietary intake of various food groups. Dietary intake was obtained by 24h-recall (food intake interview), covering the same 24h time period as urine collection. Correlations were tested by Spearman rank correlation analysis. Significantly associated food groups include soft drinks, Radler, protein shakes, and table sweeteners, explaining up to 40% of the variance. Cyclamate, saccharin and acesulfame were the main contributors. LNCS intake was highly variable, with a majority of volunteers showing low or negligible LNCS excretion. However, high concentrations of more than one sweetener were quite often detected in case of LNCS consumers.

Poster Nr. 61

Metabolomics Predicts Mode of Action of Novel Anti-Cancer Drugs

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One bottleneck in the discovery of novel anti-cancer drugs is the identification of the molecular target(s) of a drug candidate in cancer cells. In view of this, metabolomics can be an excellent tool to mechanistically investigate the responses of cancer cells upon treatment with new drug candidates. Herewith, we describe the application of ion-pairing LC-MS/MS by using a multi-targeted, MRM-based approach to profile 180 metabolites of the central carbon and energy metabolism (CCEM) in prostate cancer cells (PC3). We developed an extraction protocol for adherent cells for the reproducible assessment of the abundance of CCEM metabolites including labile CoA esters, NADPH and ATP. Treating PC3 cells with a set of anti-cancer drugs with known modes of action (MOA), we obtained metabolic fingerprints, so called “metabotypes”. Strikingly, these training data supplied distinctive metabolic patterns specific to individual MOAs, which were evaluated by hierarchical clustering of different metabotypes and descriptive statistical analyses on their predictive power. Moreover, most metabolic fingerprints allowed for plausible interpretation of inhibitory drug effects in the CCEM. Applying machine learning to predict the MOA of highly cell-toxic, plant-derived and (semi)synthetic compounds, our model promises to be a valuable tool in the developmental pipeline of novel anti-cancer drug candidates.

Poster Nr. 62

Metal assisted laser desorption ionization (LDI) analysis of metabolites

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Metabolites play a crucial role in various biological processes and their analysis provides a better understanding of biological systems. Traditional methods for metabolite analysis, such as mass spectrometry, have limitations in terms of spatial mapping, sensitivity and chemical noise for analysis of small molecules. In recent years, matrix-assisted-laser-desorption-ionization (MALDI) has emerged as a promising alternative for the analysis of metabolites. However, there are some drawbacks, as the matrix used in MALDI can interfere with the detection of the analyte itself, especially when the analyte and matrix have similar masses or ionization potentials, while also potentially forming adducts with the analyte. This interference can lead to background noise and reduced sensitivity. In addition, sample preparation for MALDI-MS analysis is crucial for obtaining high-quality data. Sample heterogeneity, impurities, and matrix degradation can affect the results and reduce the sensitivity of the analysis. In this project, LDI-MS was used to analyze metabolites in biological samples, to switch from matrix-assisted to metal assisted ionization, in order to try and mitigate the drawbacks of conventional MALDI analysis. The samples were spotted onto a copper covered substrate, and desorbed using a UV-laser. The resulting ions were measured using a Q-Exactive HF Orbitrap mass spectrometer. To optimize the sensitivity and specificity of the analysis, different matrix compositions and laser parameters were evaluated, to investigate how matrix-free laser desorption ionization can serve as a feasible approach for the analysis of metabolites. The high sensitivity allows the detection of even trace amounts of metabolites in the samples. The innovative aspect of this project is the use of a metal covered glass slide, which eliminates the need of matrix application by e.g. DHB, as it has the potential to alter the sample surface. In comparison, using a copper covered glass slide has the potential to offer a more efficient desorption of metabolites, and thus a complementary approach to matrix assisted ionization. Additionally, the sample preparation time is also reduced, making it a valuable tool for the study of biological systems. Further studies are needed to fully exploit the potential of this promising approach.

Poster Nr. 63

Profiling and quantitation of bile acids in human biofluids by LC-TIMS-MS

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Bile acids (BAs) and their conjugates are crucial players in central physiological processes related to human health [1]. Their analysis by LC-MS/MS, however, is often hampered by the occurrence of isobaric and isomeric species differing only in the position or orientation of substituents on the steroid scaffold. Co-elution of BAs and derivatives with similar or identical accurate mass and fragmentation behavior necessitates increased selectivity in MS-based workflows for these compounds.

This contribution illustrates the utilization of trapped ion mobility spectrometry (TIMS) for separation and annotation of co-eluting lipid isobars in an optimized LC-TIMS-MS method. The method, utilizing the timsTOF Pro 2 system coupled to an Elute UHPLC system via VIP-HESI source (Bruker, Bremen, Germany) provides quantitation and profiling capabilities along with increased annotation confidence for BAs extracted from human biofluids.

Mixtures of pure reference standards (Cambridge Isotope Labs) were analyzed using the optimized LC-TIMS-MS method. Applying a C8-reversed phase (RP) method [2], 71 BAs could be separated and annotated in the standard mixture using TASQ 2023 and MetaboScape 2023 software packages for targeted quantitation and non-targeted profiling, respectively. Co-eluting isomers such as lithocholic acid and allolithocholic acid could be mobility separated as [M+acetate]- ions. Furthermore, inter-technology (TIMS vs. drift-tube, [3]) and inter-laboratory assessments of CCS values using BA mix standards showed an average CCS value deviation of 0.1 % (n=6) and 0.3 % (based on 2 labs, 8 compounds), respectively.

In a qualitative profiling experiment based on the target list of 71 BAs, 25 bile acids were annotated in the SRM 1950 serum extract (NIST Standard Reference Material 1950 human reference plasma). Annotation quality (AQ) was assessed based on AQ score, a visual tool in MetaboScape incorporating multiple molecular identifiers (e.g., CCS and m/z). Data visualization in Kendrick mass defect plots facilitated annotation of isoglycocholic acid, which had not been included in the original target list.

The quantitative capabilities of the established assay were estimated based on the linear dynamic range and limit of detection (LOD) for selected BAs. Taurocholic acid was measured with a linear dynamic range of 3.7 orders of magnitude and an LOD of 300 pM. For the BAs that were detected within the dynamic range of the LC-TIMS-MS method, all determined concentrations in SRM1950 extracts were within the range of the published standard uncertainty locations [4].

[1] DOI:10.1038/s41586-020-2047-9

[2] DOI:10.1021/acs.analchem.5b01556

[3] https://mcleanresearchgroup.shinyapps.io/CCS-Compendium

[4] DOI:10.1194/jlr.M079012

Poster Nr. 64

Profiling of the Sulfur-Containing Secondary Metabolites Glucosinolates by LC-MS in Plant Species of Different Environmental Habitats

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The two plant species Arabis nemorensis and Arabis sagittata are endangered species of the Brassicaceae family. With regard to plant secondary metabolites members of the Brassicaceae family are of special interest because they contain high amounts of the sulfur-containing glucosinolates, a class of metabolites that is mostly unique to this family. Glucosinolates are recognized to play an important role to protect plants from herbivores. Upon wounding, the plant myrosinase, a specialized enzyme, hydrolyses the glucosinolates and toxic and repellent products such as isothiocyanides and nitriles are produced. The same products are appreciated in human nutrition for their taste and smell and for their anti-cancer activity, in vegetables such as broccoli, cauliflower and mustard.

The glucosinolates comprise a large variety of more than 100 identified metabolites and many others, which are as yet unidentified. It is not yet fully understood to what extend there is a biological role for this great variety, which is mostly based on different amino acid precursors early during biosynthesis and multiple modifications of the side chain later on.

Arabis nemorensis and Arabis sagittata have been isolated for thousands of years and evolved distinct ecologies in their respective habitats. Recently, the two species have been discovered in the same location, where they have begun to hybridize.

We determined the glucosinolate profiles in leaves and roots of the two plant species and their offspring, to find out more about biological and ecological function of different glucosinolate profiles. Glucosinolate molecules contain 2-3 sulfur atoms. We used the characteristic S-isotope pattern, in which the third isotope is almost higher than the second isotope for data mining to detect novel putative glucosinolate peaks. These peaks were confirmed in MS2 experiments by comparison of their fragmentation patterns with known glucosinolates from other plant species.

In a parallel approach we screened the samples for the m/z of all known glucosinolates, which had previously been described in other plant species. With both strategies we could identify several peaks which were present in both plant species and a subset of these could be unequivocally identified due to the availability of commercial standards.

For several other abundant glucosinolates a number of possible glucosinolate isomers have been described. Further MS2 experiments and comparison of the MS2 spectra with those of related glucosinolates helped narrow down the identity of these candidates.

Poster Nr. 65

Retinal degeneration is modulated by free 3-Hydroxy kynurenine. Identification and quantification of kynurenic intermediate.

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The kynurenine pathway (KP) is a metabolic pathway involved in converting tryptophan to kynurenine. KP also functions in immune and neurotransmission functions. Dysregulation or overactivation of this pathway can lead to immune system activation inflammation and Neurodegeneration.

To understand better the function of the KP we use Drosophila melanogaster. The lens system in the eye of Drosophila melanogaster are designed to focus or concentrate light. Such exposure also generates photo-oxidative stress. Under normal circumstances pigment granules, prevent damage from this stress. In certain vulnerable conditions, such as disease or mutations, this protection is lost, and retinal damage is apparent. Interestingly many intermediates of Tryptophan metabolism such as 3-hydroxy kynurenine (3OH-K) have been implicated in oxidative stress and inflammation induced damage.

We analyzed the metabolic intermediates involved in KP using shotgun analysis, which provides a quantitative snapshot of intermediates in the KP.

The metabolites were extracted from fly heads from wild-type and w, st cn1, bw1 mutants. in acidic methanol solution. Mass spectrometric analysis was performed on a Q Exactive spectrometer (Thermo Fischer) with a robotic nanoflow ion source using nanoelectrospray chips. Ionization voltage was +1.5 kV in positive mode: backpressure-1.5 psi. All samples were analyzed for 6 min. FT MS spectra were acquired at the mass resolution of R m/z 200 = 140,000; t-SIM was acquired with isolation window of 20 Th. All spectra were pre-processed using repetition rate filtering software PeakStrainer and stitched together by an in-house developed script. Metabolites were identified by LipidXplorer software.

With this method we could identified and analysed the main metabolites in the Kynurenine pathway including: Tryptophane (Trp), Kynurenine (K), 3-Hydroxykynurenine (3OH-K), kynurenic acid (KA) and Xanthurenic acid (XA).

So far our analyses revealed that cn and st loss of function inversely affect the build-up of 3OH-K +XA. Consistent with this, a combination of st and bw resulted in significantly higher levels of 3OH-K +XA and significantly reduced levels of K + KYNA in bw1; st1 as compared to cn1, bw1.

Increased 3OH-K was reported in metabolomic profiling of cerebrospinal fluid of patients of Parkinson's disease. The severity of degeneration in models of PD and Huntington's disease can be modulated by genetically inducing KP imbalances consistent with our findings in light-induced retinal degeneration. The identification and the quantification of the kynurenine intermediates could be a promising target for therapeutic development to treat inflammation and some diseases with neurological aspects.

Poster Nr. 66

Sensitive LC-MS/MS analysis of tissues and fluids gives insights into metabolism, kinetics and the related toxicity of ochratoxin A in mice models

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Ochratoxin A (OTA), a secondary metabolite of Aspergillus and Penicillium fungi species, is a widespread mycotoxin found in processed and unprocessed food like grains, nuts, coffee and cocoa products. Though OTA is known to have nephrotoxic and carcinogenic effects, the underlying mechanisms of its toxicity are not yet fully understood. OTA undergoes different ways of biotransformation in organisms including hydroxylation, dechlorination, hydrolysis and conjugation. Most of these metabolites are regarded as less toxic compared to OTA itself, although the opened-lactone form (OP-OTA) was found to have similar or even stronger toxic effects [1]. Generally, the strong binding affinity of OTA to serum albumin in blood delays its elimination process, and is an important factor for OTA's toxicity and metabolism. A recent study on the toxicity of OTA in mouse models revealed an increased tissue uptake and stronger hepatotoxic effects in heterozygous albumin deficient mice treated with OTA compared to wild type mice [2]. Following these findings, we analyzed blood, urine and bile samples of the different mouse strains in the study for multiple metabolites of OTA via a sensitive targeted LC-ESI-MS/MS method using both polarities for ionization. Sample preparation was held simple and consisted of protein precipitation and/or dilution steps, while quantification was enabled through matrix-matched calibration. First results show that next to OTA, its hydroxylated derivates 4-hydroxy-ochratoxin A (4-OH-OTA) and recently identified 7'-hydroxy-ochratoxin A (7'-OH-OTA), the dechlorinated product ochratoxin B (OTB), OTHQ and OP-OTA were found in all matrices. Most metabolites found here also seemed to be formed in higher amounts in the albumin deficient mice compared to the wild type mice. Here, we present and discuss the data from this study, comparing the quantified metabolites in the different matrices and mouse strains (wild type, heterozygous and homozygous albumin deficient mice). Furthermore, kinetics of OTA elimination from blood via urine and bile of the different mouse strains will be shown, demonstrating the relevance of albumin binding for the toxicity of OTA.

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Poster Nr. 67

Studying the reactivity of PFAS by HRMS

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Per- and polyfluorinated alkyl substances (PFAS) are currently one of the most debated topics in environmental science. They are a complex family of more than 3000 anthropogenic aliphatic compounds having at least one carbon-fluorine (C-F) bond and containing functional head group attached at the end of the chain. They have applications for example in surface coatings or fire-fighting foams. Some PFAS are widely detected in the environment and in organisms as a result of widespread applications. Due to the excessive use of PFASs, they have also been found in human blood. This is even more alarming as these substances are suspected carcinogens.

Regarding potential toxicity to humans, short-chain, long-chain PFASs and a long-chain sulfonic acid were investigated in regard of their reactivity towards amino acids, peptides and proteins. The results were obtained using high resolution mass spectrometry. This makes it possible to investigate reaction pathways and analyze possible reactions. Electrospray ionization seems to be the most suitable ionization technique, because PFAS have a polar end group which is easy to ionize.

Initial results indicate strand breakage of the longer chained carbon acid PFAS. This seems to result in further reactions that are being studied here. First results of this study are being reported.

Poster Nr. 68

21-T Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: Chemical and Structural Characterization of Complex Organic Carbon in Peat Burning Aerosol

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The combination of electrospray ioinization (ESI) and atmospheric pressure photoionization (APPI) direct-infusion 21 T Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) supported by infrared multiple photon dissociation (IRMPD) fragmentation, enables unprecedented insights into the chemical properties of climate relevant peat burning aerosol.

Peatlands are formed by the accumulation of biomass under waterlogged conditions and constitute the largest natural terrestrial carbon stock in the World. The peat composition is directly dependent on the plant communities of the respective peatlands. Large-scale peat fires can release large amounts of CO2 as well as organic carbon (OC), due to the predominant smouldering conditions in peat fires (Chakrabarty et al, 2016). Climate change and the resulting higher frequency of extreme drought events also increases the frequency and scale of peat fires, not only in tropical regions, like south-east Asia, but also in the northern hemisphere, where by far the most peat deposits are located (Langman et al, 2009).

To characterize the OC emitted from peat burning, four boreal peat samples (Finland, Siberia, Spitsbergen) and one Finnish boreal forest surface sample were dried and burned in lab experiments simulating wildfires. Among other instrumentation for aerosol characterization, the resulting particulate matter (PM) was collected on quartz fibre filters and extracted by Methanol/Dichloromethane (1:1 v%) for consequent characterization of organic compounds by APPI 21 T FT-ICR MS with IRMPD as well as ESI in positive and negative mode.

The application of the highest magnetic field FT-ICR MS system in the world, and the resulting improvement of resolving power (R > 1,200,000 @ m/z 600), mass accuracy and dynamic range for the assignment of elemental compositions based on the exact mass (Smith et al, 2018), revealed an extremely complex mixture with an average assigned compounds number larger then 30,000 of elemental compositions in the range of 180–1400 Da containing oxygen, nitrogen, sulfur, as well as phosphor. Especially, a high abundance of nitrogen-containing compounds with up to six nitrogen atoms was observed which are known to impact the atmosphere due to their potential strong light-absorbing properties.

In-cell fragmentation by IRMPD of two quadrupole-isolated mass ranges (2 Da window) at m/z 448 and m/z 560 revealed distinct structural differences of the peat-burning organic carbon. While e.g. one Finnish peat sample displayed mainly fragmentation into partially aromatic hydrocarbon ring structures with only minor abundance of heteroelements, the boreal forest surface samples showed an abundant dealkylation pattern of CHN and CH compounds, in addition to a similar hydrocarbon ring structure pattern. Additionally, the high ion capactiy and

resolving popwer of the 21 T FT-ICR MS enables the unique possibiltiy of the accurate characterization of high molecular weight compunds (m/z >1000) in a complex mixture, by isolation of a 100 Da mass range.

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Poster Nr. 69

Direct analysis in real time in combination with trapped ion mobility QTOF mass spectrometry for fast analysis of seized drugs

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Forensic analytics are facing constantly growing demands like an increasing number of targets due to emerging new psychoactive substances (NPS). Labs must keep up with increasing caseloads. Furthermore, the analyses require higher selectivity and sensitivity to minimize sample preparation and the analysis time has to be fastened to shorten response time and increase through-put. The use of Direct-Analysis-in-Real-Time Mass Spectrometry (DART-MS) as a forensic tool has been praised for its ability to generate valuable information quickly and easily, resulting in increased productivity for many users, thereby meeting demands. Therefore, this approach often eliminates the need for sample preparation and can complete an analysis in a few seconds.

For analysis with DART sampling can be distinguish between manual introduction e.g. holding the sample direct in the DART gas stream or dipping a glass capillary into a powder, swapping of the sample or introduction using the QuickStrip cards or 96 well plates. It allows analysis of samples without much sample preparation. Bruker's automated library search for DART-MS/MS data enables results from sample to report in less than 15 seconds. Therefore, homebuild libraries just as external databases, e.g. from NIST can be used. A timsTOF Pro 2 instrument using trapped ion mobility spectrometry (TIMS) as a powerful analytical technique was used for the analysis. Collision cross sections (CCS) were used as an additional identification criterium to assist in the identification of the seized drugs. Using these tools mixtures of up to 20 different drugs can be separated and identified. Furthermore, also pairs of isomeric substances such as morphine and norcodeine or hydromorphone and norhydrocodone gave clean MS/MS spectra for library search using TIMS.

The proposed workflow provides a comprehensive solution for the characterization of seized drugs in complex matrices. Trapped ion mobility and QTOF mass spectrometry solutions are used for the analysis of controlled substances thereby implementing a forensic database for expedited evidence analysis.

Poster Nr. 70

Gas-phase reactivity of alkyl- and aryl-trifluoromethyl-coinage-metal complexes

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Liu et al. published in 2020 a procedure to synthesize a [CuMe(CF3)3][NBu4] complex and there are recent Publications which focus on the investigation of similar trifluoromethyl [Cu(Alkyl)(CF3)3]– and [Cu(Aryl)(CF3)3]–-complexes. Inspired by this result, a variety of [Cu(R)(CF3)3]–-complexes forming experiments were designed to observe the gas-phase isolated reaction dynamics with ESI mass spectrometry. Through the preparation of a solution of the [Cu(bpy)(CF3)3] complex with an organometallic reagent for the transmetalation, we were reliably able to produce the spectra of [Cu(R)(CF3)3]–-complexes. We investigated the behaviour of these anionic complexes. The collision-induced dissociation experiments lead us to the conclusion that the [Cu(Alkyl)(CF3)3]– shows, with increasing potential of the radical stabilisation of the alkyl group, an increasing proportion of radical reactions. This resulted majorly in the formation of the meta-stabile [Cu(CF3)3]– anion, which decomposed further to the [Cu(CF3)2]–. In contrast the [Cu(Aryl)(CF3)3]– complexes seem to react via a presumably reductive elimination of the aryl-CF3 product. . Further we analysed the reactivity via quantum mechanical calculations. Next we analysed the influence of exchanging the central metal atom with the other coin metals silver and gold.

Poster Nr. 71

Investigation of Homemade Low Temperature Plasma (LTP) Ion Sources

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Ion sources play an important role to achieve a selective and sensitive determination of organic compounds in chromatography – mass spectrometry. Among them, ion sources based on a low temperature plasma (LTP) have recently been gaining interest as they can ionize a wide range of compounds resulting in $[M]^{+\bullet}$ and/ or $[M+H]^+$ ions with little or no fragmentation. The plasma is created by the dielectric barrier discharge (DBD) of a gas which is flowing through an insulator in contact with electrodes when fast changes of high voltages are applied. The ion source configuration itself is also thought to influence the ionization efficiency. The choice of the plasma gas is of great importance. While the ionization mechanism in helium plasmas is well understood, the species present in argon plasmas are not well elucidated yet.

For the comparison of different electrode configurations, homemade DBD-type ion sources have been constructed, optimized, and investigated, for example in a liquid chromatography – mass spectrometry (LC-MS) set-up from Hitachi High-Tech and a gas chromatography – mass spectrometry (GC-MS) set-up from Agilent. The presented homemade ion sources are characterized by better performance in the use of argon compared to helium.

In GC, the ion source was compared with other atmospheric pressure ionization sources as well as the gold standard electron ionization, showing a more sensitive and selective determination for model compounds (phthalate esters). In LC, a comparison with ion sources using established ionization mechanisms (e.g., ESI) was performed using caffeine and testosterone as model compounds.

Furthermore, coupled to a GC system, a tube plasma ionization (TPI)-configuration was used to study the effect of water and other modifiers (e.g., MeOH) in the gas phase of the ion source to shed some light on the until now unestablished argon plasma ionization mechanism. Further optical as well as mass spectrometric investigations would be useful for a further clarification of the underlying ionization mechanism, which should support in targeted optimization of the ion sources presented.

Poster Nr. 72

Ion mobility-supported hydrogen-deuterium exchange mass spectrometry analysis of p53 tetramerization mutants – An attempt to decrypt the structural dynamics of a prominent tumor suppressor

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The tumor suppressor p53, also known as the "guardian of the genome", is a transcription factor and central checkpoint in the cell cycle that is important for the cellular response to DNA damage. Mutations, mainly in the DNA-binding domain (DBD) of p53, often lead to the development of various cancers. P53 is organized as a multi-domain protein, namely the DBD and the tetramerization domain (TET), while ca. 40% of p53 consist of intrinsically disordered regions (IDRs). This classifies p53 as an intrinsically disordered protein (IDP).

Due to its nature as an IDP, a structural characterization of p53 is highly challenging as the protein is prone to aggregation.

In our lab, we have recombinantly produced two tetramerization mutants of full-length, wild-type p53 in E. coli. Wild-type p53 forms a tetramer that are formed by a dimer of dimers, while the p53 tetramerization mutants form monomers (L344P) or monomers and dimers (L344A) [1].

The structural dynamics of the two p53 mutants were analyzed using hydrogen-deuterium exchange mass spectrometry (HDX-MS). Site-specific analysis of deuterium incorporation will be conducted by on-column pepsin digestion of proteins, followed by MS analysis of deuterium incorporation of the generated peptic peptides. Base on the deuterium incorporation rates, kinetics of p53 and its mutants will be determined and structural information will be deduced, with a focus on the IDRs.

So far, we have established an LC-MS method in combination with ion mobility-MS analysis to detect p53 peptides. Data were acquired using trapped ion mobility separation on timsTOF Pro mass spectrometer (Bruker Daltonik) and data analysis was performed by the HDExaminer (Sierra Analytics) software. HDX-MS analysis of full-length, wild-type p53 and the two p53 tetramerization mutants will later be used to gain insights into the dynamic interactions of p53 with DNA as well as with other binding proteins.

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Poster Nr. 73

Proteomic signatures of inflammation-induced early pancreatic carcinogenesis

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Abstract:

In pancreatic cancer suitable diagnostic biomarkers are still lacking, particularly for the early diagnosis of (pre)invasive stages.There is however accumulating evidence that proteomic signatures, frequently modulated by complex posttranslational modifications, rather than single proteins might improve the diagnostic and predictive sensitivity and specificity of early pancreatic carcinogenesis. To enlighten the early onset of pancreatic intraepithelial neoplasia (PanIN), we are currently performing proteomic studies to obtain insights into the early stages of PanIN at the molecular level. Here, we quantitatively compared two cell lines, PanIN 4994 and PanIN 6585, representing two stages of early PanIN.

Methods:

Three biological replicates of PanIN 4994 and 6585 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10 % fetal calf serum for 96 hours. Cells were harvested and lysed by sonication in 8M urea buffer. After reduction and alkylation of proteins, enzymatic proteolysis was performed overnight with trypsin at 37 °C and stopped by acidification. For quantitative proteomics, biological replicates were analyzed as three technical replicates using a label-free data-dependent analysis (DDA) approach. Samples were analyzed by LC-MS/MS on an Orbitrap Q-Exactive Plus mass spectrometer (Thermo Fisher) coupled to a U3000 RSLC nano HPLC system. Linear 240-min gradients were applied for peptide elution and the mass spectrometer was operated using a top10 DDA method. All data were processed with MaxQuant v2.0.3.0 and statistical evaluation was done with Perseus v2.0.7.0.

Preliminary results and perspectives:

Proteome analysis allowed the comparative quantification of about 4,000 proteins for both cell lines. As expected, the expression levels of the majority of proteins quantified do not change during early stages of PanIN. Up- or down-regulation by a factor of at least 2 were observed for 221 proteins. The regulated proteins correlate well with previously acquired transcriptome data (Pearson's Correlation Coefficient = 0.41).

Specifically, the tumor suppressor p53 was found to be downregulated by a factor of 5 in PanIN 6585 cells. Other proteins found downregulated are known interactors of p53 (STAT1, Nmi) and belong to the interferon-gamma mediated signaling pathway. Proteins found upregulated (Sdhd, Aco2, Dlat) are involved in metabolic pathways. Both findings are in good agreement with the advanced cancer developmental stage of PanIN 6585 cells compared to PanIN 4994 cells. Based on our initial results, we will now further analyze protein expression levels upon interferon-mediated stimulation, both in PanIN cell lines as well as in murine pancreatic tissue.

Poster Nr. 74

Studying the Molecular Mechanisms of Liquid-Liquid Phase Separation of α-Synuclein by Mass Spectrometry

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Liquid-liquid phase separation (LLPS) has recently been recognized as a major principle for the formation of "membrane-less organelles" in cells. Proteins, such as tau or α-synuclein, involved in Alzheimer's and Parkinson's Disease (PD), undergo LLPS prior to aggregation. Aggregation of α-synuclein results in the formation of Lewy Bodies, which are a hallmark of PD. As LLPS might be a triggering factor for the formation of Lewy Bodies, a molecular understanding of the mechanisms underlying LLPS of α-synuclein is required for developing potential novel therapies to treat PD. α-Synuclein (14.5 kDa) is an intrinsically disordered protein (IDP) that consists of the membrane-binding N-terminal region and non-amyloid-component (NAC) region, as well as a C-terminal acidic region. In addition to α-synuclein, synapsin-1 is another high-abundant protein that is present in synaptic vesicles. Fluorescence microscopy has shown a colocalization of α-synuclein and synapsin-1 in LLPS, giving hints on a functional interaction between both proteins. The heat shock protein (HSP) 70-1 is one of the major chaperones that is frequently found to co-separate with α-synuclein and synapsin-1. It has been suggested that HSP70-1 is recruited in stress granules to induce protein disassembly and prevent protein aggregation.

Using three complementary techniques of structural mass spectrometry, cross-linking mass spectrometry (XL-MS), hydrogen-deuterium exchange MS (HDX-MS), and native MS, we aim to study the composition and topology of the complexes formed between α-synuclein, synapsin-1, and HSP70-1 upon LLPS.

Poster Nr. 75

Investigating non-planar structures in crude oil by high-energy CID mass spectrometry

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The analysis of crude oil brought up numerous techniques and approaches in the past decades, but none has proven to be as versatile as mass spectrometry when it comes to comprehensive composition analysis. High-resolution mass spectrometry (HRMS) left the door wide-open and allowed the investigation of completely new classes of compounds – most surprisingly fullerenes.[1] These carbon allotropes are normally formed under high-energy circumstances (lightning, meteor impacts, etc.) in comparison to relatively mild conditions during crude oil formation. This study focuses on precursors that accompany the formation of fullerenes. Their identification and structural elucidation in such a highly complex mixture is a major challenge. HRMS provides insights into individual compounds and by fragmentation also offers structural elucidation.

During this study, an asphaltene fraction of a heavy crude oil was investigated. All mass spectrometric analyses were performed on a research-type Orbitrap Elite MS (Thermo Scientific, Bremen, Germany). Collision induced dissociation (CID) was performed in the HCD cell for higher collision energy dissociation (HCD) using isolation windows of 0.8 Da and nitrogen as collision gas. The mass resolution was set to 480,000 at m/z 400, corresponding to a transient of 1.5 s. For atmospheric pressure photoionization (APPI) a Krypton-VUV lamp (Syagen, Tustin, CA, USA) was used with photon emission at 10.0 and 10.6 eV.

This study focuses on small non-IPR fullerenes (C32 to C44). Their fragmentation showed multiple C2 losses at collision energies above 200 eV indicating a typical fragmentation pattern as reported before for C60.[2] The next step comprised possible fullerene precursors (CxHy, x = 32-44, y = 2–16). These already bent, 5-membered-ring containing bowl-like hydrocarbon structures were isolated and fragmented. These results reveal a distinct fragmentation pattern in a single fragmentation step that can be associated with the standard loss of C2-units or the loss of hydrogen. The observation matches with the results of previous studies.[3] Compounds like CxH16 and precursors with higher hydrogen content show lower tendency to only lose hydrogen. These compounds have a tendency to rather lose C2H2-units. The conformation of the structure explains what happens during the fragmentation. The less hydrogen is present, the stronger the steric interference due to the bending of the structure. Therefore, CxH14 and other hydrogen deficient precursors tend to have a strong steric hindrance that leads to dehydrogenation.

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Poster Nr. 76

Isolation of oligomeric proanthocyanidins from chokeberry (Arnonia prunifolia 'Nero') and characterization via high-resolution mass spectrometry analysis

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The chokeberry, which belongs to the rose family (Rosaceae), was unknown in Germany until a few years ago and has now moved into the media spotlight as a local superfood. Chokeberry products such as juices, fruit spreads, teas or dried fruits are attributed to various positive health effects. These include antioxidant, antiproliferative or anti-inflammatory properties, thus contribute to the prevention of various chronic diseases such as diabetes mellitus, cardiovascular diseases or cancer. Such effects are associated to the presence of multiple phytochemicals in chokeberries, especially oligomeric proanthocyanidins (OPCs).

Several separation steps with different separation techniques are needed to isolate OPCs from a pre-produced extract. An initial separation of the extract into trimer-, tetramer- and pentamer-rich OPC fractions is achieved by classical column chromatography on a Sephadex LH-20 phase. To further purify the enriched fractions, a semi-preparative high-performance liquid chromatography (HPLC) via diol phase is performed. For the final characterization of the OPCs, high-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS) is used. Based on the fragment spectra of the MS2 experiments, conclusions about the fragmentation pathway and also for the structure of OPCs can be drawn. Numerous A-type and B-type OPCs are characterized so far, which may contribute to the underlying mechanisms of action of the postulated health-promoting effects of chokeberries.

Poster Nr. 77

Oxidation products of tryptophan and proline in adipokinetic hormones – artifacts or posttranslational modifications?

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Introduction

Adipokinetic hormones (AKHs) are important regulators of metabolism in insects and are characterised by a chain length of 8-10 amino acids, an N-terminal pyroglutamate residue, an amidated C-terminus, and favoured positions for some amino acids such as Pro in position 6 and Trp in position 8. Posttranslational modifications have been found in a few AKHs, including sulfation, phosphorylation, glycosylation, hydroxyproline (Hyp) and kynurenine (Kyn). Recent data from cockroaches, flies and beetles suggest that Hyp and Kyn occur much more often than originally thought.

Methods

Corpora cardiaca, the natural source of AKHs, were dissected from adult cockroaches, flies and beetles and extracted with methanol. Extracts were centrifuged, the supernatants were dried and redissolved in 10 µ l methanol followed by 10 µ l 0.1% formic acid containing 5% acetonitrile. AKH candidate peaks were identified by target mass spectrometry for eligible known peptides and by screening for the gas phase fragmentation loss of the Trp immonium ion using LC-MS/MS with Synapt G2 Si coupled to M-Class UPLC.

Results

An AKH commonly found in some cockroach species is Bladi-HrTH. There are other AKHs, which differ very little from this sequence. Hyp was detected for several of these Bladi-HrTH relatives bearing the recognition sequence of prolyl 4-hydroxylase SPG, but also for SPN- and TPG-containing peptides. PPG is the favoured recognition sequence of this enzyme, but Pro with different neighbors has also been hydroxylated. The modified form accompanies the AKH at concentrations from <1% up to 7% and is thus not always well detected. In extracts of various Nicrophorus species (burying beetles), singly and doubly oxidized Trp as well as the Kyn-containing form of the peptide hormone Nicve-AKH have been seen at similar concentrations. Both the formation of Hyp and Kyn could be the result of endogenous processes, but in particular Trp oxidation is also known to occur easily during sample handling. It is necessary to carefully differentiate artificial from endogenous processes.

Mass spectrometry related innovations

Low abundant oxidation products of Trp and Pro residues in insect hormones identified

Poster Nr. 78

Protection of biodiesel via additivation with oxygen scavengers

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Biogenic fuels can provide sustainable and clean energy for the automotive sector. In particular, biodiesel or Fatty Acid Methyl Esters (FAMEs), which can be derived from oil crops or waste vegetable oils, are already part of today's fuel mix [1]. Since these FAMEs have a lower storage stability than conventional diesel fuel, an undesirable aging of biodiesel can lead to blockages in the filter systems and fuel injectors of the engine [2]. It is therefore crucial to understand and influence the aging processes in order to increase the usefulness of these substitute fuels.

In the interest of understanding the aging processes, the samples were subjected to accelerated photo-oxidative aging. Irradiation of the samples were performed in a lab-made photoreactor consisting of four VIS lamps (Radium Ralux® Duo RX-D 26W) and eight UV lamps (Actinic BL PL-S 9W) for 168 h. The complex samples were analyzed by a 7 T ultrahigh-resolution mass spectrometry (FT-ICR MS) equipped with an ESI source. The signals detected were assigned to elemental compositions in order to understand aging on the molecular level. The biodiesel used for aging was a used cooking oil (UCOME, Used Cooking oil Methyl Ester). For a better stabilization of the biodiesel, two classes of additives were investigated. One was the class of polymerisation inhibitors represented by α-tocopherol, butylhydroxytoluene (BHT) and phenol. On the other hand, oxygen scavengers were studied (hydrazine and N,N-dieethylhydroxylamine (DEHA)).

The photo-accelerated aging of the model substance produced molecular aging patterns comparable to conventional aging. After 48 h in the photo-reactor, dimers and trimers were detected, as well as an increase in oxygen content. Neither biogenic α-tocopherol nor the two technical antioxidants BHT and phenol had any effect on reducing the aging of UCOME in the group of polymerisation inhibitors. There was no significant improvement over pure UCOME in either oxygen uptake or oligomer formation. In contrast, the oxygen scavengers showed clearer results. The uptake of oxygen by UCOME was severely limited and the formation of dimers was reduced. Trimers did not appear at all. Hydrazine was able to almost completely prevent the formation of dimers.

It has been shown that new approaches to artificial aging can give comparable results to natural aging, and that this method of accelerated aging can be used to effectively analyze potential additives.

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Poster Nr. 79

MS SPIDOC: Mass Spectrometry meets X-ray Single Particle Imaging

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Native mass spectrometry (MS) enables the ionization and transfer of structurally intact non-covalent protein complexes into the gas-phase. As such, it is a perfect tool to study proteins and their assembly intermediates in a mass and conformation specific manner. However, structure follows function, and the structural information that can be gained with techniques like top-down MS or ion mobility is limited. Accordingly, other experimental approaches such as X-ray diffractive imaging are necessary to get a full understanding of the proteins and their assemblies.

MS SPIDOC (Mass Spectrometry for Single-Particle Imaging of Dipole Oriented Protein Complexes) is a Horizon 2020 funded research and innovation program [1] aiming at the combination of both experimental techniques. In particular, well-established methods from MS like m/z selection, ion trapping or ion mobility are adapted as part of the sample delivery system for X-ray diffraction. In contrast to conventional diffractive imaging of crystallized proteins, the proteins here are delivered as single particle without the need for crystallization. This increases naturally the requirement to the X-ray source. Thus, single-particle X-ray diffractive imaging (SPI) is only conducted at X-ray free electron lasers [2], the worlds brightest X-ray sources in the world.

This contribution will highlight the ongoing efforts of the MS SPIDOC consortium to develop this sample deliverysystem for the use at beamlines of the European XFEL [3, 4]. The current state of the designing and manufacturing of the instrument prototype will be presented as well as the results of the first testing of individual component modules. Furthermore, simulations as well as experimental device design for particle dipole orientation in strong electric fields will be previewed.

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Poster Nr. 80

Comprehensive analysis of isotopologue ratios of oxyanions and small organic molecules using ESI-FTMS

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Precise analysis of the isotopic ratios of the heavy and the light isotopes can give insights to the origin and synthesis pathways of these molecular structures. For any molecular structure containing multiple elements with heavier, low abundance isotopes (for example: 2H, 13C, 18O, 15N) single and multiple substitutions with these heavier isotopes result in a variety of different isotopologue molecules. Conventional approaches to isotope ratio mass spectrometry (IRMS) of complex molecules require the conversion into simple gas molecules which isotope ratios are determined by magnetic sector field mass spectrometers. Novel approaches using electrospray ionization (ESI) or electron impact ionization and Fourier-transform mass spectrometry (FTMS) enable for the first-time routine, high precision isotope ratio determination of intact complex molecules. New developments in ESI-FTMS on the Thermo Scientific™ Orbitrap Exploris™ MS platform allow the simultaneous analysis of multiple isotope ratios with high precision and accuracy on several intact isotopologues of oxyanions and small organic molecules. Combining a soft ionization technique with the high resolution accurate mass of the Orbitrap MS offers the unique opportunity for the analysis of intramolecular isotopic information like multiple heavy isotope substitutions (isotope clumping) as well as site specific isotopic information, which is achieved through controlled collision induced fragmentation.

Similar to classical IRMS approaches, the principles of identical treatment and rigorous sample-standard bracketing have been retained for sample introduction and are the keys to achieving precise and accurate relative abundance measurements. Typical areas of applications are the analyses of oxyanions in environmental samples. Methods have been developed for nitrate ($\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$, $\Delta^{17}O$, $\Delta^{15}N^{18}O$, $\Delta^{15}N^{17}O$, $\Delta^{18}O^{18}O$), sulfate ($\delta^{33}S$, $\delta^{34}S$, $\delta^{36}S$, $\delta^{17}O$, $\delta^{18}O$, $\Delta^{17}O$, $\Delta^{33}S$, $\Delta^{36}S$, $\Delta^{34}S^{17}O$, $\Delta^{33}S^{18}O$, $\Delta^{34}S^{18}O$, $\Delta^{17}O^{18}O$, $\Delta^{18}O^{18}O$), phosphate ($\delta^{18}O$, $\delta^{17}O$, $\Delta^{17}O$, $\Delta^{17}O^{18}O$, $\Delta^{18}O^{18}O$), which achieve sub-‰ precision for isotope ratios of singly substituted isotopologues. Additionally, data for different small organic molecules such as methanesulfonic acid and caffeine will be presented. We will also touch on future directions and development opportunities in a wide range of topics in biochemical cycles, ecology and paleoclimate reconstructions.

Poster Nr. 81

Novel developments in Inductively Coupled Plasma Mass Spectrometry: How can the analysis of complex samples be made simple?

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Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is today one of the key technologies used for the determination of trace element concentrations in almost any sample type. Following on its initial development in 1980, it has seen an unparalleled ramp up, and grew from a high-end tool used in cutting edge research laboratories to a technique used in many thousand laboratories across all regions.

In 40 years of being available, there have been developments to drive detection limits lower, hyphenation of separation techniques and accessories to allow for direct sampling of solids, ramp up of productivity and sample throughput, and, most of all, better removal of interferences. One of the key highlights along the way was the introduction of collision/reaction cell (CRC) technology in traditional single quadrupole ICP-MS, which finally opened the door to fully access the superior interference removal using reactive gases in combination with triple quadrupole ICP-MS systems (TQ-ICP-MS).

However, the complexity of samples to be analyzed using ICP-MS increased at the same level, and nowadays novel challenges appear, requiring the analysis of samples containing matrix levels previously analyzed using ICP-OES, and novel sample types, creating uncommon interferences, or leading to the discovery of new interferences.

This poster will provide an overview on novel developments in ICP-MS, providing an unmatched ability to tackle samples with outstanding complexity, both in terms of matrix load and interferences. It will show how even %-levels of total dissolved solids can be handled effectively, and how the superior interference removal of a triple quadrupole ICP-MS system can be combined with the speed and simplicity of a single quadrupole ICP-MS system.

Poster Nr. 82

Fast Single Particle ICP-MS with Nanosecond Time Resolution as a New Tool for Nanomaterial Characterization

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Over the last two decades, single particle inductively coupled plasma mass spectrometry (spICP--MS) has emerged as a powerful method for nanomaterial analysis. For example, the detection and characterization of nanoparticles in environmental waters has the potential to aid in the proper conduct of toxicity studies and risk assessment. Initially, the technique utilized millisecond time resolution in a transient measurement of a colloid dispersion to enable the detection of individual particles. Recently, microsecond time resolution helped to improve the particle size detection limit of spICP--MS by reducing the continuous background signal, addressed issues of split-particle events and particle coincidence, and also provided insights into the temporal profile of individual ion clouds. [1]

In this proof-of-principle study, we present our contribution to spICP--MS developments with a novel in-house built data acquisition system with nanosecond time resolution (nsDAQ) and a matching data processing approach. The new system can continuously sample the secondary electron multiplier (SEM) detector signal and enables the detection of gold nanoparticles (AuNP) as small as 7 nm with a commercial single quadrupole ICP-MS instrument.

The nsDAQ records the SEM signal in intervals of approximately 4 ns. A tailored method was developed to process this type of transient data, which is based on determining the temporal distance between detector events (so-called event gap (EG)). The EG can be used to discriminate between particle signals and background signals in the data from nanoparticle suspension analyses. In the plasma, the colloids are transformed into ion clouds with a temporally confined high ion density, while the dissolved background analytes lead to a constant stream of ions with a lower density.

The scope of the current study is to evaluate the capabilities of the nsDAQ for fast nanoparticle characterization including particle size and particle number concentration calibration.

[1] I. Strenge, C. Engelhard, J. Anal. At. Spectrom. 31, 135 (2016), DOI: 10.1039/C5JA00177C.

Poster Nr. 83

Overcoming sample preparation challenges in the speciation analysis of gadolinium-based contrast agents in tissue

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Gadolinium-based contrast agents (GBCAs) have been used in magnetic resonance imaging (MRI) for more than three decades. Due to the toxicity of free Gd3+ ions, linear or macrocyclic aminopolycarboxylic acid chelating ligands are used to create these highly stable metallopharmaceuticals, which are usually excreted unmetabolized after MRI examination and generally considered safe. However, there is a risk of side effects associated with Gd depositions inside the human body, and the retention behavior of gadolinium after GBCA administration is not yet fully understood. Therefore, gaining species-related information about the binding form of these depositions is of high interest. After extensive preparation of tissue samples, speciation analysis of individual Gd compounds can be achieved by combining anion-exchange chromatography (IC) and highly sensitive inductively coupled plasma-mass spectrometry (ICP-MS). However, preventing species conversion during this preparation of tissues containing GBCAs and other Gd species is essential and was the focus of this work.

For this purpose, the first preparation step of bead mill-assisted tissue homogenization was evaluated by analyzing different ceramics-, glass-, metal- or garnet-based bead materials. The elemental composition of these materials was determined using micro x-ray fluorescence (μ XRF) analysis. The release of metal ions from the bead material during homogenization in water or chelating ligand solution was quantified using ICP-MS and related to the composition of the bead material. Subsequently, speciation analysis by means of IC-ICP-MS was carried out to investigate the possible transmetalation of the linear contrast agent gadodiamide with dissolved bead material in water or tissue matrix. In the following sample preparation step, the tissue homogenate was centrifuged to obtain the water-soluble fraction as the supernatant. Matrix components interfering with IC-ICP-MS speciation analysis must then be removed from the supernatant by centrifugal ultrafiltration. Centrifugal filters with membranes based on composite regenerated cellulose (CRC), cellulose triacetate (CTA), or polyethersulfone (PES) were compared regarding the recovery of macrocyclic as well as linear GBCAs.

Using μ XRF and ICP-MS, the release of metal ions during homogenization could be observed and related to the composition of the individual bead materials. In comparison of water and chelating ligand matrix, it could be shown that the latter significantly increases the concentration of certain metal ions in the solution. Transmetalation of the linear GBCA gadodiamide with metal ions dissolved from bead materials based on transition metal-stabilized ceramics was observed by speciation analysis with IC-ICP-MS. This highlights the importance of bead selection to prevent the conversion of species during the preparation of GBCA-containing samples. Regarding the recovery of GBCAs after centrifugal ultrafiltration, the CRC membrane exhibited a decreasing recovery with increasing charge of the contrast agent and additional ligand-dependent effects. Contrary to this, CTA and particularly PES showed a high GBCA recovery independent of its charge and its ligand.

Poster Nr. 84

Single particle ICP-MS method development and comparison for nanoparticles dispersed in organic and aqueous solvents

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In recent years, single particle ICP-MS (spICP-MS) has been established as an powerful method for the analysis of nanoparticles (NP). SpICP-MS allows both a versatile characterization of NPs with respect to their size, composition, and concentration as well as the discrimination between intact NPs and dissolved ionic species in one analysis. Most applications of spICP-MS have been limited to the study of aqueous systems. However, a large fraction of NPs is synthesized and stored in organic solvents, which are generally difficult to analyse by ICP-MS.

In order to compare spICP-MS analysis in organic solvents, with those in the aqueous phase, a series of measurements was performed with citrate-stabilized AuNP on a quadrupole ICP-MS using three sample introduction systems; A Scott spray chamber, a two-stage desolvation system, and a total consumption spray chamber. The sample introduction systems were compared in terms of signal intensity, sensitivity, and transport efficiency.

Using the Scott spray chamber and the desolvation system, a 2.65-fold greater sensitivity of AuNP in water was observed compared to MeCN. The decreased sensitivity in MeCN indicates an increased plasma load and consequently less available energy for atomization and ionization of the analytes. By using the total-consumption nebulizer system, the oxygen flow could be reduced and optimized due to the lower flow rates. Thus, the sensitivity for MeCN could be increased almost to the same level as with water, accompanied by an increased linear range. Furthermore, higher transport efficiencies of AuNPs in organic solvents over water could be achieved.

Poster Nr. 85

Labeling, Separation and Structural Analysis of O-Glycans by LC- and MS-Based Techniques

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O- and N-glycosylations represent major post-translational modifications of proteins. These complex oligosaccharides not only affect the structure and function of proteins but also play important roles in cellular signaling and immunity. To determine biomarkers for certain diseases and investigate further functions of glycoproteins, a robust and reliable method for the analysis of O-glycans is required.

Driven by powerful separation techniques like liquid chromatography (LC) and ion mobility spectrometry (IMS), LC-MS/MS and IM-MS/MS emerged as useful tools and represent the current state-of-the-art methods for N- and O-glycan analysis. The lack of UV-absorbance in glycans constitutes a challenge for glycan separation, analysis and/or purification by liquid chromatographic methods. Numerous protocols have been described for the derivatization or labeling of N-glycans. The case of O-glycan profiling poses a particular challenge due to the occurrence of more complex core structures and a lack of a universal glycan-cleaving enzyme. Therefore, the current method for O-glycan release is based on a reductive β-elimination, which leads to the loss of the sugars reducing end and impedes further derivatization or labeling at this position. The existence of a vast pool of structures and isomers increases the complexity of O-glycan analysis even more.

In this work, a method was established for the release of O-glycans in non-reducing conditions and for their labeling using a procainamide fluorescent label. The method was first developed on fetuin O-glycans, whose glycan composition is simple and known. Released and labeled O-glycans were separated by hydrophilic interaction liquid chromatography (HILIC) and monitored by fluorescence detection. The protocol was subsequently applied to highly glycosylated proteins such as bovine submaxillary mucins (BSM) and porcine gastric mucins (PGM) and the LC gradient was adapted to the wide variety of mucin O-glycan structures. The results show excellent separations for both simple and highly complex O-glycan mixtures and provide a perfect basis for future analyses. For the first time, a highly heterogeneous O-glycan mixture from mucins is labeled and separated using HILIC-FLD. The labeled O-glycan structures are characterized by negative ion mode MS/MS and IMS.

The established labeling and separation procedure is used to prepare single-isomer O-glycan standards by fractionation. The great advantage of this method is the possibility to generate purified O-glycan standards, avoiding the tedious and often impossible O-glycan standard synthesis. The custom-made standards will be used for further structural analysis by cryogenic gas-phase infrared spectroscopy – a technique that requires high purities in terms of isomeric structure.

Poster Nr. 86

Pipetting-free single cell analysis with the label-free proteoCHIP and the Evotip adapter for high sensitivity proteomics on the timsTOF SCP

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Single cell proteomics is a rapidly developing field with the potential to make important contributions to the understanding of cellular heterogeneity. Single cell protein extraction, minimal exposure of samples to surfaces and optimal storage and transfer conditions are crucial for loss-less single cell proteome analyses. Recent enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry established in the timsTOF SCP paired with automated single cell sorting and sample preparation realized with the cellenONE® platform allows for sensitive proteome analyses at the single cell level.

Sample pick-up directly from the label-free proteoCHIP was assessed with HeLa lysate digests (Pierce) showing excellent reproducibility at various concentrations. Injections of 250 pg of HeLa peptides on column (1 µL in well) resulted in 15,000 peptides from 2,600 proteins which was matched by 250 pg HeLa peptides injected from a vial (250 pg/µL). We then analyzed single HeLa cells which were directly isolated and prepared in the label-free proteoCHIP (48 cells) and identified in average more than 3400 proteins per single cell with good reproducibility. 3200 proteins were quantified in at least half of the analyzed samples.

Further, we assessed the Evotip adapter by first comparing manual transfer versus transfer by centrifugation. Half of the prepared cells in the Evotip adapter plate were manually pipetted onto the Evotips whereas the remaining half were transferred by centrifugation. Data analysis revealed increased precursor identification reproducibility in cells transferred by centrifugation. In a second experiment, HeLa cells were isolated according to their cell size and binned into either 18 – 20 µm, 21 – 23 µm, 24 – 26 µm or 27 – 30 µm cell size groups. Protein group identification rates across the different cell size groups were comparable with all being close to 4000 protein groups. However, the reproducibility in the smaller cells (18 – 23 µm) was better than in the larger cells (24 – 30 µm) with CVs of 4.69 % (18 – 20 µm), 2.68% (21 – 23 µm), 7.65% (24 – 26 µm), and 13.05% (27 – 30 µm).

Quantitative comparison between the protein groups identified in the different cell size groups was performed. 3922 proteins were present in at least 48 single cells of the 96 single HeLa cells. Principal component analysis of the 2018 proteins identified in all 96 single cell showed clear difference between the small cells and the larger cells.

Poster Nr. 87

Subcellular detection of PEBCA particles in macrophages: combining darkfield microscopy, confocal Raman microscopy, and ToF–SIMS analysis

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The detection of biomedical organic nanocarriers in cells and tissues is still an experimental challenge. Here we developed an imaging strategy for the label-free detection of poly (ethylbutyl cyanoacrylate) (PEBCA) particles. Experiments were carried out with phagocytic NR8383 macrophages exposed to non-toxic and non-activating concentrations of fluorescent (PEBCA NR668 and PEBCA NR668/IR), non-fluorescent (PEBCA), and cabazitaxel-loaded PEBCA particles (PEBCA CBZ). Exposure to PEBCA NR668 revealed an inhomogeneous particle uptake similar to what was obtained with the free modified Nile Red dye (NR668). In order to successfully identify the PEBCA-loaded cells under label-free conditions, we developed an imaging strategy based on enhanced darkfield microscopy (DFM), followed by confocal Raman microscopy (CRM) and time-of-flight secondary ion mass spectrometry (ToF–SIMS). Nitrile groups of the PEBCA matrix and PEBCA ions were used as suitable analytes for CRM and ToF–SIMS, respectively. Masses found with ToF–SIMS were further confirmed by Orbitrap-SIMS. The combined approach allowed to image small (< 1 µ m) PEBCA-containing phagolysosomes, which were identified as PEBCA-containing compartments in NR8383 cells by electron microscopy. The combination of DFM, CRM, and ToF–SIMS is a promising strategy for the label-free detection of PEBCA particles.