DGMS

Deutsche Gesellschaft für Massenspektrometrie

DGMS 2019

Proceedings of the 52nd Annual DGMS Conference

in Rostock

1218 HANSE- UND UNIVERSITÄTSSTADT 1419 ROSTOCK

DGMS 2019

52nd annual conference

Universität Rostock Traditio et Innovatio

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

JOINT MASS SPECTROMETRY CENTRE

Impressum
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Welcome to the 52nd annual conference of the DGMS

The annual conference of the German Society for Mass Spectrometry (DGMS) takes place from 10th to 13th of March, 2019, together with the Spring Meeting of the German Physics Society (DPG). This double conference shall be hosted by the **University of Rostock** as a main event of its 600 years anniversary celebrations.

We will start the scientific program on Sunday with four workshops which shall take place prior to officially opening the conference with the **Wolfgang Paul lecture**. The Sunday evening reception at the City Hall of the **City of Rostock** will provide an opportunity to get acquainted with the city´s history which now covers 800 years. From Monday on through Wednesday, the scientific program of the conference will provide a balanced mix of exciting plenary lectures, parallel sessions, poster presentations, and lunch seminars. In addition, information on new industrial developments will be exhibited at the booths of well-known companies.

We, the local organizers together with our teams, cordially invite everyone to join us for the **52nd annual conference of the DGMS** in Rostock. We are looking forward to welcoming you for an exciting meeting.

600 Years University of Rostock

Blücherplatz (today: University square) in Rostock 1919

The clergy in the person of Rostock region's religious leader, the Bishop of Schwerin, as well as the university's actual founders and donators, the dukes Johann IV and Albrecht V of Mecklenburg-Schwerin, and the mayor and councilors of the City of Rostock, 200 years old at that time, addressed the ecclesiastical leader in 1418. And Mecklenburgean persistence paid off. Pope Martin V issued the permission certificate in Ferrara on 13 February 1419, thus, giving his blessing to "extracurricular studies" in Rostock. The ceremonial opening of the university with a Faculty of Law, a Medical Faculty and the Facultas Artium took place on 12 November 1419 at Rostock's St. Mary's Church. Magister Petrus Stenbeke from Erfurt was elected as rector. He enrolled 160 students. A pair of small scepters for the first established faculty was handed over to the rector, who was dean of the Faculty of Arts at the same time. The later established faculties remained without sceptre. The small sceptres of the Faculty of Arts had also to serve as university sceptres as the large sceptres symbolizing the autonomy and jurisdiction granted to the university by the souvereigns were not provided.

The establishment of the missing Theological Faculty was approved by Pope Eugen IV, successor of Pope Martin, in 1433.

The University of Rostock is the Baltic Sea region's oldest university and one of the oldest universities in Germany. Today, the University of Rostock comprises of nine faculties. About 13,300 students are enrolled. The share of international students is above eleven percent. With more than 100 courses of study, the University of Rostock is one of the German universities with a particularly broad range of subjects.


University's main building, University square (Copyright: University of Rostock)

The Hanseatic and University City Rostock

With over 200.000 inhabitants, the Hanseatic and University City of Rostock is not only the largest city in the state of Mecklenburg Western Pomerania but also its economic, scientific, and cultural center. Rostock´s urban districts, the most famous may be Warnemünde, presents its beautiful historic quarters and gothic houses to all the guests from all over the world and invites to spend time at the water front and enjoy local cuisine in maritime atmosphere.
Rostock is proud of its famous emblems that come in sevens: seven doors to St. Mary's church as well as seven towers on top of the mayor´s hall; seven streets from New Market square; seven inland gates that lead out of town; seven bells in seven city churches; and seven linden trees in the city´s rose garden. During the Hanseatic time, Rostock was one of the very most important members of the Hanseatic League and of great significance as a trading post. Rostock´s majestic cathedral, St. Mary, hosts the oldest still functional astronomical clock in the world. Built in 1472 this masterpiece of handcraft and of art tells us everyday´s time ever since. The perhaps most famous Rostock citizen, Gebhard Leberecht von Blücher, should be mentioned here as well, since he was one of the very few who managed to defeat Napoleon Bonaparte - in the Battle of Waterloo. Of particular architectural value for Rostock are the extraordinary buildings from Ulrich Müther, such as the "tea pot" in Warnemünde or the catholic "Christ church". In the 1970s these were built as unique hyperbolic paraboloid shell constructions, which since decorate distinguished sites in Rostock.
Today Rostock links wealth in stories and richness in tradition with beautifully restored historical sites and with progressive development, making the Hanseatic and University City of Rostock a modern city with both, a Nordic charm and Hanseatic flair.

Rostock's Double Anniversary

With its city rights granted on the 24th of June in 1218, the Hanseatic City of Rostock celebrated its 800th birthday in 2018. This year - 2019 - Rostock University, the oldest university in the Baltic Sea region, has also joined in by celebrating its 600-year anniversary. Both dates will be commemorated by inhabitants, city partners and guests alike. With numerous impressive events, exhibitions, activities and inaugurations, 2019 will provide an abundance of cultural highlights.

800 Jahre wird die Hanse- und Universitätsstadt Rostock 2018 alt. Foto: Axel Merkel

To stay informed about all events, stay up-to-date on www.rostock800600.de.

We warmly invite you to join the celebrations and hope you enjoy your visit!

General Information

Registration and Help Desk

The registration and help desk is located in house 1, seminar room 022. Registration and information desk will be open as follows:

Sunday (March 10th): 10:30 – 18:00

Monday (March 11th): 08:00 – 16:00

Tuesday (March 12th): 08:00 – 16:00

Wednesday (March 13th): 08:00 – 14:00

Name Badge

Your personal badge is your entrance ticket to all sessions and the exhibition. Please remember always to wear your badge. Your name badge will also serve as a ticket to all lines of the public transportation system within Rostock (VVW – Verkehrsverbund Warnow).

WIFI & Mobile Phone Policy

“Eduroam” is available on the campus. If you have no access to eduroam, you can get you personal wifi code in the conference office. Access to the full conference program is available on ConfTool website or using the “DGMS 2019” app in goole playstore or iTunes store.

Delegates are advised that, mobile phones have to be switched off in the meeting rooms.

Conf Tool **Google Play Store** **iTunes Store**

Welcome evening with reception and visit of St. Mary’s Chruch

The welcome reception is scheduled for Sunday (March 10th) at 19:30 in the City hall of Rostock. (Adress: Neuer Markt 1a, 18055 Rostock). The Mayor of the City of Rostock will give insight into 800 years of the city´s history. Refreshments will be provided. The guided Cathedral Tour will take place on Sunday evening after the welcome reception in the city hall associated with a short evening prayer and organ music.

Poster Evening

For Monday evening it is planned to blur the border between science and amusement. With some music, snacks and interesting conversation we want to finish the the day in the marquee.

Conference Dinner

The Conferene Dinner is scheduled for Tuesday (March 12th) evening at 19:30 at the Nikolai church (Adress: Bei der Nikolaikirche 1, 18055 Rostock).

Instructions for Oral Presentation

Each speaker of a parallel session (PS) will be given 20 minutes for presentation. Please plan your talk for 15 minutes to allow for introduction and questions. Speakers are asked to upload their electronic presentation files (powerpoint or pdf-files; landscape orientation) from USB drives onto the presentation PC, the latest 30 min prior to session begin. Please walk up to the podium in the lecture hall which is assigned to your session to meet our technical staff for assistance, and please introduce yourself to the session chair. Each parallel session room is equipped with PC computers running Windows and Microsoft Office. Be sure to test your presentation on these platforms. Slides should be prepared in English. DGMS policy states that institutional or company logos may appear only on the title slide. Microphones and pointers are provided.

Instructions for Poster Presentation

Each poster space is A0, portrait orientation. Each board is numbered in the upper left corner. Institutional logos are restricted to one logo per institution. When authors are from multiple institutions, this means there may be one logo per each institution. Posters are to be set-up on Sunday at 2:00-5:00 PM. Posters should be removed on Wednesday at 1:00-2:00 PM the latest. All posters shall be on display throughout the duration of the conference. Poster sessions are scheduled for Monday afternoon (poster session I) and Tuesday afternoon (poster session II) from 5:15-7:00 PM. A presenter whose poster number is an odd number is asked to present his/her poster during poster session I. A presenter whose poster number is an even number is asked to present his/her poster during poster session II.

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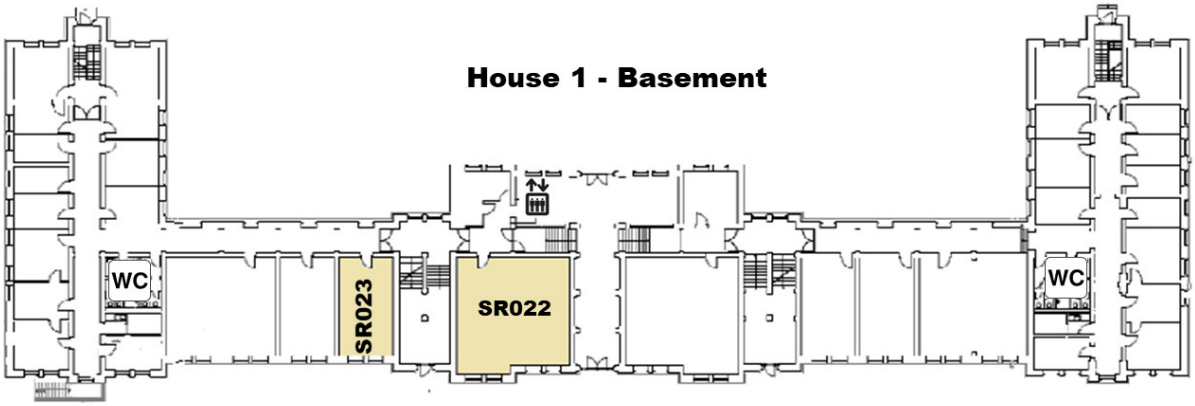
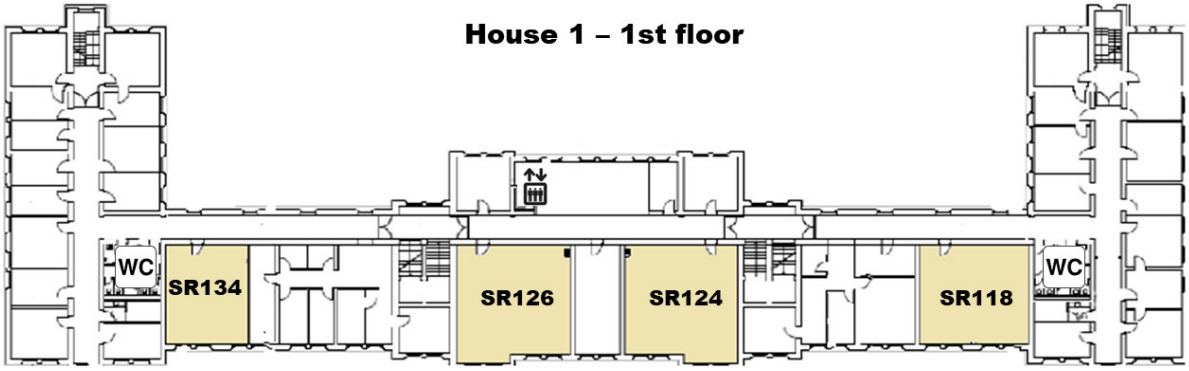
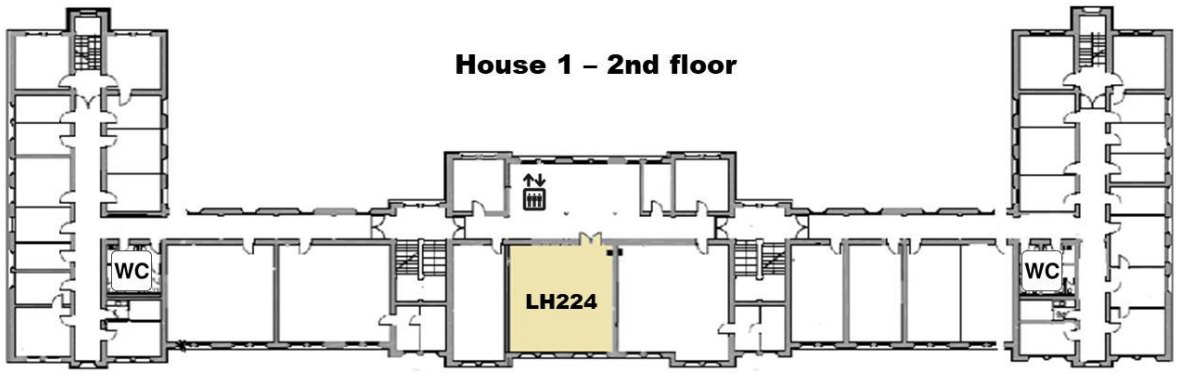
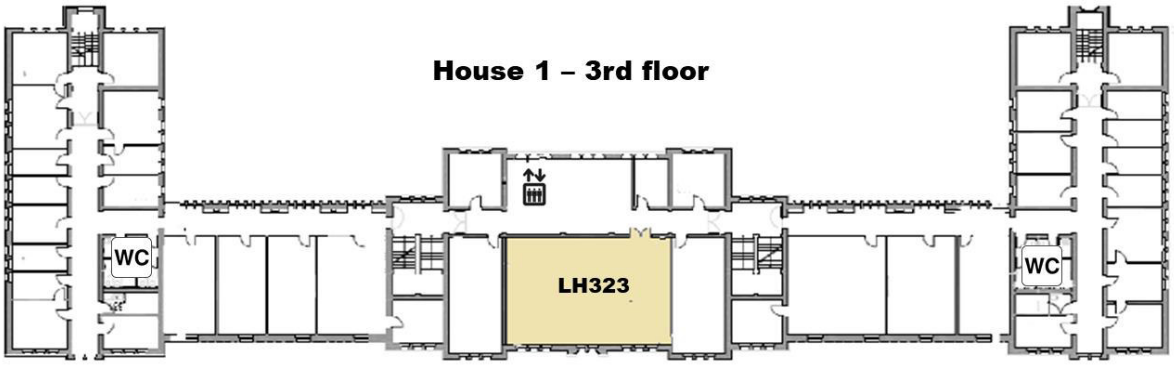
Locations / Maps

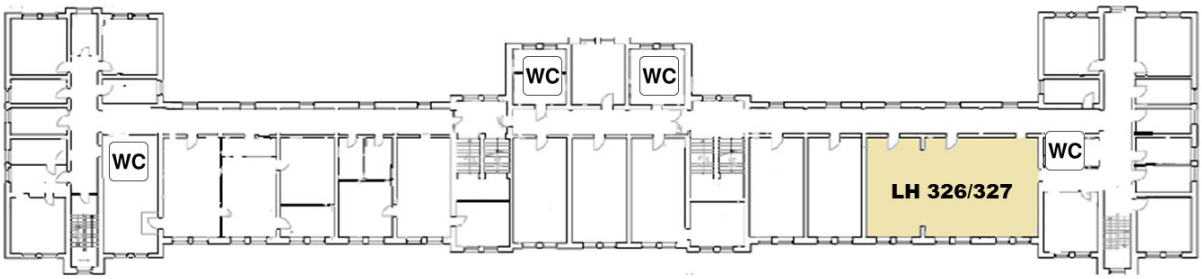




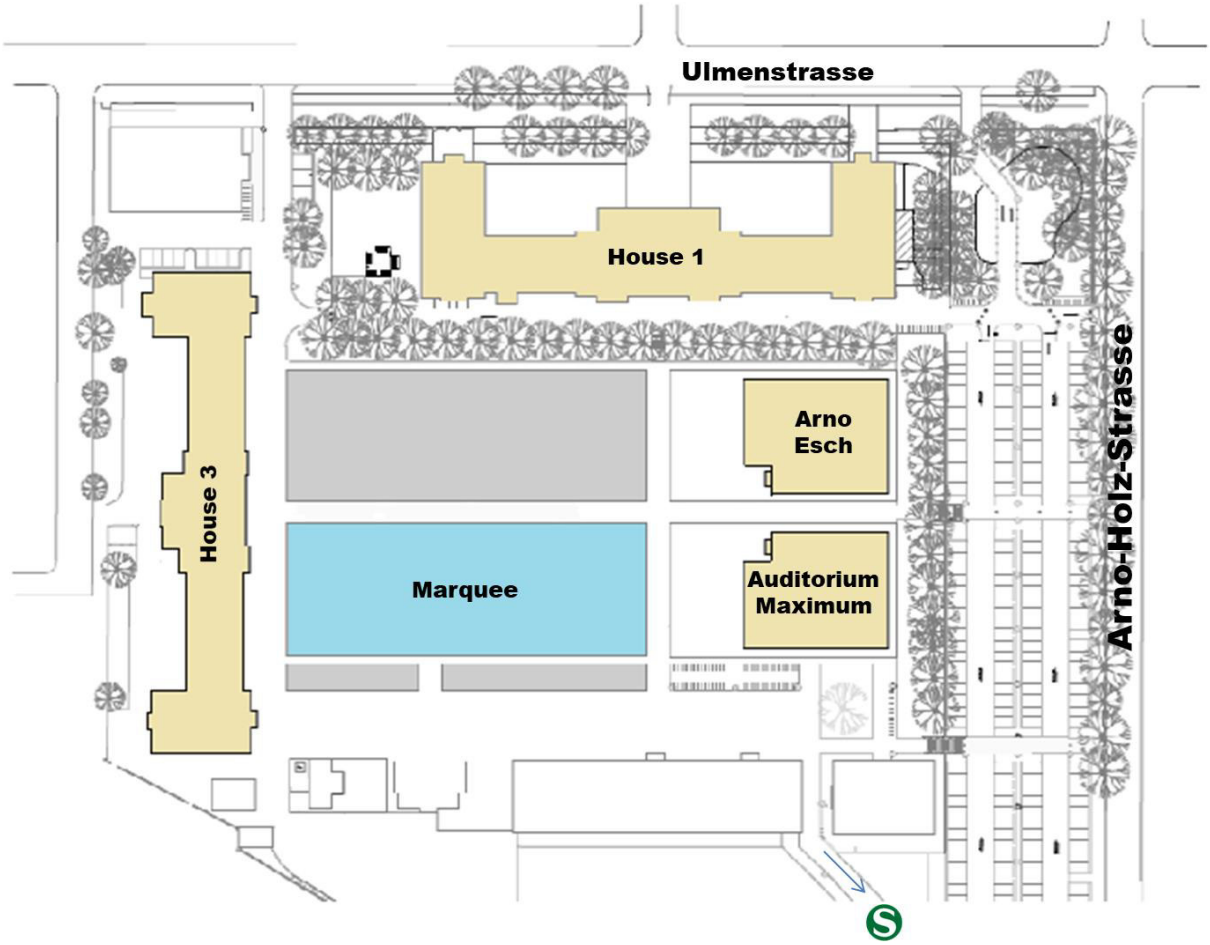




House 3 – 3rd floor



Campus



Program Schedule

Date: Sunday, 10/Mar/2019

11:30am - 6:00pm SR022/Lobby (Conference Office)	**Conference Registration**
2:00pm - 5:00pm SR118	**WS01: Workshop 01 - LC-MS** Session Chair: **Marcel Kwiatkowski**
2:00pm - 5:00pm SR124	**WS02: Workshop 02 - Affinity-MS** Session Chair: **Michael Przybylski** Session Chair: **Michael O. Glocker**
2:00pm - 5:00pm SR126	**WS03: Workshop 03 - Core-facilities** Session Chair: **Sabine Metzger**
2:00pm - 5:00pm SR134	**WS04: Workshop 04 - Lipidomics** Session Chair: **Robert Ahrends**
3:20pm - 3:40pm Marquee	**Networking (Coffee break)**
5:30pm - 6:15pm Audimax	**Welcome: Conference Welcome DGMS 2019** Session Chair: **Andrea Sinz** Session Chair: **Michael O. Glocker**
6:15pm - 7:00pm Audimax	**WP: Wolfgang Paul lecture** Session Chair: **Andrea Sinz** Session Chair: **Michael O. Glocker**
7:30pm - 10:00pm City Hall	**Welcome Reception**
10:00pm - 11:00pm City Hall	**Visit of St. Mary's Church** Guided tour through the church of St. Mary's including an evening prayer and organ music. You will have the chance to see the famous medieval astronomical clock.

Date: Monday, 11/Mar/2019

8:00am - 4:00pm SR022 (Conference Office)	**Conference Registration**
8:15am - 9:00am Audimax	**PL 01: Quantum optics and information science in multi-dimensional photonics networks *(Christine Silberhorn*)**
9:00am - 9:45am Audimax	**PL 02: Interstellar radionuclides identified in deep-sea archives (*Anton Wallner*)** Session Chair: **Michael Block**
9:45am - 10:30am Marquee	**Networking (Coffee break)**
10:30am - 12:30pm HS323	**PS 01: From fundamentals in Proteome Research to Structural Proteomics** Session Chair: **Charlotte Uetrecht**
10:30am - 12:30pm HS224	**PS 02: MS instrumentation I** Session Chair: **Bernhard Spengler**
10:30am - 12:30pm HS326/327	**PS 03: Lipidomics & Metabolomics I** Session Chair: **Heiko Hayen**
12:35pm - 1:45pm SR124	**Lunch 1: Lunch Seminar supported by Bruker Daltonik** Session Chair: **Martin Sklorz**
12:35pm - 1:45pm SR126	**Lunch 2: Lunch Seminar supported by Leco** Session Chair: **Ralf Zimmermann**
12:35pm - 1:45pm SR134	**Lunch 3: Lunch Seminar supported by Agilent** Session Chair: **Cornelia Koy**
12:35pm - 1:45pm SR118	**Lunch 4: Lunch Seminar supported by Sciex** Session Chair: **Thorsten Streibel**
2:00pm - 4:00pm HS323	**PS 04: Affinity-Mass Spectrometry** Session Chair: **Michael Przybylski**
2:00pm - 4:00pm HS224	**PS 05: MS instrumentation II** Session Chair: **Klaus Dreisewerd**
2:00pm - 4:00pm HS326/327	**PS 06: Lipidomics & Metabolomics II** Session Chair: **Dietrich Albert Volmer**
4:00pm - 4:30pm Marquee	**Networking (Coffee break)**
4:30pm - 5:15pm Audimax	**PL 03: Application of Mass Spectrometry for Detecting and Finding Cures to Brain Disorders (*Joseph A. Loo*)** Session Chair: **Michael O. Glocker**
5:15pm - 7:00pm Marquee	**Poster Session I** On **Monday** the presenters of posters with an **odd** number are asked to present their poster.
7:00pm - 10:00pm Marquee	**Poster Evening** Poster Evening with live music and finger food

Date: Tuesday, 12/Mar/2019

8:00am - 4:00pm SR022 (Conference Office)	**Conference Registration**
8:15am - 9:00am Audimax	**PL 04: Exploring Exotic Elements - all about Astatine and Actinides as accessible from Laser Mass Spectrometry (*Klaus D.A. Wendt*)** Session Chair: **Mathias Schäfer**
9:00am - 9:45am Audimax	**PL 05: Light and cavity induced new states of matter: Quantum Electrodynamical Density Functional Theory (QEDFT) (*Angel Rubio*)**
9:45am - 10:30am Marquee	**Networking (Coffee break)**
10:30am - 12:30pm HS323	**PS 07: Proteomics – Biological and Clinical Applications** Session Chair: **Claudia Röwer**
10:30am - 12:30pm HS224	**PS 08: Instrumentation and Application of MS Imaging** Session Chair: **Andreas Römpp**
10:30am - 12:30pm HS326/327	**PS 09: Environmental, natural product and forensic MS** Session Chair: **Uwe Karst**
12:35pm - 1:45pm SR124	**Lunch 5: Lunch Seminar supported by Thermo Scientific** Session Chair: **Christopher Paul Rüger**
12:35pm - 1:45pm SR126	**Lunch 6: Lunch Seminar supported by Shimadzu** Session Chair: **Claudia Röwer**
12:35pm - 1:45pm SR134	**Lunch 7: Lunch Seminar supported by Waters** Session Chair: **Cornelia Koy**
12:35pm - 1:45pm SR118	**Young Scientists: Young Scientists network of DGMS** Session Chair: **Ansgar Korf** Session Chair: **Janine-Denise Kopicki**
2:00pm - 2:40pm HS323/HS224	**PL 06: Circular Dichroism and Mass Spectrometry: An Unusual Liaison (*Ulrich Boesl*)** Session Chair: **Ralf Zimmermann**
2:40pm - 3:20pm HS323	**MS 01: Ion physics & ion chemistry** Session Chair: **Ralf Zimmermann**
3:20pm - 4:00pm HS323	**DGMS meeting: General assembly of DGMS members** Session Chair: **Andrea Sinz**
4:00pm - 4:30pm Marquee	**Networking (Coffee break)**
4:30pm - 5:15pm Audimax	**PL07: Chemistry first, Accelerator Mass Spectrometry (AMS) second (*Silke Merchel*)** Session Chair: **Bernhard Spengler**
5:15pm - 7:00pm Marquee	**Poster Session II** On **Tuesday** the presenters of Posters with an **even** number are asked to present their poster.
7:30pm - 11:59pm Nikolai Church	**Conference Dinner**

Date: Wednesday, 13/Mar/2019

8:00am - 2:00pm SR022 (Conference Office)	**Conference Registration**
8:15am - 9:00am Audimax	**PL08: The future of time: prospects for a redefinition of the SI second (*William D. Phillips*)**
9:00am - 9:45am Audimax	**PL 09: Chemical Energy Storage: a Key Element for a Sustainable Energy Future (*Ferdi Schüth*)**
9:45am - 10:30am Marquee	**Networking (Coffee break)**
10:30am - 12:30pm HS323	**PS 10: Award Lectures** Session Chair: **Andrea Sinz**
10:30am - 12:30pm HS224	**PS 11: Outside the box** Session Chair: **Ralf Zimmermann** Session Chair: **Michael O. Glocker**
12:30pm - 1:00pm Marquee	**Poster Prizes supported by Jeol**
1:00pm - 2:00pm Audimax	**Farewell** Session Chair: **Andrea Sinz**

Wolfgang Paul & Plenary Speakers

Vicki Wysocki

Vicki Wysocki received her BS in Chemistry at Western Kentucky University in 1982 and her PhD in Chemistry at Purdue University in 1987. After doing postdoctoral work at Purdue and at the Naval Research Laboratory, she joined Virginia Commonwealth University as an Assistant Professor in 1990. She was promoted to Associate Professor in 1994. Vicki joined the University of Arizona in 1996 and was promoted to Professor in 2000. Most recently she was Chair of the Department of Chemistry and Biochemistry at Arizona. Vicki joined OSU in August 2012 as an Ohio Eminent Scholar.

Anton Wallner

Assoc. Professor Anton Wallner received his Ph.D. in Experimental Physics in 2000 from the University of Vienna. He was postdoctoral fellow 2000-2003 at the Technical University (TUM) and the Ludwig-Maximilian-University of Munich (LMU), and from 2003-2010 research scientist at the Institute for Isotope Research and Nuclear Physics, University of Vienna. 2010 he moved to Australia, taking up a position as Senior Research Scientist at the Institute for Environmental Research, the Australian Nuclear Science and Technology Organisation (ANSTO) and joined 2011 the Australian National University (ANU, Canberra) as a staff scientist. Wallner is Senior Fellow and Assoc. Professor at the Research School of Physics and Engineering. Since 2014 he is AMS (Accelerator Mass Spectrometry) group leader at the Department of Nuclear Physics, ANU.

Joseph A. Loo

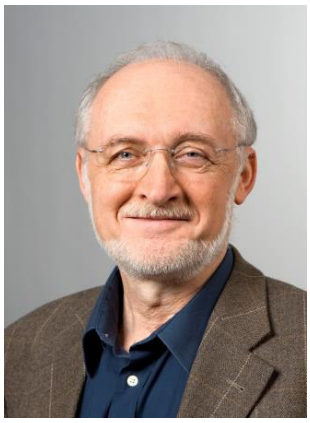
Joseph A. Loo is a Professor in the Department of Biological Chemistry, School of Medicine, and in the Department of Chemistry & Biochemistry at the University of California, Los Angeles (UCLA). His research interests include analytical chemistry, the mass spectrometry characterization of peptides and proteins and post-translational modifications, and their application for proteomics and disease biomarkers. Dr. Loo received his Ph.D. in analytical chemistry from Cornell University. He carried out research as a post-doctoral fellow, and later as a Senior Scientist, at Pacific Northwest National Laboratory (Richland, WA). He is the author of over 300 scientific publications. He is on the Editorial Boards of several scientific journals, and currently he is the Editor-in-Chief for the Journal of the American Society for Mass Spectrometry.

Klaus Wendt

Prof. Klaus Wendt studied physics at the Johannes Gutenberg-University of Mainz, where he got attracted by laser physics and quantum optics. After his diploma in 1981 he went to CERN to do a PhD on the study of nuclear structure by laser spectroscopy at the radioactive ion beam facility ISOLDE. After graduation he stayed there as CERN fellow up to 1986. Returned back to Mainz University, the Chernobyl nuclear disaster and its consequences made him establish a research group working on ultra-trace isotope determination using laser mass spectrometric techniques. Today he is professor and group leader in the field of laser resonance ionization spectroscopy and its applications. In addition, he strongly addresses teacher education and physics didactics aspects.

Silke Merchel

Silke Merchel received her diploma in chemistry and PhD at the University of Cologne. After Post-Doc positions at the Max-Planck-Institute for Cosmochemistry in Mainz and the Federal Institute for Materials Research and Testing (BAM) in Berlin, she was a Marie Curie Fellow at the CNRS institute CEREGE (Aix-en-Provence, France). She is an expert on using “big machines” like particle accelerators, neutron reactors and synchrotron facilities for analytical purposes. Since 2008, she is working at the Dresden Accelerator Mass Spectrometry facility at the Helmholtz-Zentrum Dresden-Rossendorf. Her scientific interest is in improving (radio-) chemical separation and AMS measurements for producing better radionuclide data used for Earth and Planetary Research such as astrophysics, climate and ocean sciences, cosmochemistry and geomorphology.



Ulrich Boesl

Diploma in physics in 1973; doctorate at the TUM in 1978; research scholarship at the physics department Stanford in 1982/1983; scientific employee at TUM from 1984 on; habilitation at TUM 1988; Mattauch-Herzog award in 1992; apl professorship at TUM since 1999; visiting professor at the Anhui-Institute / China since1999. Since 1988 projects on the development of new mass spectrometric methods and on fast analytical detection techniques of traces of air pollutants, e.g. from combustion engines; since 1994 research projects on mass-selective laser spectroscopy of molecules, molecular cations and anions; since 2005 research projects on the combination of laser mass spectrometry and circular dichroism; ERC-advanced grant „Asymmetric Cluster Catalysis & Chemistry“ together with U. Heiz 2010 – 2015.

Awardees

Award for “MS in Biowissenschaften”

Prof. Dr. Bernhard Küster (https://proteomics.wzw.tum.de)
Awarded for the “Characterization of the human Proteome and Kinome”

Mattauch-Herzog Award

Dr. Jürgen Hartler (http://genome.tugraz.at)
Awarded for the “High-Throughput Identification of Lipids in BiologicalMaterial Using Software-Aided Analysis of LC-MS/MS Data”

Wolfgang-Paul-Prize for the best Master thesis

Frau Maike Lettow (Prof.Dr. Kevin Pagel, FU Berlin und Fritz Haber Institut Berlin)
worked on: „Investigation of the Gas-Phase Structures of Fucosylated Glycans Employing Cold-Ion Infrared Spectroscopy.“

Wolfgang-Paul-Prize for the best PhD thesis

Dr. Mario Kompauer (Prof. Dr. Bernhard Spengler, Justus Liebig University Giessen)
worked on: „Development of autofocusing and subcellularly resolving mass spectrometry imaging and itsapplication to biological questions.”

Wolfgang-Paul-Prize for the best PhD thesis

Dr. Florian Meier (Prof. Dr. Matthias Mann, LMU München und MPI Martinsried)
worked on: „Data Acquisition Methods for Next-Generation Mass Spectrometry-Based Proteomics.“

Agilent Research Summer

Andre Knoop (Prof. Dr. Mario Thevis, Deutsche Sporthochschule Köln)
worked on: “Detection of cobalt in Sport”

Workshops

WS01: LC-MS – Two dimensional liquid chromatography in LC-MS applications

Session Chair
Marcel Kwiatkowski

Session Abstract
The LC-MS workshop is focussed on two dimensional liquid chromatography (2D-LC) in combination with mass spectrometry. The workshop covers the basic concepts of two-dimensional liquid chromatography and latest developments of online 2D-LC-MS. Examples of application in the field of proteomics are presented and discussed."

Presentations

Introduction into Liquid Chromatography coupled to Mass Spectrometry with a focus on Two-dimensional Liquid Chromatography
Hartmut Schlüter
UKE, Germany; hschluet@uke.de

Liquid chromatography (LC) coupled to mass spectrometry (MS) is a hyphenated analytical technique (LC-MS), which is more and more dominating analytical and bioanalytical areas in academic research and diverse industries for qualification and quantification of substances or whole groups of molecules, like in metabolomics or proteomics. The combination of LC with MS is allowing the relative quantification and identification of thousands of molecules in complex mixtures. Since in biological samples the total amount of molecules comprises many ten-thousand molecules being present in very different abundances a two-dimensional (2D) LC prior to MS is allowing a deeper view into complex mixtures of individual molecules. In the workshop some concepts of 2D-LC-MS will be demonstrated and discussed.

Ideas of a toolbox for multidimensional LC/MS in chemical analytics
Rainer Wolf
BASF SE, Germany; rainer.a.wolf@basf.com

In chemical analytics typical challenges are

- The chromatographic system is not compatible to mass spectrometry
- Compounds which have to be identified are too unpolar to be ionized by ESI or APCI
- In additon to mass spectrometry other spectroscopic information are necessary to make structure elucidation of unknowns more valid

The lecture will disuss practical apects and examples to overcome these difficulties and present ideas wich tools can used in addion to LC or LC/MS. Orthogonal chromatographic techniques are discussed as well as ionization techniques to ionize unpolar components in the context of LC/MS.

Recent advances in online 2D-LC-MS for proteome analysis
Marcel Kwiatkowski
University of Groningen, Germany; m.d.kwiatkowski@rug.nl

This lecture gives a brief introduction to MudPIT technology and recent developments in online 2D-LC-MS for bottom-up and Top-Down proteomics. A stronger focus is on the use of displacement chromatography in the first dimension of separation in online SCX-RP-MS including a general introduction to displacement chromatography.

Accurate processing of multidimensional liquid chromatography (LC^n-)MS/MS data

Peter Horvatovich
University of Groningen, Netherlands, The; p.l.horvatovich@rug.nl

This presentation will start with providing an overview on spectral count and single-stage based label-free quantitative pre-processing of LC-MS/MS data using unidimensional liquid chromatography (LC). This part will present the Threshold Avoiding Proteomics Pipeline (TAPP), which is a single stage LC-MS/MS workflow as well approach how to compare quantitative single-stage LC-MS/MS data processing pipelines such as mzMine, OpenMS and SuperHirn. This will be later expended on strategies that process LC^n-MS/MS using multidimensional liquid chromatography (LC^n) in both type of quantification. The presentation will also discuss data obtained with short and long second LC gradient and assessment of peak capacity of LC^n-MS/MS system and how label-free and stable isotope labeled LC^n-MS/MS data pre-processing differs. The presentation will show LC^n-MS/MS data pre-processing of real clinical and biological studies.

WS02: Affinity - Mass Spectrometry

Session Chairs
Michael Przybylski
Michael O. Glocker

Session Abstract
The Special Interest Group “Affinity – Mass Spectrometry” fosters research activities and method development of affinity enrichment strategies combined with mass spectrometry.
This workshop will focus on
- pre-fractionation methods for complex biological samples and analysis of low abundant molecules
- affinity-based separation methods in mass spectrometric proteome research
- immuno-affinity mass spectrometry using specific capture molecules
- quantitative and functional analyses of protein-protein complexes by mass spectrometry

Presentations

Affinity-Enrichment methods in PTMomics
Martin R. Larsen
Department of Biochemistry and Molecular Biology, University of southern Denmark, Denmark; mrl@bmb.sdu.dk

Over the years we have developed a large number of methods for enrichment of post-translationally modified (PTM) peptides, from complex biological matrices, in order to make it possible to study these in nature. A PTM is the attachment of a chemical group to a protein after or during protein synthesis and the global analysis of PTMs is termed PTMomics. Many PTMs are reversible and numerous enzymes for their controlled attachment and removal exist, in fact about 5% of the total amount of proteins in a cell is controlling PTMs. Often a complex interplay between these enzymes modulates the PTM level of a protein to fine-tune the protein activity, function and interaction, allowing a level of delicate control of signaling pathways not possible in any other way.
In normal proteomics proteins are digested with specific enzymes and subsequently analyzed by LC-MSMS in order to identify or quantify the peptide in the sample. However, if peptides are carrying a PTM, very often they are not identified by LC-MSMS analysis as they are either low abundant (low stoichiometry modification), hydrophilic so they do not bind to the LC column or they are not ionizing in the MS analysis. As a consequence PTMomics is not possible without robust and efficient methods for enrichment of peptides carrying PTMs. In the presentation a number of unique enrichment methods that are used widely in the proteomics society will be illustrated and their application in large scale comprehensive PTMomics strategies to characterize cellular signaling will be shown.

Molecular Epitope Determination of Aptamer Complexes of the Multi-domain Protein C-Met by Proteolytic Affinity- Mass Spectrometry
Loredana Lupu, Pascal Wiegand, Nico Hüttmann, Stephan Rawer, Wolfgang Knleinekofort, Alexander Lazarev, Maxim V. Berezovski, Michael Przybylski
Steinbeis Center for Biopolymer Analysis and Biomedical Mass Spectrometry, Germany; loredanalupu92@gmail.com

C-Met protein is a glycosylated receptor tyrosine kinase of the hepatocyte growth factor heterodimer (HGF).C-Met has been found to be aberrantly activated leading to tumorigenesis and several other diseases and has been recognized as a biomarker in cancer diagnosis. C-Met aptamers have been recently considered a useful tool for detection of cancer biomarkers. Aptamers are single-stranded DNA or RNA oligonucleotides that can be easily produced and show stabilities and affinities comparable to monoclonal antibodies. Here we report a molecular interaction study of human C-Met protein with two DNA aptamers (CLN0003 and CLN0004), derived using the SELEX procedure. Epitope peptides of the aptamers on C-Met were identified by proteolytic affinity- mass spectrometry in combination with SPR biosensor analysis (PROTEX-SPR-MS), with the use of a high pressure proteolysis as an efficient tool for digestion and proteolytic-epitope extraction mass spectrometry. High affinities were determined for aptamer-C-Met complexes, with a two-step binding pathway suggested by kinetic analyses. A linear epitope peptide, C-Met (381-393) (NSSGCEARRDEYR) was identified for CLN0004. Subsequently, the

interaction of C-Met with the CLN0003 aptamer revealed an assembled epitope comprised of two specific peptide sequences. Structure modelling studies of the protein and the two aptamers were consistent with the identified epitopes. The high affinities of aptamers to C-Met, and the specific epitopes revealed should render them of high interest for cellular diagnostic studies. In addition, the molecular epitopes identification of DNA aptamer-protein complexes could be used to characterize therapeutic antibodies and to neutralize the pathophysiological responses.

Studying drug effects on the Proteome using Affinity Methods
Marcus Bantscheff
Cellzome GmbH, Germany; marcus.x.bantscheff@gsk.com

Over the past decade, our ability to identify molecular mechanisms underlying disease and study drug action by mass spectrometry-based proteomics has progressed remarkably. Improved instrumentation and new experimental approaches allow studying cellular and tissue phenotypes in a disease context, as well as upon modulation by bioactive molecules, with unprecedented resolution and dimensionality. Furthermore, the diversification of direct and indirect target identification methodologies allows for comprehensive analysis of cellular targets of bioactive compounds in live cells and the correlation between target engagement and proteotype effects. This talk focusses on the emerging role of mass spectrometry based proteomics in drug discovery and highlights experimental approaches and applications with impact for understanding efficacy and safety of drug candidates.

Affinity-Mass Spectrometry to Determine Target Proteins of Reactive Small Molecules
Samuel M. Meier-Menches[1,2], Christopher Gerner[2]
[1]Institute of Cancer Research, Medical University Vienna, Austria.; [2]Department of Analytical Chemistry, University of Vienna, Austria.; samuel.meier-menches@meduniwien.ac.at

Anticancer agents of the platinum-class including cisplatin, carboplatin and oxaliplatin are successful therapeutic small molecules that alkylate DNA and interfere with replication. Together with arsenic trioxide they are administered in roughly 50% of all cancer chemotherapies. In contrast, next-generation metal-based drug candidates are being discovered that may engage with protein targets, but their identification and validation proved to be a challenging task, partly due to the preconception that such reactive metal-based therapeutics would be highly unspecific.
Thus, a two-dimensional proteomics approach was established combining affinity purification and response profiling as a hypothesis-generating procedure to elucidate the cellular targets of such metal-based anticancer drug candidates [1,2]. The resulting target-response networks allow selecting the most probable target proteins due to their connection to the cellular response upon drug treatment.
We found that an organometallic ruthenium drug candidate selectively targets plectin, a scaffold protein and cytolinker. The organometallic compound seems to affect the protein-protein interaction between plectin and non-mitotic tubulins with considerable effects on cell shape and migration.
References:
[1] Samuel M. Meier and Christopher Gerner et al., An Organoruthenium Anticancer Agent Shows Unexpected Target Selectivity For Plectin, ***Angewandte Chemie Int. Ed.***, 2017, *56*, 8267–8271.
[2] Samuel M. Meier-Menches and Christopher Gerner et al., Time-Dependent Shotgun Proteomics Revealed Distinct Effects of an Organoruthenium Prodrug and its Activation Product on Colon Carcinoma Cells, ***Metallomics***, 2019, *11*, 118–127.

WS03: Workshop 03 - Core-facilities

Session Chair
Sabine Metzger

Session Abstract
The workshop serves as an exchange platform for MS based core facility managers and users of core facilities. During the workshop there will be presentation from funding bodies such as the DFG and the BMBF on the funding of large equipment and networks of MS based core facilities. It will also host presentations to describe and discuss strategies for the organization, coordination and invoicing strategies for core facilities in an academic environment.

WS04: Workshop 04 - Lipidomics

Session Chair
Robert Ahrends

Session Abstract
The workshop will offer a presentation of current tools developed in the LIFS consortium (de.NBI). The user will be guided how to develop a targeted assay for lipidomics. Therefore, different strategies for MS workflows such as SRM or PRM will be presented. The attendee will also get a brief overview about the do and don'ts using this strategies. We are looking forward to your attendance!

The second half of the workshop will serve as the official meeting of the DGMS Interest Group "Lipid analysis and Lipidomics" (https://dgms.eu/en/interest-groups/interest-group-lipidanalytik-und-lipidomics/) with the following agenda:
- Introduction of the interest group "Lipid analysis and Lipidomics"
- Determination of a quorum
- Discussion of the interest group charter
- Election of the interest group speakers
- Miscellaneous

To foster lipidomics technologies in biomedical and pharmacological research, the special interest group Lipid Analytics and Lipidomics provides a platform for interaction of international acknowledged scientists, students and young researchers. Specifically, we discuss emerging MS strategies, software developments and evolving applications in lipid science. We encourage all scientists interested in lipid research to participate.

Parallel Sessions Outline:

PS 01: From fundamentals in Proteome Research to Structural Proteomics

Monday, 11/Mar/2019: *Location:* **HS323**
10:30am - 12:30pm

Session Chair: **Charlotte Uetrecht**

10:30am - 10:50am
An Integrated One-Week Protocol for Proteome-Wide Cross-Linking/Mass Spectrometry Studies Based on the MS-Cleavable Cross-linker DSBU and the MeroX 2.0 Software
Claudio Iacobucci, Martin-Luther University Halle-Wittenberg, Germany

10:50am - 11:10am
From Structural Insight into an Ene-Reductase by Ion Mobility Mass Spectrometry to Experimental Conditions of a Biotransformation
Jens Sproß, Industrielle Organische Chemie und Biotechnologie, Universität Bielefeld, Germany

11:10am - 11:30am
Triggering and Monitoring of EBV's potential G-Quadruplexes in native mass spectrometry
Kira Schamoni, Heinrich-Pette Institut, Germany

11:30am - 11:50am
Assembly studies of the soluble part F_1 of bacterial F-type ATP synthases with LILBID-MS
Khanh Vu Huu, Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt/Main

11:50am - 12:10pm
Optimization of an LC-MS targeted proteomics approach for investigation of cyclooxygenase 2
Nicole M. Hartung, University of Wuppertal, Germany

12:10pm - 12:30pm
Mass spectrometric strategies for snake venom system research [First results for the saw-scaled viper (*Echis carinatus sochureki*)]
Parviz Ghezellou, Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Germany

PS 02: MS instrumentation I

Monday, 11/Mar/2019: *Location:* **HS224**
10:30am - 12:30pm

Session Chair: **Bernhard Spengler**

10:30am - 10:50am
High precision mass measurement and separation of nuclear isomers with a multiple-reflection time-of-flight mass spectrometer
Christine Hornung, II. Physikalisches Institut, Justus-Liebig-Universität Gießen, Gießen, Germany;

10:50am - 11:10am
T-MALDI-2-Orbitrap MS: Sensitive ion imaging with sub-micrometer resolution and ppm mass accuracy
Marcel Niehaus, Institute of Hygiene, University of Münster, Germany

11:10am - 11:30am
Fast identification of metabolites in complex samples using ultra-high resolution magnetic resonance mass spectrometry
Matthias Witt, Bruker Daltonik GmbH, Germany

11:30am - 11:50am
Fast Online Separation and Identification of Electrochemically Generated Isomeric Phase-I Metabolites by means of Trapped Ion Mobility-Mass Spectrometry
Jens Fangmeyer, University of Münster, Germany

11:50am - 12:10pm
DB-nESI overcomes the ion flux problem of modern nanoLC-MS/MS
Stefan Loroch, Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V

12:10pm - 12:30pm
Detection of Anti-TB drugs in organs of mice with LC-MS
Franziska Waldow, Division of Bioanalytical Chemistry, Research Center Borstel, Germany;

PS 03: Lipidomics & Metabolomics I

Monday, 11/Mar/2019: *Location:* **HS326/327**
10:30am - 12:30pm

Session Chair: **Heiko Hayen**

10:30am - 10:50am
Does the Fenton Reaction have physiological Relevance? - An MS Study with Phospholipids
Jürgen Schiller, University of Leipzig, Medical Department, Institute of Medical Physics and Biophysics, Leipzig, Germany

10:50am - 11:10am
Relative quantification of phospholipid *sn* isomers using positively doubly charged lipid-metal ion complexes
Sven Heiles, Justus-Liebig-Universität Gießen, Germany

11:10am - 11:30am
Investigation of Oxidation Sites of Triglycerides in Food Oil Samples using Paternò-Büchi Functionalization
Patrick Esch, Justus Liebig University Giessen, Institute of Inorganic and Analytical Chemistry

11:30am - 11:50am
Lipid identification at double bond position level using customized software and data evaluation strategies
Ansgar Korf, University of Münster, Germany

11:50am - 12:10pm
Lipid profiling of beef muscle tissues by LC-MS/MS and GC analysis and possible health benefits of odd chain fatty acids
Beate Fuchs, Leibniz-Institut für Nutztierbiologie (FBN), Germany;

12:10pm - 12:30pm
Probing glycation potential of dietary carbohydrates by a combination of mass spectrometry-based approaches
Andrej Frolov, Leibniz Institute of Plant Biochemistry, Germany

PS 04: Affinity-Mass Spectrometry

Monday, 11/Mar/2019: *Location:* **HS323**
2:00pm - 4:00pm

Session Chair: **Michael Przybylski**

2:00pm - 2:20pm
Intact Transition Epitope Mapping - Targeted High-energy Rupture of Extracted Epitopes (ITEM-THREE)
Bright D. Danquah, Proteome Center Rostock, University Medicine Rostock, Rostock, Germany;

2:20pm - 2:40pm
Epitope Identification of an aptamer complex of Cathepsin D in comparison to an antibody-cathepsin D complex
Pascal Wiegand, Steinbeis Center, Germany

2:40pm - 3:00pm
MS analysis of protein complex assembly pathway for the biosynthesis of APE
Kudratullah Karimi, Institute of Physical and Theoretical Chemistry, Goethe-University Frankfurt/Main

3:00pm - 3:20pm
Epitope Identification and Affinity Characterization of Myoglobin by combination of SPR biosensor analysis and Mass Spectrometry
Delia Mihoc, Steinbeis Center of Biopolymer Analysis and Biomedical Mass Spectrometry, Germany

3:20pm - 3:40pm
Retinal Guanylyl Cyclase 1/GCAP-2 Interaction Studied by Cross-linking/Mass Spectrometry
Anne Rehkamp, Institute of Pharmacy, Charles Tanford Protein Center, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany;

3:40pm - 4:00pm
A sensitive and simple targeted proteomics approach to quantify transcription factors of the unfolded protein response pathway in glioblastoma cells
Chi DL Nguyen, Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Germany

PS 05: MS instrumentation II

Monday, 11/Mar/2019: *Location:* **HS224**
2:00pm - 4:00pm

Session Chair: **Klaus Dreisewerd**

2:00pm - 2:20pm
The big wedge for the small chunk - protein molecular ions split apart by soft X-rays
Knut Kölbel, Heinrich Pette Institute, Hamburg, Germany

2:20pm - 2:40pm
Complementarity of the Different Ionization Techniques in Environmental Analysis by GCxGC-HRMS
Viatcheslav Artaev, LECO Corporation, United States of America

2:40pm - 3:00pm
Recent advances in omnitrap technology coupled to orbitrap mass analyzers
Dimitrios Papanastasiou, Fasmatech, Greece

3:00pm - 3:20pm
Description of complex petrochemical samples by thermal analysis mass spectrometry
Christoph Grimmer, University of Rostock, Germany

3:20pm - 3:40pm
Structural analysis of heavy oil fractions by the combination of high-resolution tandem mass spectrometry and ion mobility spectrometry
Christopher Paul Rüger, University of Rouen, France

3:40pm - 4:00pm
Open LabBot – Modular Hardware and Software Platform for High-Throughput Sampling and Ambient Mass Imaging
Robert Winkler, CINVESTAV, Department of Biotechnology and Biochemistry, Irapuato, Mexico

PS 06: Lipidomics & Metabolomics II

Monday, 11/Mar/2019: *Location:* **HS326/327**
2:00pm - 4:00pm

Session Chair: **Dietrich Albert Volmer**

2:00pm - 2:20pm
Monitoring of lipid metabolism perturbation during *M. tuberculosis* infection in human sputum
Adam Wutkowski, Division of Bioanalytical Chemistry, Research Center Borstel, Germany

2:20pm - 2:40pm
Identification of small molecules by combination of ion identity networking and MS^2 molecular networking
Robin Schmid, University of Münster, Germany

2:40pm - 3:00pm
Analysis of liamocin biosurfactants by means of LC-MS and SFC-MS
Karen Scholz, University of Muenster, Institute of Inorganic and Analytical Chemistry, Germany

3:00pm - 3:20pm
Characterization of lipidomic changes in human liver samples by shotgun lipidomics: is fatty liver only about neutral lipids accumulation?
Olga Vvedenskaya, MPI-CBG, Germany

3:20pm - 3:40pm
Only with fragment intensity correction is quantification of glycerophospholipids molecular species (to isomer level directly) from MS/MS possible
Kai Schuhmann, MPI of Molecular Cell Biology and Genetics, Dresden, Germany

3:40pm - 4:00pm
Analysis and visualization of artificially oxidized cardiolipins by means of LC-HRMS and Kendrick mass plots
Patrick Olaf Helmer, University of Münster, Institute of Inorganic and Analytical Chemistry, Münster, Germany

PS 07: Proteomics – Biological and Clinical Applications

Tuesday, 12/Mar/2019: *Location:* **HS323**
10:30am - 12:30pm

Session Chair: **Claudia Röwer**

10:30am - 10:50am
Proteomics of Diatoms: Discovery of Polyamine Modifications in Biosilica-Associated Proteins
Alexander Milentyev, Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany

10:50am - 11:10am
Proteome Profile of Chronic Wounds Treated with Cold Atmospheric Plasma to Improve Wound Healing
Jan-Wilm Lackmann, Leibniz Institute for Plasma Science and Technology (INP), Greifswald, Germany

11:10am - 11:30am
PASEF for high-throughput deep shotgun proteomics analyses
Scarlet Koch, Bruker Daltonik GmbH, Germany

11:30am - 11:50am
Dried Serum Spots – An excellent means to bridge the divide between the clinics and the protein mass spectrometry laboratory
Charles Ayensu Okai, Proteome Center Rostock, University of Rostock, Rostock, Germany

11:50am - 12:10pm
A new phase to investigate the dynamics of the proteome
Nico Zinn, Cellzome GmbH a GSK company, Germany;

12:10pm - 12:30pm
Profiling of Stage I – IV colorectal carcinoma samples by quantitative proteomics for early onset biomarker detection
Christoph Krisp, Institute of Clinical Chemistry and Laboratory Medicine, Mass Spectrometric Proteome Analysis, University Medical Center Hamburg-Eppendorf, Germany

PS 08: Instrumentation and Application of MS Imaging

Tuesday, 12/Mar/2019: *Location:* **HS224**
10:30am - 12:30pm

Session Chair: **Andreas Römpp**

10:30am - 10:50am
3D-surface AP-SMALDI MS imaging reveals differential tegumental lipidomics in human pathogen *Schistosoma mansoni*
Patrik Kadesch, Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Giessen, Germany

10:50am - 11:10am
An advanced protocol for microbial MALDI-MS imaging: gaining insights into the world of bacteria on a molecular scale
Eike Ulrich Brockmann, Institute of Hygiene, University of Muenster, Germany

11:10am - 11:30am
Investigation of the distribution of food additives in processed food by MALDI MS imaging
Julia Kokesch-Himmelreich, Universität Bayreuth, Germany

11:30am - 11:50am
MALDI-Imaging for Classification of Epithelial Ovarian Cancer Histotypes from a Tissue Microarray Using Machine Learning Methods
Zhiyang Wu, Charité – Universitätsmedizin Berlin, Germany

11:50am - 12:10pm
Towards novel TB antibiotics: Drug screening and improving the predictive value of preclinical models.
Axel Treu, Universität Bayreuth, Germany

12:10pm - 12:30pm
Pulsed cold plasma for post ionization in MALDI-MS imaging
Jens Soltwisch, Institute for Hygiene, University of Münster, Germany

PS 09: Environmental, natural product and forensic MS

Tuesday, 12/Mar/2019: *Location:* **HS326/327**
10:30am - 12:30pm

Session Chair: **Uwe Karst**

10:30am - 10:50am
Complexation strategy for the analysis of protein adducts with ethylmercury in influenza vaccines *via* SEC/ICP-MS
Philipp Strohmidel, Westfälische Wilhelms-Universität Münster, Germany

10:50am - 11:10am
Effects of cosolvents in Fenton oxidation as clean-up procedure for polycyclic aromatic hydrocarbons (PAXH)-contaminated soil
Ilker Satilmis, Wolfgang Schrader
Max-Planck-Institut für Kohlenforschung, Germany; satilmis@kofo.mpg.de

11:10am - 11:30am
Remote Detection of Ship Emissions using Single-Particle Mass Spectrometry
Johannes Passig, Joint Mass Spectrometry Centre, Universität Rostock, Germany

11:30am - 11:50am
Studying protein interactions of endocrine disrupting organotin compounds using soft LC-MS techniques
Jonas Maurice Will, University of Münster, Münster, Germany

11:50am - 12:10pm
Unconventional Kendrick Mass Defect as a visualization and rapid screening tool for GC×GC-HR-ToF/MS measurements
Benedikt Alexander Weggler, The Pennsylvania State University, State College, USA

12:10pm - 12:30pm
Monitoring of Coffee Roasting by Vacuum Photoionization ToF-MS: Towards a Prediction Model for Bean Color and Antioxidant Capacity
Jan Heide, Universität Rostock, Germany

MS 01: Ion physics & ion chemistry

Tuesday, 12/Mar/2019: *Location:* **HS323**
2:40pm - 3:20pm

Session Chair: **Ralf Zimmermann**

2:40pm - 3:00pm
N-Heterocyclic Carbene (NHC) Dimerization in the Gas Phase:C-H···:C Hydrogen Bonding *vs.* Covalent Dimer Formation
Mathias Schäfer, University Cologne, Germany

3:00pm - 3:20pm
Infrared Laser Desorption in the Stress-Confinement Regime Studied by Time-Resolved Digital Interference Microscopy
Frederik Busse, Max Planck Institute for the Structure and Dynamics of Matter, Germany

PS 10: Award Lectures

Wednesday, 13/Mar/2019: *Location:* **HS323**
10:30am - 12:30pm

Session Chair: **Andrea Sinz**

10:30am - 11:20am
Characterization of the human Proteome and Kinome
Bernhard Kuester, Technical University of Munich, Germany

11:20am - 12:00pm
High-Throughput Identification of Lipids in Biological Material Using Software-Aided Analysis of LC-MS/MS Data
Jürgen Hartler, Technische Universität Graz, Austria

12:00pm - 12:15pm
Data acquisition methods for next-generation mass spectrometry-based proteomics
Florian Meier, LMU München, Germany

12:15pm - 12:30pm
Development of autofocusing and subcellularly resolving mass spectrometry imaging and its application to biological questions
Mario Kompauer, Justus Liebig University Giessen, Germany

PS 11: Outside the box

Wednesday, 13/Mar/2019: *Location:* **HS224**
10:30am - 12:30pm

Session Chair: **Ralf Zimmermann**
Session Chair: **Michael O. Glocker**

10:30am - 10:50am
HPLC-HRMS as a Tool for the Identification of Confiscated Commercial and Military Explosives
Tilo Schachel, Westfälische Wilhelms-Universität Münster, Germany

10:50am - 11:10am
Single cell analysis of *Toxoplasma gondii*- and *Besnoitia besnoiti*-infected bovine umbilical vein endothelial cells by MALDI mass spectrometry imaging
Stefanie Gerbig, Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Germany

11:10am - 11:30am
Challenges in atmospheric pressure mass spectrometry
Claudia Birkemeyer, Universität Leipzig, Institute of Analytical Chemistry, Germany

11:30am - 11:50am
Systems Proteomics: Assessment and perspectives of haploid cell systems in structural and functional proteomics
Hans-Juergen Thiesen, Institute of Immunology, University of Rostock, Germany

11:50am - 12:10pm
Speciation analysis and multimodal Imaging to trace gadolinium deposition in the brain
Uwe Karst, University of Münster, Germany

12:10pm - 12:30pm
Phospholipid profiling by LC/MS applying complementary ESI-MS and ICP-MS detection
Heiko Hayen, University of Münster, Germany

List of Posters

No.	Presenting Author	Poster Title
1	LETTOW, Maike	Investigation of the Gas-Phase Structures of Fucosylated Glycans
2	LUO, Ruoji	Characterization of Black Solid Aggregates in Highly PAH Contaminated Soil as Main Contamination Source by FT MS
3	XU, Yun	Characterization of trash fuels using GC-HR-EI-MS
4	WITT, Matthias	CID fragmentation studies of asphaltenes at different precipitation times usingMagnetic Resonance Mass Spectrometry (MRMS)
5	FARMANI, Zahra	Direct infusion ultra-high resolution mass spectrometry (UHRMS) of Saturate fractions from different crude oils
6	ACHTEN, Christine	Environmental and geochemical applications of GC-APLI-MS for sensitive und selective analysis of polycyclic aromatic compounds
7	HAMACHER, David	Generating molecular insights into biofuel/fossil fuel blends via high resolution mass spectrometry
8	PARKER, Aaron	Enhanced confidence in routine monitoring of river water quality by passive sampling with GCxGC-TOF MS with Tandem Ionisation
9	ICKERT, Stefanie	Human poisoning with the pesticide oxydemeton-S-methyl proven by detection of novel cysteine- and albumin-adduct biomarkers with HPLC/ESI-MSMS
10	BARTH, Christof	Inorganic and organic compound characterisation and quantification from an aerosol filter sample by high resolution imaging MS and ICP-MS
11	ROOCKE, Sascha	Mass spectrometry-based identification of body fluids for forensic purpose
12	CZECH, Hendryk	Single-particle Analysis from a Major Fire Incident of Discarded Metal in Rostock, Germany
13	MINTE, Olaf	A shadowgraphy technique for visualizing the plume development in MALDI/MALDI-2 at high spatio-temporal resolution
14	LOTZ, Florian	Design of a liquid-microjunction surface sampling probe for ambient mass spectrometry analysis of consumer goods
15	KÄFER, Uwe	Extended application range for a GC×GC high-resolution time-of-flight mass spectrometer platform by hyphenation to thermal analysis
16	HINZ, Klaus-Peter	Improvements and applications of the LAMPAS 3 laser mass spectrometer for on-line single particle analysis
17	YOUNG, Phoebe	LILBID-MS based method for assessing DNA binding
18	BOOKMEYER, Christoph H. M.	Single-photon ionization of head-space sampled solid and liquid food products and of breath in a dual-ion-funnel MALDI/ESI-Injector coupled to Orbitrap-MS
19	POTTHOFF, Alexander	What determines the postionisation efficiency in MALDI-2: A combined soft-/hardware-based set-up to characterise the role of relevant input parameters
20	WEIBCHEN, Gunnar	A Multi-Function Cyclic Ion Mobility – MassSpectrometry System
21	KRÄGENBRING, Julia	Advances in Orbitrap™ instrumentation for native top-down analysis of non-covalent protein complexes
22	PARKER, Aaron	Automated aroma profiling of alcoholic beverages by GCxGC-TOF MS
23	NEUMANN, Anika	Comparison of different atmospheric pressure photo ionisation techniques with atmospheric pressure chemical ionisation for gas phase ionisation high resolution FT-ICRMS

No.	Presenting Author	Poster Title
24	GEHM, Christian	Development of a membrane-introduction photoionization mass spectrometer for real-time analysis of aromatic and polycyclic aromatic hyrdocarbons in aquatic systems
25	LIEBLEIN, Tobias	Electrodynamic Droplet Levitation for the Purpose of Time-Resolved Mass Spectrometry
26	KRÄGENBRING, Julia	Evaluation of the Phase-Constrained Spectrum Deconvolution Method (Phi-SDM) for multiplex TMT application
27	VAN WASEN, Sebastian	High Resolution Mass Spectrometry of Acoustically-Levitated Droplets
28	WEYER, Christian	HILIC, Polar, and Shape Selectivity of a FluoroPhenyl Phase
29	SMYRNAKIS, Athanasios	Ion isolation, stability boundaries, and space charge effects in the omnitrap platform driven by rectangular RF waveforms
30	WEYER, Christian	Rapid Profiling and Quantification of 17 Bile Acids in Human Plasma by LC-MS/MS
31	WEYER, Christian	Using Free, High-Performance, Computer Modeling Software to Simulate Gas Chromatographic Separations
32	EHLERT, Sven	Vacuum Photoionisation ToF-MS as technique to analyze complex gas mixtures on-line and in real time
33	BOSKAMP, Marcel	A matter of composition- ion suppression effects among phospholipids in MALDI mass spectrometry imaging investigated by use of artificial tissues
34	IWAN, Volker	Adduct Suppression at Oligosaccharides and SORI-CID Fragmentation
35	STAPPERT, Florian	Effects of physical and chemical interactions in ion mobility spectrometry (IMS) depending on the reduced field strengths
36	MITROFANOV, Elena	Gas phase reactions of heptamethine cyanine dyes using femtosecond-laser-pulse induced photodissociation and collision-induced dissociation
37	KELLNER, Ina D.	Host-guest chemistry of azafullerene derivatives
38	POLACZEK, Christine	Ion-solvent interactions in nanoESI-MS: Comparison of different ion transfer settings and analyzer systems
39	THINIUS, Marco	Kinetic energy distribution measurements for ion dynamics studies
40	SCHNELL, Anne	Mechanistic studies of L-proline-catalyzed Diels-Alder reactions of unsaturated aldehydes with ESI-MS
41	RAUPERS, Björn	Performance of Cassinian ion traps in dependence of their trap length
42	HELLE, Niklas	The strong lever of phenetole: In-depth investigation of the vibronic structure in the first excited state and ionic ground state
43	OSCHWALD, Johannes	Walk-on-sphere rearrangement and retro-Bingel reaction of gas-phase fullerene malonate ions
44	HOFFMANN, Jan	Following the conformational diversity of the PYP photoreceptor with native Ion-Mobilty MS
45	LOTTES, Björn	Linewidth Pressure Measurement (LIPS) by means of protonated aminoacids in ICR-traps
46	BEHRENS, Arne	Trapped Ion Mobility Spectrometry as post-ionization separation technique for biomedical samples
47	REINERT, Thorben	Investigation of Non-Covalent Clusters of Anisole and various Aniline-Derivatives via REMPI-Spectroscopy
48	ROGGENSACK, Tim	Aufarbeitungstrategien von Krebstieren in Vorbereitung der LC-MS-Messung

No.	Presenting Author	Poster Title
49	POHLENTZ, Gottfried	Coupling of pectin-derived oligosaccharides with phosphatidylethanolamine yields side products from Amadori rearrangements
50	VON OESEN, Tobias	Entwicklung innovativer Analysenverfahren zum Nachweis von Molkenproteinen und Etablierung von Biomarkern als Qualitätsparameter bei Molkenprotein-angereichertem Schnittkäse
51	EHLERT, Sven	From cigarettes to joints - Puff resolved online investigation of conventional and new smoking products using Photoionization Mass Spectrometry
52	TARAKHOVSKAYA, Elena	LC-MS analysis of two subcellular fractions of brown algal phlorotannins
53	RUß, Manuela	Mass Spectrometric Characterization of the Zein Protein Composition in Maize Flour by SDS-PAGE and 2D Gel Electrophoresis
54	KRUSE, Stefanie	Mass spectrometric structural characterization of mucin-derived O-glycans obtained by HILIC solid phase enrichment
55	VETERE, Alessandro	Qualitative and quantitative analysis of sulfur compounds in heavy crude oil and its fractions
56	BOLL, Robert	Routine LC-MS/MS method optimization for clinically relevant metabolites of the kynurenine pathway
57	MIRANDA ACKERMAN, Eduardo Jacobo	LipidXplorer Web: An online, rapid identification and quantification tool for bottom-up and top-down shotgun Lipidomics, based on customizable queries
58	KEßLER, Barbara	Applying Trapped Ion Mobility Separation (TIMS) in combination with Parallel Accumulation Serial Fragmentation (PASEF) for analysis of lipidomics samples
59	KRAUSE, Daniel	A closer look at the porcine lung: the lipidome and changes due to inter-individual variation
60	AL MACHOT, Fadi	A Web-Tool to Compute Lipidomes Homologies based on Template SMILES
61	HOFMANN, Tommy	Absolute quantification of phospholipids using nano-ESI and short acyl chain analogues
62	LÜKE, David	Double-bond resolved shotgun MS investigations of the Schistosoma mansoni lipidome
63	AHRENDS, Robert	LipidCreator: A workbench to probe the lipidomic landscape
64	DANNENBERGER, Dirk	Long-chain n-3 PUFAs are incorporated into a series of phospholipids of the muscle in pigs fed microalgae supplemented diet
65	STRIESOW, Johanna	Oxidative modification of skin lipids by cold atmospheric plasma (CAP) - a standardizable approach using LESA and LC/MSMS
66	MOHRING, Siegrun A.I.	Robust and Sensitive LC-MS/MS Based Plasma Lipid Profiling on a Thermo Scientific™ Q Exactive™ HF-X Mass Spectrometer
67	SCHÖTT, Hans-Frieder	SIMPLEX: A multi-omics approach for screening of modified hippocampal lipid signaling pathways triggered by lifestyle condition
68	WIENKEN, Carina M.	Simultaneous determination of polar and non-polar lipids in yeast by means of heart-cut two-dimensional liquid chromatography - mass spectrometry
69	MOHRING, Siegrun A.I.	Structural characterization of complex lipids by ozone-induced dissociation and ultraviolet photodissociation on high-resolution mass spectrometers.
70	FOLBERTH, Julica	Informed unTargeted Metabolomics using LC-MS/MS
71	SCHORR, Pascal	A novel derivatization method for vitamin D metabolites to improve both sensitivity and separation ability of isomers in LC-MS/MS

No.	Presenting Author	Poster Title
72	METZGER, Sabine	A novel method for identification and quantification of sulfated flavonoids in plants byneutral loss scan mass spectrometry.
73	SCHÖTTLER, Hannah	Application of capillary ion chromatography-MS for metabolite profiling of yeast metabolites
74	WITT, Matthias	Detection of drugs and metabolites in urine by Fast Flow Injection Analysis (FIA) coupled to Magnetic Resonance Mass Spectrometry (MRMS)
75	SOBOLEVA, Alena	Formation of α-dicarbonyl compounds and advanced glycation end-products (AGEs) from blood plasma monosaccharides
76	BLANKENSTEIN, Petra	High Throughput Targeted Workflows for Metabolomics / Lipidomics Studies
77	BRUNO, Giuliana	MS-based study of the plasma liquid chemistry: effects on thiols
78	WALZ, Christina	Multiomics data integration for the identification of genotype/phenotype-interactions in non-inbred mouse strains
79	SCHMELTER, Franziska	Personalized nutrition: A metabolomics-based approach for a detailed comprehension of gut bacterial pathways by UPLC-IMS-QToF
80	BILOVA, Tatiana	Probing age-related changes in pea (Pisum sativum) root nodule metabolome by mass spectrometry
81	LUPU, Loredana	Epitope and affinity determination of recombinant Mycobacterium tuberculosis Ag85B antigen towards anti-Ag85 antibodies using proteolytic-affinity mass spectrometry and biosensor analysis
82	MARCILLO, Andrea	Fast active sampling of volatiles for field experiments
83	WENSKE, Sebastian	Mass spectrometry-based investigations of cold atmospheric plasma-induced post-translational modifications (PTM's) in peptides
84	KOY, Cornelia	Gas Phase Binding Stability Determination of RNAse S by nanoESI MS Analysis out of Solutions with Different Methanol Contents
85	BECHER, Simon	Influence of the protein charge state on 213 nm top-down UVPD results
86	HAGE, Christoph	A Biuret-derived reagent with urea-like MS-cleavability for cross-linking of proteins
87	IHLING, Christian	Evaluation of an Isotope-Labeled MS/MS-Cleavable Cross-Linker for Protein Structure Analysis
88	RÖWER, Claudia	Characterization of specificity determining positions within the HER2-Herceptin epitope using in-silico biocomputational methods
89	MARTIN, Rafaela	Application of PASEF MS/MS scans to monoclonal antibody peptide mapping
90	ARLT, Christian	Structural characterization of IDP interactions of full-length tumor suppressor p53
91	KOCH, Scarlet	PASEF for ultra-sensitive shotgun proteomics
92	KOPICKI, Janine-Denise	Structural investigations of highly pathogenic negative strand RNA viruses
93	VILLAR GAREA, Ana	Contributions of mass spectrometry to early drug discovery
94	KOCH, Scarlet	Highly reproducible and accurate label free quantification using the PASEF method on a TIMS-QTOF mass spectrometer
95	WANG, Xiaohan	Protein Interactions of the p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass Spectrometry
96	YEFREMOVA, Yelena	Investigation of the secondary structure isoforms of IgG2 type monoclonal antibody using LC-ESI-ToF-MS and orthogonal methods
97	MARTIN, Rafaela	Short LC-gradients for high throughput and deep shotgun proteomics using PASEF on a TIMS equipped QTOF

No.	Presenting Author	Poster Title
98	TSAREV, Alexander	Age-related changes in pea (Pisum sativum) root nodule: a proteomics approach
99	KOCH, Scarlet	High Sensitivity Phosphoproteomics using PASEF on a TIMS-QTOF mass spectrometer
100	KIM, Ahyoung	Comprehensive characterization of proteome changes accompanying ageing of common bean (Phaseolus vulgaris) root nodules
101	GUAN, Yudong	A Comprehensive Strategy for Deep Glycomics
102	MERKEL, Dietrich	Accelerating DIA Studies to Extend Workflow Utility, Using Fast Microflow LC Gradients
103	BAYER, Malte	Chiral analysis of hypertrehalosaemic neuropeptides of cicadas
104	KRÖSSER, Dennis	Differential Proteomic Analysis for Validation of Truffle Origin
105	MERKEL, Dietrich	Fast Microflow Chromatography for Accelerating Protein Identification Experiments
106	MERKEL, Dietrich	High-throughput phenotypic characterization of colorectal cancer tumor tissue with SWATH-MS
107	KÖNIG, Simone	Inhibitor-sensitive autophosphorylation sites of Staphylococcus aureus S/T kinase detected by target ion mobility mass spectrometry
108	ALBONY, Hasan	Phosphoproteome analysis of the near-haploid cell line HAP1 to reveal phosphorylation events originating from PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK)
109	DREYER, Benjamin	Profiling of walnut kernels: a proteomics approach
110	MIKKAT, Stefan	Stoichiometric analysis of the mitochondrial glycine decarboxylase multienzyme complex using isotope-labeled concatenated peptides
111	HEIKAUS, Laura	Tracing the Proteolytic Cascade of the Contact System by Migration Profiles
112	KEßLER, Barbara	Rapid identiy assays for mAB development, production control and release
113	BRDAR, Biljana	Clinical Diagnostics of Neuronal Ceroid Lipofuscinoses (NCL-1, NCL-2 and NCL-10) using New Substrates for Tandem Mass Spectrometry and Fluorimetry
114	BÜCKING VILCHEZ, Maria Isabel	Investigation of post-biopsy degradation processes with laser ablation sampling and differential proteomics
115	KOCH, Scarlet	Maximized throughput and analytical depth for shotgun proteomics using PASEF on a TIMS equipped QTOF and a novel LC system
116	MÜLLER, Bernd	Multi-attribute monitoring (MAM) to identify differences in Trastuzumab from 2 manufacturers.
117	VOß, Hannah	Quantitative LC-MS/MS proteomics of FFPE Medulloblastoma tissue reveals new molecular signatures for different cancer subtypes
118	KRUTILIN, Andrey	Sampling of Tissues with Laser Ablation for Proteomics: Comparison of Picosecond Infrared Laser (PIRL) and Microsecond Infrared Laser (MIRL)
119	DREISBACH, Domenic	Visualizing chemical defense mechanisms of Asclepias curassavica against herbivores using autofocusing MALDI mass spectrometry imaging
120	KEßLER, Barbara	Integrating MALDI imaging and ESI metabolomics for broadband identification and validation
121	ORTHEN, Julian	Comparison of MALDI-MS imaging and quantitative HPLC-FLD of Pseudomonas quinolone signal molecules
122	KLAUS, Florentine	Development and characterisation of novel MALDI-matrices based on polycyclic aromatic hydrocarbons

No.	Presenting Author	Poster Title
123	BRUNGS, Corinna	Analysis of tattoo pigments in human skin tissue with µXRF and LDI-MS
124	WALETZKO, Michael Thomas	Lipid analysis on tissue with improved lateral resolution using nanospray desorption electrospray ionization (nano-DESI)
125	BIEN, Tanja	MALDI-2-mass spectrometry and multimodal imaging of globotriaosylceramide (Gb3Cer) and further lipids in human colorectal cancer tissue
126	MÜLLER, Max Alexander	Metabolic changes in the brain of sleep-deprived and rested Drosophila melanogaster flies, investigated by high-resolution atmospheric pressure MALDI-MSI
127	HOFFMANN, Nils	LipidCompass

Exhibitors

Advion Ltd.

https://www.advion.com

Advion's nearly three-decade dedication to serving scientists yields customer-focused life science solutions. Our deep scientific, engineering and customer workflow knowledge spawns an unrivaled solution portfolio. We work directly with, train, and passionately advocate for our customers to ensure their success.

The Advion product portfolio includes the expression Compact Mass Spectrometer, the AVANT (U)HPLC, the SOLATION ICP-MS and many other novel sample introduction systems including the Plate Express TLC Plate reader and the TriVersa NanoMate nano-electrospray ionization technique.

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Agilent Technologies Sales & Services GmbH & Co. KG

https://www.agilent.com

Agilent Technologies Inc. ist ein weltweit führender Anbieter in den Bereichen Life Sciences, Diagnostik und angewandte Chemie. Das Unternehmen blickt auf mehr als 50 Jahre Innovation und neue Erkentnisse zurück und liefert, in Form von Geräten, Software-Programmen, Dienstleistungen, Lösungen und kompetentem Personal, zuverlässige Antworten auf die anspruchsvollsten Fragen seiner Kunden. Agilent Technologies erzielte im Geschäftsjahr 2018 einen Umsatz von 4,91 Milliarden US-Dollar und beschäftigt weltweit 14.800 Mitarbeiter. Informationen zu Agilent finden Sie unter www.agilent.com, dem Agilent Newsroom, Linked In, Twitter und Facebook.

Bruker Daltonik GmbH

https://www.bruker.com

Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels.
In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology.

Fasmatech SA

http://fasmatech.com/

Fasmatech offers a variety of products and custom engineering solutions to the mass spectrometry research and development community. Advanced ion optical designs, mass selective RF transfer lines, ion trapping technology, time-of-flight mass analyzers and ion mobility spectrometers can be combined to produce unique instrumentation platforms, supported by state of the art electronics and user friendly interfaces. Fasmatech has produced unique instrumentation for a wide range of application extending from top-down proteomics and fundamental research in protein chemistry to the study of ion-molecule reaction kinetics, imaging of biological and extraterrestrial material and detection of intact viruses. Fasmatech has introduced the Omnitrap platform, a unique ion processing system designed to provide access to an arsenal of ion activation-dissociation techniques all embedded in the same unit. This unique technology is currently available as a retrofit to the Q Exactive instrument series (ThermoFisher Scientific) and also with Fasmatech's high performance oTOF mass analyzer.

JEOL (Germany) GmbH

https://www.jeol.de

JEOL is the world's largest supplier of electon-optical and analytical systems, carrying out research and development in the fields of materials sciences, nanotechnology, medicine, life sciences and biotechnology. We offer systems for the analysis of chemical compositions in the micro- and nanoscale and systems for microscopic imaging with resolutions spanning to the atomic range. JEOL has been supplying mass spectrometers for many fields of application for more than 50 years. The instruments are used for both simple, routine tasks, as well as for extremely demanding analyses. Developing these devices, particular attention was paid to robustness and use of innovative technologies.

With extensive decades of experience, JEOL is your reliable partner for biological and medical research as well as for development, characterization and quality control of materials. We offer, amongst others, GC-Q, GC-TOF, HPLC-TOF, DART-TOF, GC-TripleQ and MALDI-TOF. Come meet us at our booth for more information!

KR Analytical Ltd

https://www.kranalytical.co.uk

KR Analytical are suppliers of ambient ion sources including AP-MALDI , SICRIT and ASAP. We also provide MALDI Image preparation equipment from SunChrom, Chemyx syringe pumps and Apex nitrogen generators.

AP-MALDI gives your existing LC-MS the capability to perform standard MALDI experiment or imaging MALDI at a fraction of the cost of a dedicated MALDI-MS instrument.

SICRIT is a versatile soft ionisation source that can be connected to any LC-MS and also enables chromatographic and thermal instruments such as GC, LC, SPME and TGA to be connected to your existing LC-MS. This hyphenation of techniques is unique and gives access to high-resolution measurements on a variety of samples either directly – or from a separation technique.

LECO Instrumente GmbH Deutschland

http://de.leco-europe.com/

Seit mehr als 80 Jahren maßgeschneiderte Lösungen für die Elementaranalyse - seit 25 Jahren hochwertige Analytik im Bereich Chromatographie und Massenspektrometrie. LECO bietet erstklassigen Service und die Qualität eines Marktführers.
LECO verfügt über ein breit gefächertes Angebot fortschrittlicher Analysengeräte, einschließlich der Elementbestimmung, Thermogravimetrie, Kalorimeter, GDS-Spektrometer, metallographischer Lösungen und Verbrauchsmaterial. Der Geschäftsbereich Separation Science bietet umfassende Lösungen für die Chromatographie und die Massenspektrometrie. Mit seinen leistungsstarken Time-of-Flight-Massendetektoren und dem Know-How und der Erfahrung in der zweidimensionalen Gaschromatographie können auch komplexe Fragestellung ausführlich und grundlegend analysiert werden. Anwendungsgebiete in der Nahrungsmittel-, Geschmacks-/ Duftstoff-, Öl- und Umweltindustrie, Forensik, Werkstoffkunde oder Metabolomikforschung werden mit verlässlicher Qualität und hervorragendem Service abgedeckt.

Linden ChroMasSpec GmbH

https://www.linden-cms.de/

Linden CMS markets LIFDI, one of the softest ionization techniques of Mass Spectrometry. It is sensitive and easy to use even for air/moisture sensitive samples. It is performed within minutes. LIFDI gives meaningful mass spectra from difficult compounds inaccessible to established techniques.
LIFDI forms intact molecular ions of e.g.

- organometallic complexes including: highly reactive samples, oily (not crystallizable) compounds, NMR or X-ray silent samples, unpurified mixtures
- hydrocarbons, crude oils etc.
- sterically hindered samples

Merck KGaA

https://www.merckgroup.com

Merck is a leading science and technology company in healthcare, life science and performance materials.
Around 50,000 employees work to further develop technologies that improve and enhance life. With a catalog of more than 300,000 products, our Life Science business delivers many of the most highly-respected brands in the industry, such as Millipore, Milli-Q, Supleco and BioReliance. Our offering covers every step of the biotech production chain, creating a complete end-to-end workflow with enhanced customer service, a simplified interface and a leading distribution platform. Our innovative portfolio, well-balanced geographic reach, and industry leading capabilities, uniquely positions us to anticipate and deliver on customer needs. Our Life Science business brings together the legacy expertise of Merck's life science portfolio and Sigma-Aldrich, which was acquired by Merck in 2015.

MS Vision

https://msvision.eu

MS Vision ist seit 2004 ein europaweit tätiger Servicedienstleister für LC-MS-Systeme der Hersteller Waters, Sciex und ThermoFisher. Mit unserem ISO9001-zertifizierten Service und einem hervorragenden Serviceteam unterstützen wir Sie dabei bei allen Fragen rund um Ihre Hardware von ad hoc-Reparaturen über jährliche Wartungen bis hin zu Schulungen, Umzügen und Qualifizierungen. Entscheidender Unterschied zu den Herstellern ist hierbei zum einen unsere Unabhängigkeit und zum anderen unser klarer Fokus auf den Service anstatt diesen als Nebengeschäft zu betrachten. Bei uns steht Ihr Serviceanliegen im Vordergrund!
Daneben bieten wir Ihnen neben geprüften Gebrauchtgeräten, speziellen Benches für LC-MS-Systeme, Systemen zur Probenvorbereitung auch dezidierte, speziell angepasste Systeme für Hochmassen-Anwendungen im Bereich von m/z >10.000. In diesem Bereich betreiben wir auch eigene Entwicklung und laufend unsere Kompetenzen in diesem Bereich.

Pfeiffer Vacuum GmbH

https://www.pfeiffer-vacuum.com

Mit dem Smartphone die neuesten Informationen immer sofort erfahren, besser sehen dank hochwertigen Brillengläsern, unabhängig und mobil im Auto die Welt entdecken – diese und viele weitere Annehmlichkeiten des alltäglichen Lebens sind ohne Vakuum nicht möglich. Pfeiffer Vacuum bietet umfassende Lösungen, die für die Herstellung dieser Produkte unentbehrlich sind.
Seit mehr als 125 Jahren steht der Name Pfeiffer Vacuum für hochwertige Vakuumtechnik, ein umfassendes Komplettangebot in höchster Qualität und erstklassigen Service. Dank der engen Zusammenarbeit mit unseren Kunden und der kontinuierlichen Ausrichtung an ihren Bedürfnissen optimieren und erweitern wir unser Portfolio ständig. So können wir unseren Kunden auch in Zukunft immer die beste Lösung für ihre individuelle Anwendung bieten. Unser Leistungsportfolio reicht von Vakuumpumpen über Mess- und Analysegeräte bis hin zu kompletten Vakuumsystemen.
Pfeiffer Vacuum beschäftigt weltweit etwa 3.200 Mitarbeiter und hat mehr als 20 Tochtergesellschaften. Das Unternehmen ist im TecDAX an der deutschen Börse notiert.

PharmaFluidics

https://www.pharmafluidics.com/

PharmaFluidics introduces the silicon revolution in liquid chromatography. The micro-Chip Pillar Array (µPAC™) column overcomes the physical limits of any packed bed alternative. The µPAC™ columns feature a perfectly ordered separation bed of free-standing pillars ensuring excellent separation power, unprecedented reproducibility and unrivalled robustness.
Whether you are analyzing trace amounts of compounds in complex biological samples for proteomics, metabolomics or lipidomics, or whether you are looking for subtle modifications in monoclonal antibodies: use plug-and-play µPAC™ columns to boost your biomarker and life sciences research. Our µPAC™ columns are compatible with all standard nano-LC equipment.

Photonion GmbH

https://www.photonion.de

Photonion GmbH is an innovative company providing customized solutions for monitoring of chemicals using mass spectrometry with soft ionization methods such as photo ionization. Different photo ionization sources, including VUV-lamps or Lasers are used for single photon ionization (SPI) or resonance enhanced multi photon ionization (REMPI) for the generation of nearly fragment free mass spectra.

The products are used e.g. for online puff by puff analysis of toxic compounds in cigarette smoke, e-cigarette vapor and vapor of tobacco heating products (Smoke-Photo-TOF-MS) or for the Thermal Analysis of polymers, crude oil or consumer products (TG-PIMS) and for general research such as coffee roast gases or gases of biomass pyrolysis with our flexible standalone systems (Photo-TOF-MS). New developments are also focusing on single particle analysis of environmental samples and dose monitoring for Vitrocell® Air-Liquid Interface (ALI) and Automated Exposure Station.

Plasmion GmbH

https://www.plasmion.de

Die Plasmion GmbH ist ein mit dem „ACHEMA-Gründerpreis 2018“ ausgezeichnetes Startup, welches eine neuartige Ionenquellen-Technologie für die Massenspektrometrie entwickelt und patentiert hat. Die sogenannte SICRIT®-Technologie revolutioniert nicht nur getreu dem Motto „Simple. Smart. Sensitive“ die chemische Analytik im Laborumfeld, sondern soll zukünftig auch das Leistungsspektrum eines analytischen Labors in einem automatisierten Messsystem für die Industrie zur Verfügung stellen.

Als wesentliches Alleinstellungsmerkmal entkoppelt die SICRIT® (Soft Ionization by Chemical Reaction In Transfer)-Technologie die Probenvorbereitung von der Probenmessung, so dass hochsensitive Echtzeit-Messungen bei gleichzeitig sehr geringen Verbrauchskosten möglich werden. Dank des flexiblen plug&play-Designs kann die Ionenquelle als Upgrade an allen LC-MS-Geräten installiert werden und ermöglicht direkte SPME-MS- genauso wie GC-Soft Ionization-MS-Analysen.

Restek GmbH

https://www.restekgmbh.de

Restek bietet Säulen, Zubehör und Verbrauchsmaterialien für die bestmögliche Chromatografie an. Von der Probenahme und -aufgabe über die Trennung bis hin zur Qualitätskontrolle liefert Restek nicht nur einzelne Produkte, sondern komplette Lösungen. Das Ergebnis sind über 16.000 Produkte für fast alle Geräte und Systeme, die mit einer ausgefeilten Logistik kurze Lieferfristen ermöglichen. Restek ist von keinem Gerätehersteller abhängig. Für alle Gerätetypen liefern wir optimale Lösungen.

Shimadzu Deutschland GmbH

https://www.shimadzu.de/

Shimadzu Deutschland GmbH bedient den deutschen Markt mit Beratungs- und Produktlösungen sowie Serviceleistungen für Massenspektrometrie,
Chromatographie (LC-MS/MS, HPLC, GC-MS/MS, GC), Life Sciences (Maldi, Accuspot, ChiP), Spektroskopie (UV-Vis, FT-IR, AAS), Summenparameter (TOC) sowie für Geräte in der Materialprüfung. Die LabSolution Softwarefamilie integriert Chromato- und non-Chromato- Geräte in einer Client/Server Umgebung. Neben seiner Vertriebszentrale in Duisburg unterhält Shimadzu Deutschland sechs Technische Büros und Service-Stützpunkte in regionalen Einzugsgebieten und gewährleistet damit schnelle und kurze Wege zu den Kunden.

SunChrom GmbH

https://www.sunchrom.de

SunChrom GmbH is located in the Rhein-Main-Area near Frankfurt am Main in Germany. Funded over 30 years ago, SunChrom has specialized on the development and marketing of LC system solutions for food and beverage analytics as well as front-end applications and sample preparation systems for mass spectrometry, in particular for MALDI-Mass Spectrometry.
The instrumentation provided by SunChrom covers a wide range of sample preparation applications for MALDI-MS and MS-Imaging. The product portfolio includes the multifunctional MALDI-Spotter and -Sprayer SunCollect, that can be used as an interface for liquid chromatography (LC) and MALDI-MS as well as for matrix and enzyme application high resolution for MALDI Imaging. The incubation chamber SunDigest allows fully-controlled and absolutely reproducible “on-tissue” digestion of proteins. The latest product SunPrep is used for deparaffinisation of and antigen-retrieval in FFPE-tissue sections as well as for HE-staining of the tissues after MALDI analysis.

Thermo Fisher Scientific GmbH

http://www.thermoscientific.com

Thermo Fisher Scientific Inc. ist der weltweit führende Partner der Wissenschaft mit einem Umsatz von 23 Mrd. $ und über 70.000 Mitarbeitern in 50 Ländern. Unsere Mission ist es, unsere Kunden in die Lage zu versetzen, die Welt gesünder, sauberer und sicherer zu machen. Wir helfen unseren Kunden dabei, die Life-Science-Forschung voranzutreiben, komplexe analytische Probleme zu lösen, die Diagnostik am Patienten zu verbessern und die Produktivität der Labore zu steigern. Mit unseren Hauptmarken – Thermo Scientific, Applied Biosystems, Invitrogen, Fisher Scientific und Unity Lab Services – liefern wir eine einzigartige Kombination aus innovativen Technologien, Anwenderfreundlichkeit beim Einkauf und umfangreichem Support.

Waters GmbH

https://www.waters.com

Since 1958, Waters Corporation has been in the business of making innovative analytical instruments that assist scientists in reaching their scientific goals, increase productivity, and earn laboratory-based organizations a higher return on their investments in research, development, and quality control.

Whether at work discovering new pharmaceuticals, inventing new and more effective ways to treat diseases, assuring the safety of the world's food and drinking water supplies, monitoring and controlling pollution, or conserving the world's greatest art treasures, scientists worldwide rely on Waters liquid chromatography and mass spectrometry products.

60+ years in business, Waters is now one of largest companies in the analytical instruments industry, supporting scientists working thousands of laboratories around the world. With a hard-earned reputation for applications expertise, dedication to customer success, and post-sales support, Waters stands out among its peers and, year after year, is one of the best-performing companies in the industry.

Abstracts

Plenary Lecture Abstracts

Wolfgang Paul lecture

Native MS: A Structural Biology Tool

Vicki Wysocki

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Characterization of the overall topology, inter-subunit contacts of protein complexes, and their assembly/disassembly and unfolding pathways, is critical because protein complexes regulate key biological processes, including processes important in understanding and controlling disease. Tools to address structural biology problems continue to improve. Native MS is becoming an increasingly important component of the structural biology toolbox. When the mass spectrometry approach is used early or mid-course in a structural characterization project, it can provide answers quickly using small sample amounts and samples that are not fully purified. Examples will be presented to illustrate the role MS and surface-induced dissociation can play in guiding a structural biology workflow and will include designed protein complexes and isolated or recombinant protein and nucleoprotein complexes.

Plenary Lecture 01

Quantum optics and information science in multi-dimensional photonics networks

Christine Silberhorn
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Classical optical networks have been widely used to explore a broad range of transfer phenomena based on coherent interference of waves, which relate to different disciplines in physics, information science, and even biological systems. At the quantum level, the quantized nature of light gives rise to genuine quantum effects that can appear completely counter-intuitive.
Photonic quantum systems with many optical modes have been investigated intensively in various theoretical proposals over the last decades. However, their implementation requires advanced setups of high complexity, which poses a considerable challenge on the experimental side. The successful realization of controlled quantum network structures is key for many applications in quantum optics and quantum information science.
Here we present three differing approaches to overcome current limitations for the experimental implementation of multi-dimensional quantum networks: non-linear integrated quantum optics, pulsed temporal modes and time-multiplexing.

Plenary Lecture 02

Interstellar radionuclides identified in deep-sea archives

Anton Wallner
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The Interstellar Medium (ISM) is continuously fed with new nucleosynthetic products. The solar system moves through the ISM and collects dust particles. Therefore, direct detection of freshly produced nuclides on Earth provides insight into recent and nearby nucleosynthetic activities. ISM radionuclides trapped in deep-ocean archives include ^{60}Fe (t1/2=2.6 Myr), ^{26}Al (0.7 Myr) and ^{244}Pu (81 Myr). These nuclides can be measured with Accelerator Mass Spectrometry (AMS) with high sensitivity.
Recent measurements, which continued pioneering work at TU Munich, demonstrate a global ^{60}Fe influx and is evidence for exposure of Earth to recent supernova explosions. Unknown is still the site where the heaviest elements are made in nature. Very low concentrations measured for ^{244}Pu, however, disfavours supernovae as the predominant producing site for heavy-element nucleosynthesis.

I will present new results for ^{60}Fe measured at the ANU and ^{244}Pu at ANSTO with unprecedented sensitivity. These data provide new insights into their concomitant influx and their ISM concentrations over a time period of the last 11 Myr.

Plenary Lecture 03

Application of Mass Spectrometry for Detecting and Finding Cures to Brain Disorders

Joseph A. Loo
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Bioanalytical mass spectrometry can play important roles to positively impact our ability to detect human health disorders and to develop potential therapies. Our laboratory has been engaged in research to determine the mechanistic basis of compounds that inhibit protein aggregation found in neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases. Protein "native" mass spectrometry, ion mobility, and tandem mass spectrometry are key techniques used in these studies. The role of mass spectrometry and quantitative liquid chromatography/MS to discover new protein biomarkers for head trauma, i.e., traumatic brain injury (TBI) and how these markers perform in clinical studies will also be highlighted in this presentation.

Plenary Lecture 04

Exploring Exotic Elements - all about Astatine and Actinides as accessible from Laser Mass Spectrometry

Klaus D.A. Wendt
Johannes Gutenberg-Universität, Mainz, Germany; kwendt@uni-mainz.de

The UN has proclaimed 2019 as the International Year of the Periodic Table of Chemical Elements, celebrating the 150th anniversary of this significant discovery by Dmitry Mendeleev. Today the table with its broad implications in astronomy, biology, chemistry, geosciences, physics, and even medical research extends up to element 118. Nevertheless, for a number of elements listed, which either have no stable isotopes or are produced only artificially, still today fundamental relevant quantities have not been determined precisely or are entirely missing. These gaps concern atomic and nuclear structure, isotope and isomer effects, and include even basic parameters like ionization potential or electron affinity. Resonant laser mass spectrometry is the method of choice for investigations on rare species. In the range around proton numbers Z=85-95 a variety of results has been reported during the last years, not only filling these blanks but also enabling analytical lowest-level determination of radiotoxic contaminations.

Plenary Lecture 05

Light and cavity induced new states of matter: Quantum Electrodynamical Density Functional Theory (QEDFT)

Angel Rubio, H. Appel, M. Ruggenthaler, H. Hübener, U. de Giovannini, M. Sentef, J. Flick, C. Schafer, V. Rokaj, D. Welakuh
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Computer simulations that predict the light-induced change in the physical and chemical properties of complex systems usually ignore the quantum nature of light. Recent experiments at the interface between materials science and quantum optics have uncovered situations where both the molecular system and the photon field have to be treated in detail. In this talk, we show how the effects of quantum-photons can be properly included in the newly developed quantum electrodynamics density-functional formalism (QEDFT). We provide an overview of how well-established concepts in the fields of quantum chemistry and material sciences have to be adapted when the quantum nature of light becomes important. We identify fundamental changes in Born-Oppenheimer surfaces, conical intersections, spectroscopic quantities, and quantum control efficiency. We also show how periodic driving of many-body systems allow to design Floquet states of matter with tunable electronic properties on ultrafast time

scales (and cavity induced-topology). This work paves the road for the development of two new fields, namely QED-materials and QED-chemistry.

Plenary Lecture 06

Circular Dichroism and Mass Spectrometry: An Unusual Liaison

Ulrich Boesl
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Precondition of this liaison (CD-MS) is CD-spectroscopy (CDS) in the gas phase. This is a restriction on the one hand, but opens new possibilities such as two-photon CDS, molecular ion CDS, and cold-molecular-beam CDS on the other hand.
In comparison to conventional electronic CDS, two-photon CDS allows access to so-called dipole-forbidden electronic states and may exhibit considerable CD-enhancement. CDS on molecular ions opens ways to study internal molecular dynamics and it may allow mass selective CDS of biomolecules from a MALDI source. Molecular cooling in supersonic beams enables selective excitation of special vibrations which are expected to show CD enhancement in electronic transitions. This effect is congested at room temperature by overlapping bands.
Draw-backs of CD-MS and measures to overcome them as well as recent developments of CD-MS beyond the above topics will be discussed. For review see *U. Boesl, A. Kartouzian, Annual Rev. Anal. Chem. 2016, 9, 343.*

Plenary Lecture 07

Chemistry first, Accelerator Mass Spectrometry (AMS) second

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Accelerator mass spectrometry (AMS) is the most sensitive analytical method to measure long-lived radionuclides. The detection limits are generally several orders of magnitude better, i.e. as low as 10^{-16} (radionuclide/stable nuclide), than any other mass spectrometry or decay counting method. AMS needs smaller sample sizes and measurements are finished within a few minutes to hours; though after performing chemical separation of the radionuclide from the sample matrix (ice, snow, rain, ground water, marine sediments, soil, meteorites, deep-sea nodules, lava, rocks). Hence, AMS is right from the start, from sample taking over chemistry and measurements to data interpretation, true interdisciplinary research.
Users at the DREAMS (DREsden AMS) facility [1] apply AMS to most diverse projects, e.g. the proof and dating of multiple supernovae during the last 10 Ma [2] and dating of a boulder from a rock fall triggered by a medieval Earthquake in the Nepal Himalaya [3].
Ref.:
[1] www.dresden-ams.de
[2] Nature 532 (2016) 69
[3] Science 351 (2016) 147

Plenary Lecture 08

The future of time: prospects for a redefinition of the SI second

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The reform of the International System of Units (the SI) in which the kilogram, ampere, kelvin, and mole are all defined by fixing the values of four fundamental constants of nature, gives us a measurement system in which all of the base units are defined in terms of natural constants. While one might have hoped that this reformed SI would serve us into the indefinite future, the SI unit of time, the second, remains in serious need of redefinition. This talk will describe the history and current status of the SI second, and speculate about a possible redefinition.

Plenary Lecture 09

Chemical Energy Storage: a Key Element for a Sustainable Energy Future

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Our energy systems are facing fundamental changes, caused by the depletion of fossil fuels and climate change. This requires increased use of renewable energy, which are typcially intermittent, such as solar radiation and wind energy. Storage of energy could thus become a key question in future energy systems, and methods for storage and different time and size scales are necessary. Chemical storage, including electrochemical systems such as batteries, have advantages compared to purely physical methods, since only chemical methods reach the required storage densities. The presentation will address the conditions, which future storage systems will have to meet and discuss different systems and their integration into the energy system. Main develeopment lines and the research needs associated with them will also be addressed.

Parallel Session Abstracts

PS 01: From fundamentals in Proteome Research to Structural Proteomics

10:30am - 10:50am

An Integrated One-Week Protocol for Proteome-Wide Cross-Linking/Mass Spectrometry Studies Based on the MS-Cleavable Cross-linker DSBU and the MeroX 2.0 Software

Claudio Iacobucci, Michael Götze, Christian Ihling, Andrea Sinz
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We developed and applied a fast and robust integrated workflow to perform cross-linking/mass spectrometry experiments of highly complex protein mixtures, such as cell lysates and intact cells. Our protocol only requires one week to complete and is based on the commercially available MS-cleavable cross-linker disuccinimidyl dibutyric urea (DSBU). The workflow can be employed by every lab having access to a mass spectrometer with tandem MS capabilities. We provide the version 2.0 of the freeware software MeroX (www.StavroX.com) that allows a fully automated analysis to deliver insights into protein interaction networks and protein conformations on a proteome-wide scale. We demonstrate the successful application of our workflow for *Drosophila* embryo extracts as well as intact *E.coli* cells and human embryonic kidney cells.

10:50am - 11:10am

From Structural Insight into an Ene-Reductase by Ion Mobility Mass Spectrometry to Experimental Conditions of a Biotransformation

Jens Sproß, Yasunobu Yamashita, Harald Gröger
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In the last decades, an increasing tendency to apply biocatalysis in organic synthesis was observed. Among redox enzymes, the ene-reductase from *Gluconobacter oxydans* (GOX-8) turned out to represent a versatile biocatalyst, useful for the reduction of different types of activated C=C double bonds.[1-3] Encouraged by the success of ion mobility mass spectrometry for studying protein structures in the absence of bulk water[4], we chose this methodology to obtain information about the folding of this protein in dependence of the amount of acetonitrile used in the buffer system. The results about the folding properties in the gas phase are expected to give an insight into the solvents effects of the enzyme in the condensed phase.
The ene reductase GOX-8 was analysed using *off-line* nanoESI-Q-IMS-ToF mass spectrometer (Synapt G2Si, Waters, Manchester). Data analysis was performed using and Driftscope™ and BioLynx™ (Waters, Manchester). The results show a strong tendency of the protein to unfold in aqueous solutions of acetonitrile. In contrast, in the presence of ammonium acetate as buffer, the folding of the protein keeps stable in acetonitrile contents up to 25%.
In parallel, studies on the impact of acetonitrile content and buffer in "*in-solution* biotransformation" using a micro-titerplate-assay are currently in progress to enable a comparison of the two methodologies for the determination of the impact of reactions parameters such as cosolvent and buffer on the enzyme folding, and thus, activity.

11:10am - 11:30am

Triggering and Monitoring of EBV's potential G-Quadruplexes in native mass spectrometry

Kira Schamoni[1], Boris Krichel[1], Charlotte Uetrecht[1,2]
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Putative G-Quadruplex forming sequences (PQS) are present in all species of life playing important regulatory roles. In Epstein-Barr Virus (EBV), G-Quadruplexes (GQ) regulate translation of maintenance proteins for the protein EBNA1. This mechanism preserves EBV's latency. QGRS mapper, a prediction tool, revealed several overlapping PQS in EBNA1. For studying EBV's GQs, five PQS were chosen.
The aim is to characterize biophysically these sequences via circular dichroism (CD) and native mass spectrometry. Folding behavior of potential GQs should be analyzed by triggering GQ formation with increasing potassium ion concentration to monitor ion complexation.
This study shall gain new insights into EBNA1's G-rich region and putative GQ formation – in the broader sense to understand translational repression and virus' host immune evasion.

11:30am - 11:50am

Assembly studies of the soluble part F_1 of bacterial F-type ATP synthases with LILBID-MS

Khanh Vu Huu[1], Volker Müller[2], Nina Morgner[1]
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F-type ATP synthases are multiprotein complexes composed of two coupled motors (F_1 and F_O) providing ATP as the universal major energy source in a variety of relevant biological processes. However, the precise assembly pathway of F_1F_O-ATP synthases is still unclear. To analyze the subunit assembly in the soluble part F_1 of *Acetobacterium woodii*, we express all subunits of F_1 and determine binding steps by *Laser Induced Liquid Bead Ion Desorption Mass Spectrometry* (*LILBID-MS*).
In LILBID-MS a droplet generator generates droplets of an aqueous sample. These are transferred into vacuum and irradiated with an IR laser, leading to an explosive expansion of the droplets, ionization and detection by a time-of-flight analyzer.
With LILBID-MS experiments we finally propose a model assembly pathway and investigate conditions for the formation of the F_1 complex from single subunits.

11:50am - 12:10pm

Optimization of an LC-MS targeted proteomics approach for investigation of cyclooxygenase 2

Nicole M. Hartung[1], Stephan Immenschuh[2], Nils Helge Schebb[1]
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Oxylipins are potent mediators of many physiological functions such as intracellular signaling, inflammation and pain, and are formed in the arachidonic acid (ARA) cascade via cyclooxygenases (COX), lipoxygenases (LOX) and cytochrome P450 (CYP) enzymes. They are analyzed dominantly by LC-MS/MS, where their concentrations are directly quantified. However, this approach allows no detailed insights into the mechanisms responsible for changes in the oxylipin pattern. For this reason, we developed a targeted proteomics LC-MS/MS method to quantify COX-2 expression levels, allowing to comprehensively monitor the COX-2 branch of the ARA cascade together with an established targeted oxylipin method.
Based on *in silico* tryptic digestion and data from proteomics platforms (e.g. sites of natural variants, post-translational modifications, cleavage probability) unique "proteotypic peptides" (PTP) were selected. MS-detectability of the candidate peptides was evaluated on a QTRAP instrument in positive-electrospray (ESI+) mode and MS parameters were optimized, increasing the signal up to 20-fold and enabling detection in the low ng/mL range. For analysis of COX-2 expression levels in cells a standard sample preparation workflow including protein precipitation and desalting via soild phase extraction (SPE) columns was optimized, further enhancing the sensitivity of the method. It was then applied to monitor COX-2 expression levels in different human colon cancer cell lines and challenged macrophages in parallel to COX-2 derived oxylipins. Inclusion of the remaining ARA cascade enzymes into the method will help to further understand the mechanisms responsible for a modulation of the ARA cascade by drugs and food ingredients.

12:10pm - 12:30pm

Mass spectrometric strategies for snake venom system research [First results for the saw-scaled viper (*Echis carinatus sochureki*)]

Parviz Ghezellou[1], Wendell Albuquerque[2], Vannuruswamy Garikapati[1], Patrik Kadesch[1], Seyed Mahdi Kazemi[3], Alireza Ghassempour[3], Bernhard Spengler[1]
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Proteinaceous components of snake venom have been shown a wide range of biological functions. Venoms facilitate snake survival but venom compounds are also very potent medicinal compounds. An in-depth understanding of venom systems (venom gland and secreted proteomes) can help to 1) develop anti-venoms, 2) discover new drug candidate molecules, and 3) provide insight into the evolution of venom mechanisms. Here we present the comprehensive methods for complex venom systems in saw-scaled viper employing SEC-RP-LC proteomics and MS imaging strategies. Top-down and bottom-up results for the venom`s protein composition will be presented and help to assign and quantify venom constituents, whereas MSI of the venom gland reveals first hints on the storage and secretion mechanism of the venom.

PS 02: MS instrumentation I

10:30am - 10:50am

High precision mass measurement and separation of nuclear isomers with a multiple-reflection time-of-flight mass spectrometer

Christine Hornung[1], Samuel Ayet San Andrés[1,2], Daler Amanbayev[1], Julian Bergmann[1], Timo Dickel[1,2], Hans Geissel[1,2], Israel Mardor[3,4], Ivan Miskun[1], Wolfgang R. Plaß[1,2], Christoph Scheidenberger[1,2], And the FRS Ion Catcher Collaboration[1,2]

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The multiple-reflection time-of-flight mass spectrometer (MR-TOF-MS) is an ideal tool to investigate masses of short-lived (millisecond) exotic nuclei and their nuclear isomers. It is part of the FRS Ion Catcher at GSI in Darmstadt, where mass measurements and ion separation are performed in a short measurement duration (~20ms), with mass resolving powers up to 620,000 and an uncertainty better than 0.1 ppm. The masses of more than 40 short-lived ground and isomeric states, produced at relativistic energy, with half-lives down to 17.9ms and count rates as low as 11 events per nuclide were determined. Among them 9 nuclides were measured for the first time directly, e.g., helping to understand nuclear structure phenomena around the double magic nucleus ^{208}Pb.

10:50am - 11:10am

T-MALDI-2-Orbitrap MS: Sensitive ion imaging with sub-micrometer resolution and ppm mass accuracy

Marcel Niehaus[1], Jens Soltwisch[1,2], Mikhail Belov[3], Klaus Dreisewerd[1,2]

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MALDI-MS imaging is an emerging label-free technique for visualizing the distribution of numerous biomolecular classes in tissue sections and in cell cultures. Here we combined transmission (t-) mode MALDI-MSI with laser-induced postionization (MALDI-2) and a dual-ion funnel/Orbitrap MS analyzer. In this way, we achieved (i) a sub-micrometer resolution, (ii) a crucially enhanced sensitivity and chemical coverage, and (iii) a high mass accuracy in the 1 ppm range. We demonstrate and discuss the performance characteristics of our t-MALDI-2-MSI technique with sub-confluently grown Vero and Renca cell cultures and by visualizing the neuronal organization of Purkinje cells in mouse cerebellum. Via the used high-N.A. objective and a CCD camera our set-up also enabled parallel optical inspection of the tissues at high magnification.

11:10am - 11:30am

Fast identification of metabolites in complex samples using ultra-high resolution magnetic resonance mass spectrometry

Matthias Witt[1], Theodora Nikou[2], Maria Halabalaki[2], Markus Godejohann[3], Nikolas Kessler[1], Aiko Barsch[1], Christopher Thompson[4]

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Identification of metabolites in complex food, urine or blood plasma samples is typically performed by LC/MS in 15-20 minutes per sample. We present a fast approach in < 3min in Flow Injection (FIA) combined with ultrahigh resolution FTMS after a simple purification step.Food and clinical samples were analyzed using the solariX FTMS (Bruker) in ESI and MALDI. Data evaluation was done by PCA (MetaboScape, Bruker) and by OPLS-DA (SIMCA-P, Umetrix). Molecular formula calculation was based on accurate mass, isotopic fine structure.Olive oils and wines could be clustered according to geographical origin and the type of wine using OPLS-DA. Hundreds of metabolites responsible for grouping of samples could be identified. MRMS data was in good agreement with quantitative 1H-NMR.

11:30am - 11:50am

Fast Online Separation and Identification of Electrochemically Generated Isomeric Phase-I Metabolites by means of Trapped Ion Mobility-Mass Spectrometry

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The hyphenation of electrochemistry and mass spectrometry (EC/MS) has emerged as a powerful tool in mimicking the phase-I metabolism of xenobiotics catalyzed by cytochrome P450 enzymes. Combined with high resolution mass spectrometry, EC enables identification of unknown metabolites and short-lived intermediates via accurate mass detection. Moreover, fragmentation experiments allow structural elucidation. The distinction between electrochemically generated isomers requires conventional LC separation techniques coming along with increased method complexity and prolonged analysis times. Trapped ion mobility spectrometry (TIMS) offers a time-efficient separation enabling fast analysis of short-lived metabolites after their generation.

Oxidative metabolites were generated in an electrochemical thin-layer cell equipped with a boron-doped diamond working electrode. In order to obtain three-dimensional mass voltammograms, the EC cell was coupled online to an ESI-TIMS-ToF-MS applying a potential ramp by an external potentiostat. Oxidation products were identified via accurate mass detection. In the same analysis run, mobilo voltammograms for each *m*/*z* were recorded to distinguish between isomeric metabolites. Additionally, MS/MS experiments were carried out for each mobilogram signal.

Identification of the different metabolites of the model compound metoprolol was achieved via accurate mass detection provided by mass voltammograms. TIMS separation revealed different isomeric hydroxylations taking place depending on the applied potential. Additionally, subsequent reactions of formed metabolites were detected occurring at higher potentials by overlaying different mobilo voltammograms revealing possible metabolic reaction pathways. MS/MS results after TIMS separation were in good accordance to previous HPLC based experiments. Therefore, the analysis time can be reduced by at least a factor of five.

11:50am - 12:10pm

DB-nESI overcomes the ion flux problem of modern nanoLC-MS/MS

Sebastian Brandt[1], Irina Reginskaya[1], Michael Schilling[1], Albert Sickmann[1,2,3], Joachim Franzke[1], Stefan Loroch[1]
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Question

With increasing scan rates of modern MS instruments, efficient ion transfer becomes a major bottleneck of sensitivity. In fact, Kelstrup et al. have recently shown that the newest quadrupole-Orbitrap instrument (Q Exactive HF-X) does not benefit from the 41Hz-acquisition mode, if low sample amounts are injected because of the insufficient ion flux of nano-electrospray ionization (nESI). To overcome the emerging ion flux problem, we developed a novel dielectric barrier nESI (DB-nESI) interface, which allows injection of dense ion packages within a few milliseconds.

Methods

We compared DB-nESI and nESI as interface for LC-MS in bottom-up proteomics using an LTQ Orbitrap Elite (Thermo Scientific) online-coupled to nanoHPLC (reversed phase). The MS was operated in data dependent acquisition-mode with short maximum ITs (0.5, 6ms for MS, MS/MS) to mimic the high scan rate of modern instruments. 50 ng HeLa digests were analyzed in quadruplicates and database search was conducted using Mascot.

Results

In comparison, our DB-nESI interface improved identification 40% on peptide and 29% on protein level, predominantly attributed to higher precursor intensities (1.7-fold) and improved S/N. Relative intensities did not correlate with physicochemical peptide properties indicating no complementarity. Accordingly, 88% of all peptides identified with nESI were also detected by DB-nESI.

Conclusion

DB-nESI allows for considerable improved protein identification in bottom-up LC-MS with short ion injection times. Our results render DB-nESI as next-generation ionization method, especially in the light of most recent instrumental improvements, as it overcomes the ion flux problem of modern instruments operated at high scan rates.

12:10pm - 12:30pm

Detection of Anti-TB drugs in organs of mice with LC-MS

Franziska Waldow[1], Kerstin Walter[2], Michael Weinkauf[1], Ann-Kathrin Stoltenberg[2], Hande Karaköse[1], Christoph Hölscher[2], Dominik Schwudke[1]

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An increased incidence of multidrug resistant tuberculosis (MDR TB) worldwide made it necessary to develop and apply combination regimens with up to 5-7 antibiotics. Such antibiotic therapy may last a prolonged period of time up to 24 months. Lack of adherence or incorrect dosages can lead to development of further resistances during treatment, while high doses could lead to increased side effects. Animal models reflecting human pulmonary pathology (e.g. interleukin-13-transgenic (IL-13^{tg}) mice) are of particular importance for an improved preclinical validation of novel drug candidates. To validate the effectiveness of Anti-TB drugs, mouse lung tissue was analyzed by mass spectrometry. Therefore, we developed a high-performance liquid chromatography-mass spectrometry (HPLC-MS^2)-based multi-analyte assay, which enables us to quantify 20 antibiotics in all medical applied combination regimens in one HPLC run. For the determination of the drug concentration in murine lung tissue mice were treated five times a week with clofazimine, rifampicin and pyrazinamide. The lung tissues were extracted by liquid-liquid extraction with acetonitrile and 1% formic acid in water. Measurements are performed on an 1100 HPLC system utilizing a Milipore SeQuant ZIC-HILIC column which was coupled with a Quattro Premier XE triple quadrupole MS applying electro spray ionization. The LC-MS^2-based multi-analyte assay is based on multiple reaction monitoring (MRM) for 20 antibiotics and five internal standards. The runtime is only 20 minutes and was also established for drug monitoring in plasma. We determined the drug concentration in lung tissues and observed an accumulation in dependence to their half-life.

PS 03: Lipidomics & Metabolomics I

10:30am - 10:50am

Does the Fenton Reaction have physiological Relevance? - An MS Study with Phospholipids

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Reactive oxygen species (ROS) play an enormous role in medicine. In particular, lipid (phospholipid) oxidation needs to be explored in more detail because many important diseases (such as atherosclerosis) are accompanied by increased levels of lipid peroxidation products. The Fenton reaction, i.e. the transition metal (cuprous or ferrous ions) catalyzed decomposition of H_2O_2 is often used in the laboratory to generate hydroxyl radicals (HO•) and the Fenton reaction is also assumed to occur under in vivo conditions. Lipids with unsaturated fatty acyl residues are primarily converted by HO• radicals into (hydro)peroxides. These primary products are afterwards converted into chain-shortened aldehydes and carboxylic acids. In contrast, chlorohydrins are generated by the treatment of unsaturated phospholipids with HOCl, i.e. an addition of HOCl to the double bonds occurs.
We will show by means of MALDI-TOF and ESI mass spectrometry that halogenated products (particularly chlorohydrins) are the main products if the Fenton reaction is performed in the presence of NaCl. In contrast (hydro)peroxides and products derived thereof are generated in the absence of NaCl. Therefore, it also makes a significant difference whether $FeCl_2$ or $FeSO_4$ is used to initiate the generation of HO•. These results question the in vivo relevance of the Fenton reaction and of the HO radicals because in physiological systems there is always an excess of NaCl which readily converts HO• into HOCl. A mechanism which explains the related pathways will be suggested.

10:50am - 11:10am

Relative quantification of phospholipid *sn* isomers using positively doubly charged lipid-metal ion complexes

Simon Becher, Patrick Esch, Bernhard Spengler, Sven Heiles
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Assignment of phospholipid *sn* isomers, permutational isomers of the fatty acid (FA) positions, is challenging. Only few techniques allow for untargeted assignment of *sn* isomers. Here, we present results for a simple tandem-MS strategy that allows phospholipid *sn* isomer assignment by fragmentation of phospholipid metal ion complexes. ESI of solutions containing authentic phospholipid standards and divalent metal cations was employed to optimize metal-lipid complexation yields for different phospholipid classes. CID, HCD and 213 nm UVPD were used to fragment resulting doubly-charged phospholipid complexes. The tandem mass spectra were dominated by two fatty-acid-associated fragment ions for every FA moiety, whereas head group loss channels were suppressed compared to results for protonated phospholipid or phospholipid alkali metal adduct ions. For lipids containing two different FA moieties, activation of lipid-metal complexes resulted in preferential dissociation of the FA moiety located at the *sn*-2 position of the glycerol backbone. Intensity ratios of the pairs of mass spectrometric FA fragment ion signals were used to deduce relative *sn*-isomer abundances. Results for phosphatidylcholines were compared to in-solution PLA_2 digestion and revealed that CID and UVPD facilitate *sn*-selective fragmentation, whereas for HCD *sn*-scrambling can occur. Consequently, CID and UVPD were utilized in order to infer *sn*-isomer abundances of phosphatidylcholines in complex lipid extracts.

11:10am - 11:30am

Investigation of Oxidation Sites of Triglycerides in Food Oil Samples using Paternò-Büchi Functionalization

Patrick Esch, Sven Heiles
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Lipids play crucial roles in cell function and signaling and are ubiquitously found in organisms. Oxidation processes can alter fatty acid moieties close or at C=C double bond positions by forming alcohols, ketones or other functional groups and therefore change the fluidity and chemical reactivity of these molecules. To pinpoint oxidation sites and unveil functional groups of the oxidized lipids, we developed a Paternò-Büchi reaction scheme using 3-acetylpyridine and triglyceride standards and food oils. Experiments with internal standards reveal that UV light oxidation does not occur. We will present first

results for the oxidation sites of triglycerides derived from Paternò-Büchi measurements as a function of reaction time. These findings will be interpreted in the context of known lipid oxidation processes.

11:30am - 11:50am

Lipid identification at double bond position level using customized software and data evaluation strategies

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Technical advances regarding liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) enable fast measurements of full lipid extracts. However, the interpretation and evaluation of the resulting multidimensional data sets is challenging and still the bottleneck in terms of the total analysis time. Therefore, new evaluation tools for the automated analysis of HRMS and tandem mass spectrometry (MS/MS) data sets have been developed and implemented in the open-source metabolomics software MZmine 2.

To evaluate the developed tools, membrane lipids of the green alga *Chlamydomonas reinhardtii* were analyzed by LC-HRMS/MS in combination with a Paternò-Büchi post-column derivatization. This derivatization is a photoinduced functionalization of C-C double bonds in lipid acyl chains with acetone. Data-dependent MS/MS experiments of the resulting reaction products allow localization of the double bond position based on diagnostic fragments. Through an optimized MZmine 2 workflow in combination with a newly developed module for the annotation of lipids, Paternò-Büchi products and respective diagnostic fragments were identified. To confine the candidates of potential lipid classes and species, data mining with 3D Kendrick mass plots was previously performed, which enables fast graphical spotting of lipids due to their homologous appearances on horizontal lines.

The developed tools for MZmine 2 have reduced the total evaluation time for a highly complex real sample data set of the green alga *Chlamydomas reinhardtii* to about one day. Due to this extensions MZmine 2 is the only available software to support the identification of lipids at the double bond position level.

11:50am - 12:10pm

Lipid profiling of beef muscle tissues by LC-MS/MS and GC analysis and possible health benefits of odd chain fatty acids

Beate Fuchs, Dirk Dannenberger
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Wagyu beef fat is often observed to be softer than other breeds, implying more unsaturated fat in Wagyu animals. However, it seems that Wagyu cattle have a greater amount, but not greater percentage of these because of the higher fat content.

Odd chain fatty acid (FA) metabolism might have an underestimated role in beef muscle and for human health. Historically, odd chain saturated fatty acids (esterified in intact lipids) were used as internal standards in GC-MS (or LC-MS) methods, as it was thought their concentrations were insignificant in humans and in other mammals. Later on, increased consumption of dairy products was associated with the increased blood plasma levels of odd chain fatty acids. Nowadays it is known that odd numbered FAs more likely accumulate in the adipose tissue than even numbered FAs.

LC-MS/MS and GC profiling of lipids and fatty acids will compare Wagyu beef muscle with other beef breeds. Results suggest that odd chain FAs increase softness of fat to a similar degree as PUFAs, whereby it is known that even chain saturated FAs decrease softness.

12:10pm - 12:30pm

Probing glycation potential of dietary carbohydrates by a combination of mass spectrometry-based approaches

Viet Duc Nguyen[1,2], Nadezhda Frolova[2], Alena Soboleva[1,3], Tatiana Mamontova[1,3], Gerd U. Balcke[4], Uta M. Herfurth[5], Claudia Birkemeyer[2], Andrej Frolov[1,3]
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Type two diabetes mellitus (T2DM) is one of the most widely spread metabolic diseases, accompanied with enhanced glycation of blood proteins with glucose and formation of advanced glycation end-products (AGEs). However, other monosaccharides, despite their lower (in comparison to glucose)

plasma concentrations, might contribute to glycation patterns of blood proteins. Moreover, the concentrations of these sugars can vary much depending on individual dietary habits, affecting glycation patterns, characteristic for T2DM. Therefore, here we address relative reactivity and glycation potential of five major plasma sugars using *in vitro* glycation models based on synthetic peptides.For this, the peptides Ac-AFGSAKASGA-NH_2 and Ac-AFGSARASGA-NH_2 were incubated with equimolar amounts of the corresponding sugars under the buffer conditions, mimicking plasma (0 – 28 days, 37° C, pH 7.2). The patterns of the peptide-derived AGEs were characterized by RP-UHPLC-QqTOF-MS in parallel to quantitative profiling of glycation carbonyl intermediates, i.e. carbohydrates and α-dicarbonyls, with GC-EI-Q-MS and RP-HPLC-IT-MS, respectively, after appropriate derivatization. The analysis revealed totally 28 and 14 oxidative and glycoxidative modifications in total, identified in incubations with lysine- and arginine-containing peptides, respectively. Thereby, incubations with ascorbic acid and fructose resulted in the highest peptide degradation rates and intense formation of arginine-derived AGEs that might be related to enhanced production of α-dicarbonyls – glyoxal and methylglyoxal. Verification of this result in model incubations with human serum albumin revealed, however, site-specific formation of the corresponding protein AGEs.

PS 04: Affinity-Mass Spectrometry

2:00pm - 2:20pm

Intact Transition Epitope Mapping - Targeted High-energy Rupture of Extracted Epitopes (ITEM-THREE)

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Identification of protein epitopes is essential for modern design of therapeutics and diagnostics. Mass spectrometric epitope mapping has been shown to be extremely powerful. However, complicated solution handling limits its application for epitope mapping.

We developed an affinity-mass spectrometry method which is capable of identifying epitopes with very little sample manipulation. Immune complexes are formed without any immobilization or additional purification procedures. Upon mixing the antibody of interest with antigen peptides and nano-electrospraying them into the mass spectrometer, epitope peptides are extracted from unbound peptides, immune complexes are dissociated, and complex-released peptides are fragmented. Resulting mass lists are then submitted unto data base search to retrieve the amino acid sequence of the epitope and to reveal the antigen.

2:20pm - 2:40pm

Epitope Identification of an aptamer complex of Cathepsin D in comparison to an antibody-cathepsin D complex

Pascal Wiegand[1], Loredana Lupu[1], Maxim V. Berezovski[2], Andre R. A. Marques[3], Paul Saftig[3], Michael Przybylski[1]

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Cathepsin D (CD) is an aspartic protease with pepsin like cleavage behavior that processes, degrades and activates proteins and hormones. Besides its natural function a loss in CD activity is related to neuronal ceroid lipofuscinosis (NCL) and especially to NCL-10, a neuronal lysosomal storage disorder. Patients with such diseases are treated with enzyme replacement therapy (ERT) by infusion of recombinant active CD for compensation of the own CD. During ERT frequently autoimmune response is observed with formation of antibodies against the new CD which binds to the enzyme and renders the treatment ineffective. In order to inhibit autoimmune antibodies we are investigating the use of aptamers against CD produced by the SELEX procedure. Aptamers are single stranded DNA (or RNA) oligonucleotides which are able to bind proteins. To evaluate the use of aptamers for the inhibition of antibodies we compared the binding properties of an antibody-CD complex to an aptamer-CD complex. For comparison we determined the epitope of the aptamer and the antibody on the CD by proteolytic epitope extraction mass spectrometry; in addition we determined the binding kinetics and affinities by a surface plasmon resonace (SPR) assay. For investigation of the epitopes an enzymatically inactive pro-Cathepsin D (pCD) is used which has the same amino acid sequence like the CD and an additional pre-sequence which makes it enzymatically inactive.

2:40pm - 3:00pm

MS analysis of protein complex assembly pathway for the biosynthesis of APE

Kudratullah Karimi[1], Gina L.C. Grammbitter[2], Helge B. Bode[2], Nina Morgner[1]

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Aryl polyenes (APE) are a class of polyketides found in many bacteria and are biosynthesized by the polyketide synthase II (PKS II) like mechanism. Ten proteins are known to be involved in the PKS II system, but the exact process of the biosynthesis of APEs is mostly unknown. We analyzed the APE enzymes by nESI-MS and identified stable protein complexes, which play a role during the APE elongation. We could determine the oligomeric states and binding behavior of the proteins that are involved in APE biosynthesis and the regulating effect of the acyl carrier protein on this process. From these results we are on the way to understanding the pathway of APE biosynthesis.

3:00pm - 3:20pm

Epitope Identification and Affinity Characterization of Myoglobin by combination of SPR biosensor analysis and Mass Spectrometry

Delia Mihoc[1], Loredana Lupu[1,2], Pascal Wiegand[1], Michael Przybylski[1]
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Myoglobin(MB) is a biomarker for muscle injury making it a potential target protein for early detection of myocardial infarct. However, elevated myoglobin levels alone have low specificity for acute myocardial infarction (AMI), and thus together with cardiac troponins is taken into account for diagnosis. Myoglobin is a monomeric heme protein with a molecular weight of 17 kDa that is found in skeletal and cardiac tissue as intracellular storage unit of oxygen. Myoglobin consists in 8 α- helices connected by loops and a heme group responsible for the binding of oxygen. Monoclonal antibodies are widely used analytical tools in biochemical research. The knowledge of the corresponding epitope structure recognized by antibodies, is of major interest as a biomarker. One major approach for epitope identification and characterization is the application of proteolytic epitope extraction/excision-affinity methods in combination with mass spectrometry and surface plasmon resonance (SPR) biosensor analysis. In this study immobilization of an MG antibody, followed by affinity incubation of proteolytic digestion mixture and elution of the epitope. The method was developed in a model study consisting of horse heart myoglobin and commercially available antibodies. The aim of this study was the identification of the myoglobin epitope. The results show the epitope region to be located in the C-terminal sequence [148-153] [ELGFQG] of the protein. SPR kinetic evaluation of myoglobin provided a K_D of 450 nM. The identification and knowledge of epitope structure is of major interest for the development of MB as a diagnostic biomarker.

3:20pm - 3:40pm

Retinal Guanylyl Cyclase 1/GCAP-2 Interaction Studied by Cross-linking/Mass Spectrometry

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The retinal guanylyl cyclase 1 (ROS-GC 1) is a transmembrane protein that is regulated by guanylyl cyclase-activating proteins (GCAPs) upon changes in intracellular Ca^{2+} concentration. The exact mechanisms of how GCAP-1 and -2 activate their target proteins have not been fully elucidated yet. To gain insights into GCAP's binding sites at ROS-GC, we conducted cross-linking/MS studies with three ROS-GC peptides: The first peptide comprises the binding motif of GCAP-2, the second one contains an *N*-terminal extension, while the third peptide contains the GCAP-1 binding motif. The majority of cross-links were identified between GCAP-2 and second ROS-GC peptide confirming the interaction site of GCAP-2 with the catalytic domain of ROS-GC. Additionally, surface plasmon resonance and fluorescence spectroscopy were performed.

3:40pm - 4:00pm

A sensitive and simple targeted proteomics approach to quantify transcription factors of the unfolded protein response pathway in glioblastoma cells

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Many cellular events are driven by changes in protein expression, measurable by mass spectrometry or antibody-based assays. However, using conventional technology, the analysis of transcription factor or membrane receptor expression is often limited by an insufficient sensitivity and specificity. To overcome this limitation, we have developed a high-resolution targeted proteomics strategy, which allows quantification down to the lower attomol range in a straightforward way without any prior enrichment or fractionation approaches. The method applies isotope-labeled peptide standards for quantification of the protein of interest. As proof of principle, we applied the improved workflow to proteins of the unfolded protein response (UPR), a signaling pathway of great clinical importance, and could for the first time detect and quantify all major UPR receptors, transducers and effectors that are not readily detectable via antibody-based-, SRM- or conventional PRM assays. As transcription and translation is central to the regulation of UPR, quantification and determination of protein copy numbers in the cell is important for

our understanding of the signaling process as well as how pharmacologic modulation of these pathways impacts on the signaling. These questions can be answered using our newly established workflow as exemplified in an experiment using UPR perturbation in a glioblastoma cell lines.

PS 05: MS instrumentation II

2:00pm - 2:20pm

The big wedge for the small chunk - protein molecular ions split apart by soft X-rays

Knut Kölbel[1], Alan Kadek[1,2], Boris Krichel[1], Steffi Bandelow[4], Kristina Lorenzen[2], Florian Trinter[3], Jens Buck[3], Charlotte Uetrecht[1,2]

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Native mass spectrometry – especially when combined with ion mobility – provides a powerful tool to study non-covalent protein assemblies without disrupting crucial interactions. Time-honored methods as collisional activation or electron attachment for deliberate dissociation of the precursor ions as well as newly emerging ones like photodissociation are available to obtain information about components, stoichiometry and internal organization in addition to overall mass and conformation. However, none of these approaches is entirely suited to disassemble very large protein complexes as, for instance, virus shells conveniently.

With this in mind, we coupled native MS with highly energetic soft X-ray radiation provided by PETRA III synchrotron's P04 beamline in Hamburg. The results hint at alternative subunit dissociation and backbone fragmentation mechanisms alike.

2:20pm - 2:40pm

Complementarity of the Different Ionization Techniques in Environmental Analysis by GCxGC-HRMS

Viatcheslav Artaev[1], Georgy Tikhonov[1], Dmitrii Mazur[2], Albert Lebedev[2]

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Good science starts with good reliable data. The untargeted screening of environmental samples presents significant challenges in reliable identification of the detected compounds. Large variety of chemical classes, hundreds or even thousands of analytes at very wide range of concentrations present in the single sample – all that demand the most advanced analytical approaches to deliver trusted results. The High-Resolution TOFMS (HRTOFMS) coupled with Comprehensive Two-Dimensional Chromatography (GCxGC) is a powerful analytical tool for efficient separation and analysis of complex samples. The GCxGC increases separation capacity versus one-dimensional GC up to 5-10 times. Accurate mass measurements provided by HRTOFMS allowed correct compounds assignment even when the library similarity match score is low. It is also very helpful in the presented challenging cases, when manual structural elucidation and reconstruction of mass chromatograms is nessesary. A novel ion source capable of producing EI, positive and negative CI (PCI and NCI) spectra without changing the hardware or GCxGC methods was installed on the GCxGC-HRTOFMS. This novel instrument produces EI, PCI and NCI data of the same samples to expand coverage of compounds of interest, increase number of reliably assigned analytes and improve sensitivity of analysis. The complementarity of different ionization techniques helps in identification of particular species. For example, alignment of EI and PCI data provides reliable assignment including structural elucidation of such classes of chemicals widely present in environmental samples as phthalates, fatty acids, alkanes, etc. Important complementary information is also obtainable with NCI data. Examples demonstrate improved detection of the halogenated compounds.

2:40pm - 3:00pm

Recent advances in omnitrap technology coupled to orbitrap mass analyzers

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The omnitrap is a novel platform for tandem mass spectrometry (MSn) sustaining a highly diverse ion activation-dissociation network. The functionality of the omnitrap combines enhanced slow-heating and high-energy CID under gas pulse transients and highly-efficient ion-electron interactions extending from the low energy range for electron capture to the high energy range for radical ion formation and electron induced dissociation. The ion activation arsenal is further equipped with a new pulsed hydrogen atom gun and optical ports for UV-IR photo-dissociation. Activation techniques can be applied sequentially and/or simultaneously to enhance fragmentation efficiency. Combined with rectangular RF waveform technology, the effective mass range of the omnitrap becomes unlimited offering unique capabilities for top down sequencing of proteins in the gas phase. Unique ion activation-dissociation pathways are currently explored involving electron meta-ionization of insulin chain B and ubiquitin ions and subsequent

MS3 CID experiments of the radical ions revealing the formation of new c, z and a,x complementary fragment pairs. Complete sequence coverage of ubiquitin ions is obtained in ECD and EID MS/MS of ubiquitin ions. Experiments with a new H atom gun are also reported and recent advances in software development for analyzing complex top-down spectra are outlined. The design of a new ion mobility drift cell combined with the omnitrap platform for the simplification of congested spectra is also discussed.

3:00pm - 3:20pm

Description of complex petrochemical samples by thermal analysis mass spectrometry

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Introduction: Characterisation of heavy petrochemical fractions is a challenging analytical problem as heavy crude oils, oil sands, asphaltenes etc. are extremely complex mixtures with low volatility/solubility. Thus insight into these molecular systems will only be possible by combining results from different high-end methodologies such as LC-MS/MS, ultra-high mass resolution MS or IMS-MS. This aspect is particularly true if molecular motives are targeted, that cause certain physical properties such as unwanted precipitation or corrosion. In this context thermal analysis coupled to laser-based photoionisation mass spectrometry represents a complementary and beneficial tool for the characterisation of asphaltenes or fouling samples from refineries.
Method: A thermal analyser (TA) is coupled to a photoionisation time-of-flight mass spectrometer (TA-PIMS). Ionisation is performed by laser-generated VUV-light pulses (118 nm, 10.5 eV) in a single photon ionisation process (SPI, universal soft ionisation) or with 266 nm UV-photons in a resonance enhanced multi photon process (REMPI, selective soft ionisation for aromatic compounds). Chemical information is retrieved by the characteristic homologue rows and temperature-resolved comparison of SPI and REMPI.
Results: The presented work focuses on data obtained by TA-PIMS measurements of heavy petrochemical fractions such as fouling samples and asphaltenes. Mass loss, DSC as well as soft and selective mass spectrometric information revealed a polymer like structure of the fouling sample. Hetero atom containing species, Diels-Alder or polystyrene monomers are released temperature resolved. A variety of additional analytical methods such as pyrolysis gas chromatography or high resolution mass spectrometry was used to support the findings.

3:20pm - 3:40pm

Structural analysis of heavy oil fractions by the combination of high-resolution tandem mass spectrometry and ion mobility spectrometry

Johann Le Maître[1,2,3], Christopher Paul Rüger[1,3], Marie Hubert[1,3], Benoît Paupy[2,3], Sabrina Marceau[2,3], Pierre Giusti[2,3], Carlos Afonso[1,3]
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Heavy petroleum fractions are highly complex mixtures. Structural and compositional knowledge is a requisite for the development of the processing unit as changes in the chemical composition have a direct impact on physical properties and, thus, on all refining processes. This study aimed to identify and characterize N_1 compounds, in particular, those in vacuum gas oils.
Aside from broadband direct injection electrospray ionisation (ESI) ultra-high resolution analysis (12T solariX XR Bruker FTICR MS), automated tandem mass spectrometry deploying collision-induced dissociation (CID) with a narrow selection window (1u) was performed. This information was combined with results from ESI ion mobility spectrometry tandem mass spectrometry (Waters Synapt G2-Si HDMS) to allow for a more in-depth structural elucidation within the complex mixture. Self-written Visual Basic and Matlab scripts automatically treated the MS/MS-FTMS spectra (100 selected windows per sample) for recognition of the overlapped pattern.
The characteristic dealkylation pattern at fixed CID energy was used for structurally grouping similar QCID FT mass spectra and allowed a comparison between the various vacuum gas oils. Thus, MS/MS experiments allowed to gather valuable structural information, such as the presence of “island” (a central nucleus branched by alkyl chains) structures. On the other hand, the IMS-MS/MS experiments allowed adding valuable complementary information on the isomeric diversity and the conformation of the N_1 compounds.
The combination of IMS-MS/MS and ultra-high resolution tandem mass spectrometry open up exciting and promising prospects for studies in the structural determination of complex mixtures, in particular, compounds that are problematic in refining.

3:40pm - 4:00pm

Open LabBot – Modular Hardware and Software Platform for High-Throughput Sampling and Ambient Mass Imaging

Robert Winkler[1,2], Abigail Moreno-Pedraza[1], Cesaré Ovando-Vázquez[1,3], Héctor Guillén-Alonso[1], Ignacio Rosas-Román[1,4]

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We designed the Open LabBot as a flexible hardware and software framework for the quick integration of new analytical components and the rapid development of applications.

The Open LabBot platform is based on 3D printer technology (MakerMex, León, MX). Movements are controlled by simple G-Code, which is an industry standard and used for example in CNC production and 3D printer control. New hardware modules, such as an ion source, laser or lens, can be easily integrated using 3D printed adaptors. We provide the open software RmsiGUI (https://bitbucket.org/lababi/rmsigui) for programming the system and data evaluation. RmsiGUI is written in the powerful statistics and graphics language R.

High-throughput screening of essential oils was possible by mounting a commercial PlasmaChip (NovionX GmbH, Lindau, DE) to the Open LabBot. Using a 3D printed low-temperature plasma (LTP) probe [1] allowed for automated chemical fingerprinting and classification of spirits (tequila and mezcal) [2]. Coupling a continuous wave UV laser and a 3D-LTP probe enabled the fast ambient mass imaging (~500 ms/pixel) of plant metabolites from native plant tissue with adequate lateral resolution (50 µm) [3].

These three application examples demonstrate the high potential of the Open LabBot for the fast implementation of novel analytical tools.

[1] Martínez-Jarquín *et al.. Anal. Chem.* **88**, 6976–6980 (2016). **[2]** Martínez-Jarquín *et al.. Anal. Meth.* **9**, 5023–5028 (2017). **[3]** Moreno-Pedraza *et al. Anal. Chem.* in press (2019).

PS 06: Lipidomics & Metabolomics II

2:00pm - 2:20pm

Monitoring of lipid metabolism perturbation during *M. tuberculosis* infection in human sputum
Adam Wutkowski[1], Christoph Leschczyk[2], Ulrich E. Schaible[2], Dominik Schwudke[1]
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The lipid metabolism can be used as an important read out to study the inflammatory host response to microbial infection. Host-directed Therapies (HDT) is a supporting approach for antibiotics therapies, e.g. tuberculosis, as caused by *M. tuberculosis* (*Mtb*). To study the perturbations of the lipid metabolism by *Mtb* infected neutrophils, we applied two analytic approaches using a Q Exactive Plus (Thermo, Bremen, Germany) mass spectrometer: 1) Liquid chromatography (LC-MS^2) applying parallel reaction monitoring used for identification and quantification of PUFAs and their derivative lipid mediators (LM) and 2) Shotgun lipidomics using a DIA approach focusing on Gycerolipids, Glycerophospholipids, Sphingolipids, Cardiolipins and Cholesterol derivatives.
For the study, human sputum of four different patient was used. The sample collection began with the onset of treatment at day zero and was repeated weekly until successful *Mtb* treatment. Afterwards the samples were homogenized and diluted with methanol and butyl-hydroxy-toluol, to stop metabolic activity and inactivate *Mtb*.
We were able to quantify up to 21 LMs from total lipid extracts using LC-MS^2. Pro-inflammatory LM like LTB4 and PGE2 could be identified in all sputum samples. However, due to strong variation in the sputum sample quality, normalization of the LM concentration to the protein content was not possible to provide a clear correlation to the therapy status.
The shotgun lipidomic experiments were analyzed using LipidXplorer. In this case we were able to identify 277 different lipid species of 19 different lipid classes. Human sputum is characterized by high amounts of Phosphatidylcholine, Cholesterol esters and Triacylglycerol species.

2:20pm - 2:40pm

Identification of small molecules by combination of ion identity networking and MS^2 molecular networking
Robin Schmid[1], Daniel Petras[2], Louis-Felix Nothias[2], Ming Wang[2], Ansgar Korf[1], Birgit Arndt[1], Florian Hübner[1], Hans-Ulrich Humpf[1], Pieter Dorrestein[2], Uwe Karst[1]
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The separation of complex samples with high performance liquid chromatography (HPLC) and the detection with high resolution mass spectrometry (HRMS) generates gigabytes of information in a single study. Multiple open source tools and workflows have been developed to provide a flexible selection of algorithms for data preprocessing, feature identification and statistics. Due to the possible generation of multiple different ions per analyte, the data needs to be handled cautiously.
Here we present a new comprehensive collection of feature identification algorithms, which were fully integrated into the widely used open source software MZmine 2. The core idea is to bundle and reduce all MS signals that originate from the same analyte into one descriptor for this molecule. Step one is the grouping of all features within a retention time range, that share a similar peak shape and correlated peak height across all samples. Subsequently, all groups are annotated by checking for isotopologues, adducts, in-source fragments, multimers, clusters and different charge states. If available, these ion type annotations are then automatically verified and refined with tandem mass spectrometry (MS^2) fragmentation data. All ions which describe the same molecule are then displayed in a clustered ion identity network with the molecule as its centre. Based on an MS^2 spectra comparison and a library query, structurally similar molecules are being connected and annotated. The molecular formulae of all possible molecules is then predicted based on their respective ions.
For demonstration, the new identification workflow was applied to bile acid standards and samples.

2:40pm - 3:00pm

Analysis of liamocin biosurfactants by means of LC-MS and SFC-MS
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Biosurfactants are more eco-friendly than conventional petrochemical surfactants because of their lower toxicity and good biodegradability with comparable effects regarding to physical and chemical properties

like surface activity or critical micelle concentration. Thus, the applications of biosurfactants contain many different industrial fields like medicine, foods, cleaning materials or cosmetics. Liamocins are biosurfactants produced by the fungi *Aureobasidium pullulans* and belong to the glycolipid class. Their structural diversity is based on a polar polyol head group and a polyester rest consisting up to five 3,5-dihydroxydecanoic ester groups.
Structural characterization of liamocins is limited to MALDI-MS and nuclear magnetic resonance spectroscopy (NMR). These methods have a common trait in that they require time-consuming sample handling and separation steps. Therefore, different chromatographic techniques were hyphenated to mass spectrometry to separate the individual liamocin species and elucidate their structures by MS/MS experiments. It was possible to separate 10 liamocin species as well as structural related compounds and to confirm the structure proposals by means of MS/MS experiments.

3:00pm - 3:20pm

Characterization of lipidomic changes in human liver samples by shotgun lipidomics: is fatty liver only about neutral lipids accumulation?
Olga Vvedenskaya, Oskar Knittelfelder, Andrej Shevchenko
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Nonalcoholic fatty liver disease (NAFLD) affects up to 35% of the world population and, from the histological point of view, manifests with the accumulation of neutral lipid in hepatocytes. Without proper treatment NAFLD may lead to the development of cirrhosis or liver cancer. We applied quantitative shotgun profiling to analyse the full lipidome of liver biopsies from patients screened for known and suspected NAFLD risk markers: PNPL3, MBOAT7, TM6SF2, HSD17B13, SERPINA Z and SERPINA S. The 366 samples span a wide range of conditions such as BMI, age, various disease stages and comorbidities.
Preliminary analysis was performed on a pilot cohort consisting of 38 patients: 19 NAFLD patients and 19 control (non-NAFLD) patients (age 50+/-17, 9 males, 27 females). We quantified absolute (molar) content of 18 lipid classes covering 264 lipid species. Expectantly liver of NAFLD patients was enriched in neutral lipids. In particular, the amount of both diacylglycerols and triacylglycerols were elevated by 3 and up to 20-fold, respectively. At the same time, cholesteryl esters levels were ca. 2.5 times higher in fatty liver than in healthy control. Interestingly, in non-NAFLD samples the amounts of several lysophospholipids, such as LPC and LPE increased by 1.5 to 2 times.
We have performed the MS analysis of the full cohort, and currently apply statistical analyses, to discover a network of biomarkers and their dependency on mutation statuses that would allow the prediction based on risk factors, an early detection and differential diagnosis of NAFLD or nonalcoholic steatohepatitis.

3:20pm - 3:40pm

Only with fragment intensity correction is quantification of glycerophospholipids molecular species (to isomer level directly) from MS/MS possible
Kai Schuhmann[1], HongKee Moon[1], Henrik Thomas[1], Michael Groessl[2], Nicolai Wagner[1], Markus Kellmann[3], Andre Nadler[1], Andrej Shevchenko[1]
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Carboxylate anions (CA) fragments are used for quantifying molecular species of glycerophospholipids (GPL) by MS/MS. Yet, CA of saturated and polyunsaturated fatty acids (FA) have distinct fragmentation properties. Thus, a strong bias is introduced into lipid quantification through MS/MS depending on the structure of the analyte but moreover on collision energy and instrument parameters.
We therefore developed a generic model of GPL CA intensities in HCD FT MS/MS and a software to adjust corresponding biases. Associated with the correction became the CA intensity ratios of GPL a quantitative readout for determining their isomeric composition.
In the end, the software was validated with a mixture of synthetic standards (including isomers) on multiple QExactive instruments. We observed that our correction can be applied to other instruments data. We met expected values with abundances adjustments ranging from -100 to +30% and we were able to quantify glycerophospholipids on isomer level directly from the MS/MS.

3:40pm - 4:00pm

Analysis and visualization of artificially oxidized cardiolipins by means of LC-HRMS and Kendrick mass plots

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Cardiolipins (CL) are anionic phospholipids, which are exclusively located in mitochondrial membranes. Especially in the inner mitochondrial membrane, this lipid class plays significant role in energy metabolism. While it is essential for stability of protein complexes of the respiratory chain, it is also involved in various pathologies like neurodegenerative diseases. Oxidation of CL is known to destabilize these protein complexes and is associated with apoptotic events. The analysis of CL and their oxidation products is important to understand these processes. Due to four partly different acyl chains with various levels of saturation, CL themselves are very complex phospholipids. Furthermore, this diversity increases by oxidation and the formation of numerous oxidized species. Analyzing this diverse lipid class is a very challenging task. Therefore, we developed a method based on high-performance liquid chromatography (HPLC) and high-resolution mass spectrometry (HRMS), complemented by software-assisted data processing for CL characterization.

The CL species of an artificially oxidized lipid extract were separated by reversed phase HPLC. Using an Orbitrap MS with negative electrospray ionization, the CL species were identified by their accurate masses. Additionally, homologs CL species and their oxidation products were visualized using Kendrick mass plots. By plotting the Kendrick mass defect against the Kendrick mass, CL species have been arranged based on their structural characteristics, for example number of added oxygen atoms after oxidation. The Kendrick mass plot capturing additional chromatographic data is a novel tool for graphical lipid identification such as CL homologs and homologs of their oxidation products.

PS 07: Proteomics – Biological and Clinical Applications

10:30am - 10:50am

Proteomics of Diatoms: Discovery of Polyamine Modifications in Biosilica-Associated Proteins

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Diatoms are unicellular algae that use highly specialized proteins to produce nano silicified cell walls. These proteins, termed *silaffins*, share no homology across diatom species. Silaffins are heavily modified and their lysine residues bear ε-polyamine chains. However, their structure and biological role remain elusive. To this end, we developed a method to identify and quantify ε-polyamine-modified lysines and map them back to silaffin proteins from three diatom species (*Thalassiosira pseudonana*, *T. oceanica* and *Cyclotella cryptica*). In total, 17 novel modifications were discovered, including acid-resistant phosphoester-containing polyamines. We demonstrated that the pattern of polyamine modifications reflects the phylogenetic proximity of the diatom species. Modified lysine residues were identified by polyamine-specific fragments in MS/MS spectra followed by iterative searches and deconvolution of raw MS/MS spectra. We localized 130 polyamine-modified sites in 26 proteins from the three diatom species and revealed three consensus motives common to all three diatoms, despite full sequences of the modified proteins were not conserved. Additionally, we developed an approach for the systematic identification of ε-polyamine-modified peptides in total biosilica extracts that relied on the polyamine-specific fragments and applied all-ions fragmentation (AIF) LC-MS/MS. For this purpose, Arcadiate software was customized for recognition of characteristic fragments originating from the same peptide precursor by alignment of their XIC traces, thus bypassing recognition and fragmentation of very low abundant peptide precursors by the conventional data-dependent acquisition (DDA) approach.

10:50am - 11:10am

Proteome Profile of Chronic Wounds Treated with Cold Atmospheric Plasma to Improve Wound Healing

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Cold atmospheric plasmas (CAPs) are investigated in various fields of industry and medicine, one of the most promising being the improvement of wound healing. However, the underlying molecular mechanisms are barely understood. Elucidation of mechanisms of action of CPPs would allow for a better tuning of plasma conditions to affect desired processes in the wound.

Patients were treated with the kINPen med and wound exudates taken. To elucidate the impact of treatment, a proteomics approach was chosen with a special focus on chemical post-translational modifications known to be introduced by kINPen treatment. Proteins were grouped and could be matched to certain wound healing processes, *e.g.* inflammatory response. Furthermore, chemical modifications were observed know to modulate certain processes in the wound.

11:10am - 11:30am

PASEF for high-throughput deep shotgun proteomics analyses

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With the previously introduced parallel accumulation - serial fragmentation method (PASEF) on the timsTOF Pro mass spectrometer (Bruker Daltonics), five to ten times faster data dependent acquisition (> 120 Hz) without any loss in sensitivity became possible, enabling the identification of hundreds of peptides per minute. Measurements of 40 up to 200 samples/day can be performed with remarkable depth by using very short gradients. TIMS increases the sensitivity by time and space focusing of ions and the limit of quantitation can be reduced by directing multiple PASEF MS/MS scans to low abundant species, which is perfectly suited for measurements of sample amounts in the low ng range. More than 2400 proteins can be identified by using a 60 min gradient and a sample amount of around 6 ng. Reproducible ($R > 0.98$) and accurate label free quantification as well as highly reproducible collisional cross sections ($R > 0.99$) can be obtained on a large scale over a high dynamic range. We conclude that

analysis on short gradients with the timsTOF Pro provides both, high throughput measurement for a large number of samples and the possibility to dig deep into the proteome.

11:30am - 11:50am

Dried Serum Spots – An excellent means to bridge the divide between the clinics and the protein mass spectrometry laboratory

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Mass spectrometric profiling of intact serum proteins, i.e. determination of relative protein abundance differences, was performed using deposited and dried serum. Prepared as such, proteins can be stored and shipped at room temperature. After resolubilization of proteins from “dried serum spots” mass spectra of high quality have been recorded. Proteins have also been subjected to immunoanalytical assays such as Western blot and ELISA which proved full functionality of resolubilized proteins.

Having at hand a robust method for serum storage and shipment bridges the divide between the clinics and the protein analysis laboratory. Our novel serum handling protocol reduces costs for both, storage and shipping, and ultimately enables clinical risk assessment based on mass spectrometric determinations based on intact protein profiles

11:50am - 12:10pm

A new phase to investigate the dynamics of the proteome

Nico Zinn[1], Konstantin Aizikov[2], Dmitry Grinfeld[2], Arne Kreutzmann[2], Daniel Mourad[2], Oliver Lange[2], Maria Fälth-Savitski[1], Markus Queisser[3], Alexander Makarov[2], Marcus Bantscheff[1]

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Multiplexed proteome dynamics profiling (mPDP) enables a comprehensive investigation of perturbation effects on protein homeostasis. The combination of dynamic SILAC and TMT 10-plex allows to differentiate effects of treatments on mature and nascent proteins in tissue culture but the analytical depth and proteome coverage is compromised by the increased sample complexity presented to the mass spectrometer. Phase constrained spectrum deconvolution (PhiSDM) reduces the required minimum detection time for Orbitrap mass spectrometers to resolve neutron encoded TMT 10-plex thus improving MSMS cycle time and analytical depth in LC-MS experiments. We evaluated the benefit of PhiSDM and applied the combination with mPDP to investigate effects of inhibiting NEDD8 which regulates protein degradation mediated by the family of Cullin-Ring-Ligases.

12:10pm - 12:30pm

Profiling of Stage I – IV colorectal carcinoma samples by quantitative proteomics for early onset biomarker detection

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Colorectal cancer (CRC) is one of the most common cancer types worldwide. It predominantly develops from benign polyps and is frequently caused by lifestyle choices. Early detection and treatment results in 5-year survival rats of greater 95% however decreases dramatically to about 10% for stage IV. Availability of reliable biomarkers for CRC is sparse. Here we want to use mass spectrometry to profile stage I-IV CRC specimen for early onset biomarker detection.

Fresh frozen CRC specimen, with at least 10 patients per group (71 in total), were analyzed by fixed window DIA on a QExactive mass spectrometer. Pools of stage I-II and stage III-IV were fractionated by basic reversed phase (RP) chromatography for spectral library generation. Additionally, micro particle

enrichment from serum samples of the same patients and 10 serum samples from healthy individuals was performed and analyzed by data dependent acquisition on a Fusion mass spectrometer.
Basic RP chromatography resulted in a spectral library including ~4600 proteins. Data extraction with Skyline revealed 1900 proteins quantifiable across all specimen. Statistical analysis was performed which identified stage-specific marker proteins. Especially for Stage I compared to the other stages, significant differences were observed. For later stages (III & IV) malignancy markers were significantly increased.
For the micro particles, label-free MS1 quantification was performed which quantified ~1400 proteins. Micro particles from patients with cancer drastically varied from the healthy individuals. With increasing stage, the number of detectible proteins associated to cancerous alteration increased which may be used for blood-based colon cancer diagnosis.

PS 08: Instrumentation and Application of MS Imaging

10:30am - 10:50am

3D-surface AP-SMALDI MS imaging reveals differential tegumental lipidomics in human pathogen *Schistosoma mansoni*

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Schistosomiasis is a waterborne disease caused by *Schistosoma spp.*, endemic in the subtropics and tropics worldwide. Due to globalization and climate change, schistosomes are spreading and threatening about 1 billion people, often living in poverty. To focus research capacities and to facilitate anti-schistosomal strategies, the World Health Organization classifies Schistosomiasis as a neglected tropical disease. The only drug against schistosomes is Praziquantel, commonly used in mass drug administration in endemic regions. To gain fundamental knowledge on schistosome biology, we investigated the lipidome of adult *Schistosoma mansoni* worms on their tegumental surface and inner worm body.

Thin cryosections of adult schistosome couples were prepared for investigating the worm body. Additionally, freshly separated schistosomes (males and females) were selected for surface analysis. After drying in a desiccator and microscopic assessment, dihydroxybenzoic acid was applied by pneumatic spraying using SMALDIprep (TransMIT, Giessen, Germany) robot. MS imaging data was acquired using an AP–SMALDI5 AF autofocusing ion source (TransMIT GmbH, Giessen, Germany) coupled to a Q Exactive HF (Thermo Fisher Scientific, Bremen, Germany) mass spectrometer. This setup enabled MS imaging at 5 µm spatial resolution with a mass resolution of 240,000. A LC-MS^2-based database in combination with Metaspace online repository served for lipid annotation. Putative differences were assessed by multivariate statistical analysis.

We found differential lipid composition of surface and worm body on the lipid class level for sphingomyelins, (lyso–) phosphatidylcholines, phosphatidylethanolamines (PE) and phosphatidylserines (PS). Additionally, we found differences in PEs regarding fatty acyl substituent chain length.

10:50am - 11:10am

An advanced protocol for microbial MALDI-MS imaging: gaining insights into the world of bacteria on a molecular scale

Eike Ulrich Brockmann[1,2], Daniel Steil[1,2], Jens Soltwisch[1,2], Klaus Dreisewerd[1,2]
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Microbial MALDI-MS imaging is an emerging technique with the potential to provide important insights into the chemical communication within bacterial colonies. However, current protocols typically use cultivation on agar and are associated with notable drawbacks like a missing inactivation step. Here we present a mixed cellulose ester membrane-based protocol for cultivation, rapid steam-inactivation, and subsequent MALDI-MS imaging at high lateral resolution. Laser-induced postionization (MALDI-2) with Synapt and Orbitrap mass spectrometers was used to crucially boost the analytical sensitivity. This enabled identification of more than hundred metabolites including signaling molecules and lipids with specific distributions across single and interacting Gram-positive and Gram–negative colonies, including highly virulent pathogens (EHEC). MALDI-2-MSI of cryosections was, moreover, used to visualize the 3D-organization of colonies.

11:10am - 11:30am

Investigation of the distribution of food additives in processed food by MALDI MS imaging

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Food additives such as preservatives agents are used in processed food to prevent the growth of microorganisms or slowing the oxidation of fats. Some food preservatives bear a health risk and are therefore regulated and need to be monitored. For example, natamycin is used as a preservative for cheese and sausages but was introduced as an antifungal drug. The cheese is usually dipped into namatycin or natamycin is brushed onto the cheese. Therefore, a European regulation stipulates not only the permitted concentration but also the penetration depth from the surface. The commonly used method

for the analysis of natamycin is LC-DAD and does not provide any lateral information. MALDI mass spectrometry imaging has been successfully applied for the investigation of non-processed food, wherein sugars, amino acids and lipids were analyzed. Processing significantly alters the physico-chemical properties/consistency of food and thus poses specific challenges for MS imaging analysis (e.g. sample preparation). In this study we developed a high resolution MALDI imaging method to investigate the penetration of natamycin into cheese. Sectioning and matrix application were optimized and a data analysis tool was developed. None of the investigated Gouda samples exceeded the penetration limit but different penetration depths of natamycin could be found in different Gouda samples. Learning more about the relation between the application method and penetration process can improve the application method for natamycin and thus, could lead to less exposure for the customers. The developed approach can be used to study diffusion processes via MALDI MS imaging.

11:30am - 11:50am

MALDI-Imaging for Classification of Epithelial Ovarian Cancer Histotypes from a Tissue Microarray Using Machine Learning Methods

Oliver Klein[1], Frederic Kanter[2], Herbert Thiele[3], Jan Lellmann[2], Carsten Denkert[1], Silvia Darb-Esfahani[4], Ioana Braicu[1], Jalid Sehouli[1], Zhiyang Wu[1], Eliane T. Taube[1]

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The epithelial ovarian cancer (EOC) differing in pathogenesis, molecular changes and clinical behavior with diverse chemotherapeutic sensitivity and prognosis was classified into five subtypes: High-grade serous ovarian cancer (HGSOC), low-grade serous ovarian cancer (LGSOC), serous borderline tumors (sBOT), endometrioid and clear-cell carcinoma (OCCC). The correct recognition of EOC histotype has a great significance in clinical management. Because of the limitation of conventional clinical routine relying on histology and sparsity of novel biomarkers the misdiagnosis of EOC ranges around 15%. In face of this medical challenge the pilot study verified the potential of proteomic signatures derived from matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) in combination with machine learning methods to classify EOC histological subtypes from tissue microarray.

Formalin-fixed-paraffin-embedded tissue microarray made up of 20 OCC, 14 LGSOC, 19 HGSOC and 14 sBOT was analyzed by using MALDI-IMS. The classifications were computed by linear discriminant analysis (LDA), support vector machines with linear (SVM-lin) and radial basis function kernels (SVM-rbf), a neural network (NN) and a convolutional neural network (CNN). The best overall EOC histotype classifier was developed by CNN. Dataset yields in a mean accuracy of 85%, mean specificity of above 90% for all four subtypes and mean sensitivity of above 90% for OCCC, sBOT and HGSOC, only 69% for LGSOC. In conclusion, MALDI-IMS combined with machine learning algorithms is an impactful aid of histological assessment of EOC subtypes.

11:50am - 12:10pm

Towards novel TB antibiotics: Drug screening and improving the predictive value of preclinical models.

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Tuberculosis (TB) is an infectious disease of the pulmonary system caused by *Mycobacterium tuberculosis*. Presently it remains as one the top ten causes of premature death worldwide. To study the distribution of TB antibiotics in tissue, MALDI-MSI is the molecular imaging technique of choice. In order to improve upon the predictive value of preclinical trials and accelerate the development of future TB antibiotics, a scanning microprobe AP-MALDI-MSI workflow featuring high mass resolution, spatial resolution and mass accuracy was adapted for the detection of TB antibiotics at therapeutic concentrations in γ ray sterilised lung sections of a novel mouse model closely resembling human TB pathology. A sample preparation protocol encompassing sample collection, cryosectioning and subsequent λ ray sterilisation of sections was established to manage analyte delocalisation, which is especially severe when dealing with water soluble compounds. Furthermore, a standardised approach to determine the matrix substance best suited for the detection of the target drugs was developed. To enable reliable detection of low dosed drugs MALDI-MSI measurements were conducted with multiple alternating acquisition modes and subsequently converted into individual imzML files for data analysis. In tissue signal identification was performed based on accurate mass identification and MS/MS. The MS imaging workflow presented here is capable of measuring the distribution of drug compounds in γ ray

sterilised lung sections of IL 13tg mice at therapeutic concentrations with previously unreported spatial resolution and mass accuracy.

12:10pm - 12:30pm

Pulsed cold plasma for post ionization in MALDI-MS imaging

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In MALDI, only a minor fraction of desorbed molecules is concomitantly ionized and, therefore, available for mass spectrometric analysis. To mitigate this shortcoming, we here present a new strategy that utilizes a gas discharge for post ionization (PI) inside an intermediate pressure ion source. The RF-driven dielectric barrier discharge (DBD,13.6 MHz) is pulsed and timed to directly interact with the MALDI plume. Careful optimization of laser and plasma parameters resulted in PI of phospho- and glycolipids with low degree of fragmentation and mass spectra that largely resembled those produced by laser-induced PI in a MALDI-2 setting. A strong boost of protonated/deprotonated lipids and further metabolites is obtained. First DBD-MALDI imaging results obtained with mouse brain sections will be presented.

PS 09: Environmental, natural product and forensic MS

10:30am - 10:50am

Complexation strategy for the analysis of protein adducts with ethylmercury in influenza vaccines *via* SEC/ICP-MS

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Ethylmercury ($EtHg^+$) exposure is strictly anthropogenic and derived from the use of ethylmercury thiosalicylate (Thiomersal, THI) as preservative in multi-dose vials of vaccines. THI decomposes in aqueous media and the following release of $EtHg^+$ leads to antimicrobial properties. Banned in the EU since 2001, multi-dose vials are still used in the USA and developing countries and contain approximately 25 µg Hg as $EtHg^+$ per dose. Adduct formation of $EtHg^+$ with blood proteins has already been indentified to contribute to the elucidation of $EtHg^+$-toxicokinetics. For these investigations, it is necessary to preserve molecular information of mercury-protein adducts during analysis, while separation of protein adducts and non-bound Hg has to be achieved. Size discriminating techniques, like size exclusion chromatography (SEC), coupled to elemental detection, like ICP-MS, are particularly well suited for this purpose.
This study investigates the adduct formation of $EtHg^+$ from THI with the active ingredient in influenza vaccines, hemagglutinin (HA), which is a membrane protein of virions. SEC/ICP-MS was applied to obtain information on the size of the protein involved in adduct formation. Since non-bound $EtHg^+$ adhered on the stationary phase of the SEC column and subsequent injections of thiol-containing compound showed false-positive results on the contaminated system, gradient elution using a thiolate as complexing agent was developed. Using this approach, it was possible to show adduct formation of $EtHg^+$ and HA. The use of a complexing agent allowed for external quantification with THI and revealed that 0.56% of $EtHg^+$ binds to HA in influenza vaccines under the selected conditions.

10:50am - 11:10am

Effects of cosolvents in Fenton oxidation as clean-up procedure for polycyclic aromatic hydrocarbons (PAXH)-contaminated soil

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The environmental pollution of soil with contaminants is a critical and important health issue. Polycyclic aromatic hydrocarbons and heterocycles (PAXHs) are one of the most abundant contaminants in polluted soil, which lead to toxic, mutagenic and carcinogenic health effects. Depending on the number of the condensed aromatic rings, PAHs are divided into low molecular (LMW, 1-3 rings) and high molecular weight (HMW, ≥ 4) compounds.
Nowadays, there are different remediation techniques for soil clean-up. For this, the combination of biodegradation and chemical (pre)treatment is needed for transforming poorly bioavailable pollutants into oxygenated and more bioremediable products. But due to solubility issues the use of cosolvents is necessary, because PAHs have a poor availability, because of the hydrophobicity and strong sorption on soil.
Understanding the chemical reaction processes of non-selective Fenton oxidation with over a thousand available compositions in such a complex matrix like PAH-contaminated soil is a major challenge. Therefore, a combination of high resolution mass spectrometry with non-target analysis is necessary for getting a comprehensive characterization of various oxidized products obtained during the reaction.
In this study, we present for the first time the Fenton reaction with addition of different cosolvents. Our aim deals with exploring possible reaction mechanism during the treatment of an unknown complex mixture and improving the degradation of PAXHs in soil. The results show, that using toluene as cosolvent leads to notable removal of hydrocarbons by forming multiple oxygen-containing compositions.

11:10am - 11:30am

Remote Detection of Ship Emissions using Single-Particle Mass Spectrometry

Johannes Passig[1,2], Julian Schade[1], Thomas Kröger-Badge[1], Robert Irsig[1], Hendryk Czech[1], Martin Sklorz[1], Benjamin Stengel[3], Bert Buchholz[3], Thorsten Streibel[1], Ralf Zimmermann[1,2]

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Ship emissions play an important role in the global concern of air pollution, with strong effects on climate and public health. Up to 400,000 annual deaths as well as several million cases of childhood asthma were attributed to air pollution from ships. They emit large amounts of particulate matter, especially if low-grade heavy fuel oils (bunker fuels) are used. These particles contain high loads of organics, sulfur and metals. Travelling over large distances, the aerosols undergo (photo)chemical and physical modifications in the atmosphere. Innovative on-line methods for aerosol analyses are needed to establish efficient risk assessment and mitigation strategies. Herein, single-particle mass spectrometry (SPMS) is a promising technique, allowing on-line characterization and classification of individual airborne particles. It is also sensitive to both metals and sulfur, thus suitable to measure ship emissions in ambient air. Optimizing laser desorption/ionization of particles in SPMS, we substantially enhanced the methods sensitivity to marker substances for bunker fuel emissions in air. We demonstrate the detection of residual metals in ship emissions, even hours after switching from bunker fuel to diesel. Based on this findings, we established a method to detect ship plumes from >100km distance and to attribute them to ship passages. Furthermore, our approach allows the evaluation of sulfur emissions, indicating remote violations against sulfur limits in emission control areas.

11:30am - 11:50am

Studying protein interactions of endocrine disrupting organotin compounds using soft LC-MS techniques

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Organotin compounds (OTCs) have been used as biocides since the 1970s, which has led to their wide distribution in the environment. Despite their classification as endocrine disruptors and their subsequent ban as biocides in most of Europe and North America in the 2000s, triorganotin compounds and their degradation products are still present in aquatic ecosystems due to their persistence in sediments. Additionally, diorganotin compounds are still applied as catalysts or PVC stabilizers in the chemical industry with potential contact to food or drinking water. Since the molecular mechanisms of OTC toxicity are still not fully understood, further research on their interactions with biomolecules is required. Especially proteins with several nucleophilic side chains of varying Lewis basicity represent potential targets for both triorganotins and diorganotins.

In this study, beta-lactoglobulin A (LGA) was used as a model protein to investigate the interactions with different OTCs. The lipocalin-type protein was selected because of its ability to bind small lipophilic molecules. After incubation with OTCs, the protein samples were analyzed by means of size-exclusion chromatography coupled to electrospray ionization-mass spectrometry (ESI-MS) and inductively coupled plasma-mass spectrometry (ICP-MS), respectively. Structural insight was gained via protein labeling and enzymatic digestion experiments.

The complementary MS approach enabled the identification and characterization of OTC-LGA adducts. The adduct formation was observed to strongly depend on the number and nature of organic ligands of OTCs. Additionally, non-denaturing conditions during incubation and LC-MS analysis proved to be necessary to study OTC-LGA adducts, which is important for further research.

11:50am - 12:10pm

Unconventional Kendrick Mass Defect as a visualization and rapid screening tool for GC×GC-HR-ToF/MS measurements

Benedikt Alexander Weggler[1,3], Beate Gruber[1,2], Delphine Zanella[3], Jean-Francois Focant[3], Frank Dorman[1]

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Characterization of complex matrices commonly implicates scientific challenges such as wide concentration ranges of various compound classes and/or the limited, available sample volume. Applying cutting-edge, discovery based separation techniques such as multidimensional gas chromatography

coupled to high-resolution time-of-flight mass spectrometry (GCxGC-HRToF/MS) facilitate such analytical challenges. Nevertheless, the majority of studies is still focused on targeted analysis, which tend to disregard important details of the sample of interest.
GCxGC-ToF/MS provides in-depth chemical insight in the molecular fingerprint of analyzed matrices. However, such analysis produces high amounts of data generally containing several thousands of compounds per experiment. The amount of data will further increase by coupling GCxGC to high-resolution mass spectrometry (HRT), which requires advanced data reduction and mining techniques. So far, GCxGC-HRToF/MS information is evaluated by focusing either on the chromatographic separation for e.g. group type analysis, or utilizing exact mass data applying Kendrick Mass Defect (KMD) analysis or van Krevelen.
This study integrates high-resolution mass information directly into the multidimensional separation space, combining KMD data and knowledge-based rules. Combining of these approaches allows for fast, visual data screening as well as a first quantitative estimation of the samples composition. Additionally the obtained classification drastically reduces the number of variables allowing a clear and distinct chemometric analysis in e.g. environmental and forensic studies such as for detailed hydrocarbon analysis (DHA).

12:10pm - 12:30pm

Monitoring of Coffee Roasting by Vacuum Photoionization ToF-MS: Towards a Prediction Model for Bean Color and Antioxidant Capacity

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Being one of the most popular beverages, coffee has a high economic value. One of the most significant factors in determining taste and pricing of coffee is the roasting process. This process is controlled by a roast master, who takes especially sensory and physical parameters like weight loss, smell or bean color into account. For an improvement in batch consistency and on-line roast control, these parameters are not useful.
Photoionization mass spectrometry (PIMS) allows monitoring the chemistry of coffee roasting at appropriate time resolution of 1 s or less. PIMS combined with partial least square regression modelling allows an on-line prediction of bean color and content of health-promoting antioxidants. This technique is strengthening the chain of process understanding, monitoring and finally on-line process control for product/process optimization.

MS 01: Ion physics & ion chemistry

2:40pm - 3:00pm

N-Heterocyclic Carbene (NHC) Dimerization in the Gas Phase:C-H···:C Hydrogen Bonding *vs.* Covalent Dimer Formation

Mathias Schäfer[1], Mathias Paul[1], Eric Detmar[1], Maria Schlangen[2], Martin Breugst[1], Jörg-Martin Neudörfl[1], Helmut Schwarz[2], Albrecht Berkessel[1]

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N-Heterocyclic carbenes (NHCs; **:C**) can interact with azolium salts (**C-H$^+$**) by either forming an H-bonded aggregate (**CHC$^+$**), or a covalent C-C bond (**CCH$^+$**). In this study, intramolecular NHC-azolium salt interactions of aromatic imidazolin-2-ylidenes and saturated imidazolidin-2-ylidenes were investigated in the gas phase by travelling wave ion mobility mass spectrometry (TW IMS) and DFT calculations. The TW IMS experiments provided evidence for the formation of these important intermediates in the gas phase, and they identified the predominant aggregation mode (H-bond *vs.* covalent C-C) as a function of the nature of the interacting carbene-azolium pairs.

M. Paul, E. Detmar, M. Schlangen, M. Breugst, J.-M. Neudörfl, H. Schwarz, A. Berkessel, M. Schäfer, *Chem. Eur. J.* **2019***, 25*, accepted. http://dx.doi.org/10.1002/chem.201803641

3:00pm - 3:20pm

Infrared Laser Desorption in the Stress-Confinement Regime Studied by Time-Resolved Digital Interference Microscopy

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Infrared Laser Desorption is a promising approach for bio-diagnostics, laser surgery, and mass spectrometry imaging: it provides high spatial resolution, requires minimal sample preparation, and can be easily coupled to purification and separation techniques such as liquid chromatography. Desorption by impulsive vibrational excitation (DIVE) with a picosecond infrared laser (PIRL) has recently been demonstrated to achieve high sensitivity and preserve molecular integrity when coupled to an ion-trap MS [1, 2]. DIVE eliminates the necessity of a light-absorbing chemical matrix by targeting the absorption band of water at a wavelength of 3 um. The fast (picosecond) timescale of energy deposition prevents thermal as well as acoustic damage to both the ablated material and the remaining substrate, leaving large biomolecules intact for analysis.

Here, we developed a time-resolved digital interference microscopy (DIM) setup to probe the optical density of ablation plumes at various laser fluences [3]. Although DIM offers a lower spatial resolution than typical imaging techniques, such as dark-field or Schlieren imaging, it provides an absolute optical phase shift with which the absolute refractive index and a density map of the primary shock front can be reconstructed. We apply this technique to water and glycerol droplets and to droplets confined in microwell structures. We identify and characterize different fluence regimes and new ablation dynamics for DIVE.

[1] K. Franjic and R.J.D. Miller, Phys. Chem. Chem. Phys. 12, 5225–5239 (2010)
[2] Y. Lu et al., Anal. Chem. 90, 4422–4428 (2018)
[3] F. Busse et al., J. Appl. Phys. 124, 094701 (2018)

PS 10: Award Lectures

10:30am - 11:20am

Characterization of the human Proteome and Kinome
Bernhard Kuester
Technical University of Munich, Germany; kuster@tum.de
Award for "MS in Biowissenschaften"

11:20am - 12:00pm

High-Throughput Identification of Lipids in Biological Material Using Software-Aided Analysis of LC-MS/MS Data
Jürgen Hartler
Technische Universität Graz, Austria; juergen.hartler@tugraz.at
Mattauch-Herzog Prize 2019
Mass spectrometry is currently one of the most sensitive methods for the detection of proteins and metabolites. As such, it has advanced the scientific fields of proteomics, metabolomics and lipidomics by providing detailed information on molecular composition. Specifically for lipidomics, distinctive patterns of lipid molecular compositions offer clues about biochemical mechanisms – or in case of dysregulation, insights on pathophysiological processes. While MS^1 spectra typically yield information about the intact lipid, fragmentation spectra obtained by MS/MS or MS^n provide additional structural information about the lipid species of interest. However, in lipidomics there are currently only a few tools available that utilize both MS^1 and MS/MS information. Moreover, most lipid annotations rely on spectral libraries and the similarity between library spectra and experimentally obtained spectra. Yet, variables such as the type of mass spectrometer, the collision energy applied, the type of adduct, and the charge state can profoundly influence the pattern of lipid MS/MS spectra.
To solve these problems, we have created a universal, flexible and automated solution, which has been integrated in the user-friendly open-source Lipid Data Analyzer software (LDA). For the MS^1 level, the software provides an intuitive 3D algorithm that confines the peak borders in m/z and time direction, and the usage of the theoretical isotopic intensity distribution to reliably remove ^{13}C peaks. The flexibility on the MS/MS level is achieved by *decision rule sets*. Lipid annotation is based on well-defined fragments (fragment rules) and their intensity relationships (intensity rules), allowing for routine-profiling of known lipid targets as well as the detection of novel lipid molecular species. Platform independence was proven in experiments with eight different mass spectrometric platforms, comprising low- and high-resolution instruments at various collision energies and use of several adduct ions. With LDA, both the number of correctly identified lipid molecular species and the reliability of the results increased compared with presently available software. Moreover, this approach is easily extendable to other metabolite classes by a graphical user interface providing direct visual feedback on acquired spectra. Taken together, we provide now a comprehensive, platform-independent and easily adaptable package for lipid profiling based on hyphenated MS/MS data for a variety of lipid classes.

12:00pm - 12:15pm

Data acquisition methods for next-generation mass spectrometry-based proteomics
Florian Meier[1,2]
[1]LMU München; [2]MPI Martinsried; fmeier@biochem.mpg.de
Wolfgang-Paul-Prize for the best PhD thesis:
Mass spectrometry has become the method of choice to study proteomes in a global and unbiased manner. Yet, it still trails other omics technologies in terms of coverage, throughput and sensitivity. I present three MS acquisition strategies that break through longstanding technological limitations and facilitate comprehensive and high-throughput proteomics. EASI-tag enables interference-free multiplexed proteome quantification on the MS2 level. The BoxCar method divides the full mass range into multiple narrow segments and thereby increases the dynamic range of Orbitrap mass analysis by one order of magnitude. A core subject of my PhD was trapped ion mobility spectrometry (TIMS) as an additional dimension of separation in proteomics. Building on TIMS, parallel accumulation – serial fragmentation (PASEF) multiplies peptide sequencing speed without any loss in sensitivity. Importantly, the fundamental concepts and methods developed here are generic and can be seamlessly applied in other MS-based omics fields facing similar challenges.

12:15pm - 12:30pm

Development of autofocusing and subcellularly resolving mass spectrometry imaging and its application to biological questions

Mario Kompauer, Sven Heiles, Bernhard Spengler
Justus Liebig University Giessen; Mario.Kompauer@anorg.Chemie.uni-giessen.de

Wolfgang-Paul-Prize for the best PhD thesis:

One of the major demands in science, in particular in the life sciences, is the advancement of imaging techniques to visualize and determine the precise location of molecules within the sample. In a single experiment matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) allows the visualization of hundreds of different biomolecules in various tissue types without the need of labeling. In today's routine instruments a lateral resolution of 5 µm is feasible but this is insufficient to study intracellular processes. We developed a MALDI MSI instrument performing measurements at a sub-cellular resolution of 1 µm, at atmospheric pressure with high resolving power and high mass accuracy. We characterized the improved imaging capabilities and developed a general method for sub-cellular MSI, including sample preparation and matrix application. MS images of high quality were obtained with significant improvements over the state-of-the-art MALDI MSI instruments. Nevertheless, high-lateral-resolution MSI systems suffer from a shallow depth of field and thus generate geometry-related artifacts when imaging samples of insufficient flatness. To analyze non-flat tissue sections or three-dimensional (3D) sample surfaces, it is essential to constantly refocus the desorption/ionization laser. We therefore developed an autofocusing MSI system which allows to simultaneous analyze the topographical surface and molecular composition on sample objects with low micrometer resolution. The new system allows to study compound distributions on various irregular 3D surfaces and improves common 2D tissue section MSI analysis by avoiding ion signal intensity variations across large samples of imperfect flatness.

PS 11: Outside the box

10:30am - 10:50am

HPLC-HRMS as a Tool for the Identification of Confiscated Commercial and Military Explosives
Tilo Schachel[1,2], Rasmus Schulte-Ladbeck[2], Uwe Karst[1]
[1]Westfälische Wilhelms-Universität Münster, Germany; [2]Bundeskriminalamt, Germany; tilo.schachel@uni-muenster.de
Forensic scientists constantly develop improved methods for the detection and identification of hazardous materials employed by criminals and terrorists. In this field, explosive compounds are of particular interest. The identification of confiscated explosives that do not appear to be homemade is an important step toward uncovering and revealing distribution pathways of these illicit materials.
Commercially available explosives, as well as military explosives, contain numerous additives that influence the performance and properties of each formulation. As a result, these formulations pose complex samples, which do not allow for a straight forward analysis. So far, mainly two different approaches have been pursued to facilitate the analysis of such samples. The identification was either achieved on the basis of a few selected compounds in each sample, or by “fingerprint analysis” of the samples.
In this work high performance liquid chromatography (HPLC) in combination with high resolution mass spectrometry (HR-MS) was employed to analyze commercially available explosives. A relatively simple and effective dissolution and extraction strategy was developed to minimize possible sources of error. Several commercial explosives were analyzed regarding their composition with a focus on explosive compounds as well as by-products and additives. Molecular formulae for various additives and by-products could be derived from exact masses, taken from HR-MS spectra. Pronounced differences in the amount and character of components between a set of samples could be shown, and thus a differentiation was possible.

10:50am - 11:10am

Single cell analysis of *Toxoplasma gondii*- and *Besnoitia besnoiti*-infected bovine umbilical vein endothelial cells by MALDI mass spectrometry imaging
Stefanie Gerbig[1], Patrik Kadesch[1], Tobias Hollubarsch[1], Lars Schneider[1], Carlos Hermosilla[2], Anja Taubert[2], Bernhard Spengler[1]
[1]Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Germany; [2]Institute of Parasitology, Justus Liebig University Giessen, Germany; stefanie.gerbig@anorg.chemie.uni-giessen.de
Coccidian parasites are a subclass of obligate intracellular apicomplexan parasites which induce a variety of human and veterinary diseases. Given that their metabolism is barely investigated on the molecular level, the aim of this study was to analyze the lipidome of *Besnoitia besnoiti*- and *Toxoplasma gondii*-infected host cells by MALDI mass spectrometry imaging (MSI).
Cell pellets and cell monolayers were analyzed using an AP-SMALDI10 ion source (TransMIT GmbH, Giessen, Germany) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).
Infection markers were deduced from cell pellet measurements by analyzing technical and biological triplicates of infected and non-infected cells. Single infected cells in monolayers were identified by detection of marker signals and comparison to optical images.

11:10am - 11:30am

Challenges in atmospheric pressure mass spectrometry
Andreas Kiontke, Claudia Birkemeyer
Universität Leipzig, Germany, Institute of Analytical Chemistry; birkemeyer@chemie.uni-leipzig.de
Modern technical evolution made mass spectrometry (MS) the leading technology of analytical chemistry in terms of application range, detection limits and speed. When it comes to mass spectrometric detection, one of the critical steps is to ionize the analyte and bring it into the gas phase. Several ionization techniques were developed for this purpose among which electrospray ionization (ESI) and atmospheric pressure ionization (APCI) are two of the most frequently applied atmospheric pressure methods to ionize target compounds from liquid matrices or solutions. In addition to that, recent efforts in the emerging field of “ambient” MS enable the applicability of newly developed atmospheric pressure techniques to solid matrices greatly simplifying the analysis of samples with MS and anticipating to ease the required, or even leave out any sample preparation and enable analysis at ambient conditions, outside the instrument itself.

We review relevant compound characteristics for ionization with the two traditional methods ESI and APCI and compare those with one of the most frequently employed representatives of the plasma-based methods, i.e. low temperature plasma ionization. We present a detailed analysis which compound characteristics are most beneficial for the response of aromatic nitrogen-containing compounds with these three methods and provide evidence that desorption characteristics appear to have the main common, general impact on signal response. In conclusion, we discuss the optimization of instrumental conditions not only with respect to most important requirements of the three ionization techniques but, at the same time, for future developments in the field of ambient ionization.

11:30am - 11:50am

Systems Proteomics: Assessment and perspectives of haploid cell systems in structural and functional proteomics

Mohammed Alchiblak[1], Hasan Albony[1], Felix Steinbeck[1], Dirk Koczan[1], Michael O. Glocker[2], Peter Lorenz[1], Hans-Juergen Thiesen[1]

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With the advent of CRISPR technologies including CRISPR/i and CRISPR/a approaches, molecular mechanisms can be characterized on the transcriptional and posttranslational/proteomic level from genetic alterations via epigenetic modifications to responses to small compounds. However, the major concern is whether results obtained within one specific cell system can be generalized or are just events being unique to the cell system being studied. Reproducibility of experimental results has become an issue in the scientific community (reproducibility crisis). Our lab has been investigating the suitability and potential of haploid HAP1 cell models in studying the functionalities of KRAB ZNF proteins in HAP1 cells including haploid HAP1 C631 knockout models and diploid C665 cells. Addressing issues of standardization and reproducibility, the advantage of working with nearly haploid genomes is: besides being commercially available, all HAP1 cell models should nearly have the same haploid genetic background. Our combined HAP1 studies of epigenetic, transcriptional and proteomic events e.g. concerning phylogenetic variants of TRIM28 interacting with KRAB domains (present in about 400 human proteins) including the identification of targets addressed by small compounds that interfere with transcriptional repression describe molecular mechanisms based on one nearly haploid SNP pattern. Working with HAP1 cells might complement the tool box in systems proteomics.

11:50am - 12:10pm

Speciation analysis and multimodal Imaging to trace gadolinium deposition in the brain

Uwe Karst

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Metallopharmaceuticals find increasing use as therapeutics and as diagnostic agents in recent years. While platinum-based cytostatics likely are the most important examples, gadolinium-based magnetic resonance imaging (MRI) contrast agents have become a focal point of researchers and governmental agencies in the last decade. The compounds are known to be tolerated well, and to exhibit only few side-effects due their fast excretion kinetics with a half-life of only two hours.

However, in 2006, these compounds started to be associated with a newly discovered disease, nephrogenic systemic fibrosis (NSF), which is only observed for dialysis patients. This disease has only been described for contrast agents with linear ligands, but not for those with macrocyclic ligands, indicating an influence of the lower kinetic stability of the former. While the general cause of the disease with a deposition of the gadolinium in the lower parts of the skin has been identified, many details of the pathogenesis are still unknown.

Very recently, small residues of the contrast agents have been discovered to remain in the human brain, and many related questions are currently under investigation. Even despite any current indication for respective pathogenic effects, scientists, manufacturers and regulatory agencies are concerned about these findings. To investigate this situation, liquid chromatography (LC) coupled to inductively coupled plasma-mass spectrometry (ICP-MS) and electrospray mass spectrometry (ESI-MS) are used, combined with spatially resolved analysis by laser ablation (LA)-ICP-MS imaging.

The development of the respective analytical methods and their application to address the challenges raised above are presented here.

12:10pm - 12:30pm

Phospholipid profiling by LC/MS applying complementary ESI-MS and ICP-MS detection

Vosse Christian, Heiko Hayen
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Phospholipids (PL) are important lipids with multiple functions in biological systems. The PL classification is based on different polar head groups, and the diversity within each PL class is generated by combination of different fatty acids with variation of chain length and number of double bonds. Powerful analytical methods for identification and quantification of PL are required, and thus, different LC/MS methods were developed in this study.

Head group-based separation of PL classes was achieved by hydrophilic interaction liquid chromatography (HILIC). The structural characterization of phospholipid species was performed by electrospray ionization-high resolution-MS and MS/MS. Additionally, PL classes were quantified by universal phosphorus detection using inductively coupled plasma (ICP)-MS. An inverse gradient system was implemented for steady mobile phase composition after HILIC separation. Thus, constant plasma and ionization conditions were provided and ensured consistent detector response. Polyatomic interferences were decreased by using the oxygen reaction mode of the triple quadrupole instrument.

Applicability of the complementary methods was demonstrated by analysis of a total lipid extract of baker‘s yeast, i.e. *Saccharomyces cerevisiae*.

Poster Abstracts

Poster: 1
Investigation of the Gas-Phase Structures of Fucosylated Glycans
Maike Lettow[1,2], Eike Mucha[1,2], Daniel A. Thomas[1], Gerard Meijer[1], Gert von Helden[1], Kevin Pagel[1,2]
[1]Department of Molecular Physics, Fritz Haber Institute of the Max Planck Society, Faradayweg 4–6, 14195 Berlin; [2]Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustraße 3, 14195 Berlin; maikelettow@fhi-berlin.mpg.de
Fucose migration is a reoccurring issue in mass spectrometry of naturally occuring glycans that can lead to false sequence assignments. The reaction involves an intramolecular transfer of a fucose monosaccharide to adjacent or remote sites. In preceding research, fucose migration was strictly associated with the fragmentation process in collision-induced dissociation in tandem mass spectrometry. The fucose migration reaction and subsequent fragmentation is therefore often termed internal residue loss. Comparable to the investigation of peptide scrambling in the early 2000's, multidimensional approaches are needed to shed light on the reaction. Cold-ion infrared spectroscopy and ion mobility-mass spectrometry are employed to investigate the gas-phase structures of fucosylated tri- and tetrasaccharides of biological relevance. In the cold-ion infrared spectroscopy experiment, glycans are softly ionized with nano-electrospray ionization and mass-to-charge selected before trapped in a linear ion trap. In the ion trap, ions are picked-up by traversing superfluid helium nano-droplets and immediately cooled to 0.4 K. With light supplied by the Fritz Haber Institute's free electron laser, the encapsulated ions are irradiated and in an action spectroscopy approach, infrared spectra are recorded with the ejection of ions. The results show that fucose migration can occur in intact glycan ions and is catalyzed by a mobile proton. The observation indicates a possible low activation energy barrier for this migration reaction. Thus, fucose migration is generalized to an issue that may affect any mass spectrometry experiment and most techniques are blind to the phenomenon.

Poster: 2
Characterization of Black Solid Aggregates in Highly PAH Contaminated Soil as Main Contamination Source by FT MS
Ruoji Luo, Wolfgang Schrader
Max-Planck-Institut für Kohlenforschung, Germany; luo@kofo.mpg.de
Highly PAH contaminated soil from industrial sites can contain coal-tar like black solid aggregates (BSA), which contribute the majority of the solvent extractable organics (SEO) from the soil. The Soxhlet extract of BSA using dichloromethane was characterized using FT-Orbitrap-MS with various atmospheric pressure ionization (API) methods including electrospray (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) in both polarities. The results show that BSA primarily consist of highly aromatized hydrocarbons and polycyclic aromatic nitrogen heterocycles (pyridine- and pyrrole-like structures), whose maximum carbon count and double bond equivalent (DBE) value exceeded 90 and 70, respectively. The unique pattern of the assigned compositions in the DBE vs. carbon count per molecule plot revealed, that PAHs and PANHs found in BSA contained highly aromatic core structure with short side chains. Another character of these BSA discovered is the lower density in comparison to common soil constituents. Therefore, a density separation was developed for highly contaminated soil. After separation, the light fraction, which consisted of mainly BSA, recovered over 80% of the SEO from the contaminated soil. Accordingly, the amount of SEO from the heavy fraction was reduced to only 15% compared to the original soil. Use this density separation, a "hot-spot" of heavily PAHs contaminated soil from industrial areas can be treated and the majority of contaminants pre-separated before other chemical or biological remediation techniques are applied.

Poster: 3
Characterization of trash fuels using GC-HR-EI-MS
Yun Xu, Wolfgang Schrader
Max-Planck-Institut für Kohlenforschung, Germany; yxu@mpi-muelheim.mpg.de
In daily life humankind is producing a large amount of garbage including household and industrial trash, which creates a great problem on global environment by dumping them into ocean or landfillings. On the other side, garbage consists of high amounts of carbon based materials, which can make it a very useful resource. One easy way to use it is to produce transportation fuel by a pyrolysis process, which is basically carried out at high temperature without oxygen. This may help to achieve the goal towards a zero waste scenario and reduce the demand of fossil fuel at the same time. Trash can initially be separated into paper, organic waste, etc. Previously we have done some research work about biomass pyrolysis oil containing a high amount of oxygen, which requires special upgrading processes before it can be used as transport fuel. One advantage of using waste plastics as starting material for fuel production is that it is mainly consist of hydrocarbons, which makes it possible to be directly used. In this project, we tried to understand how the

pyrolysis process works for plastic materials. TGA studies of single plastics (such as PP, PS, HDPE and LDPE) show that almost complete loss could be observed, which means a highly efficient transformation to gas and liquid fuel. A high amount of liquid fuel derived from pyrolysis process of PP and PS, more than 70%, was obtained. Using GC-high-resolution-EI-MS allows detailed study of pyrolysis liquid fuel products.

Poster: 4

CID fragmentation studies of asphaltenes at different precipitation times usingMagnetic Resonance Mass Spectrometry (MRMS)

Matthias Witt[1], Estrella Rogel[2]

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The structures of asphaltenes are one of the hot topics in asphaltenes analysis because they might have an influence on aggregate formation. Since years the existence of archipelago and/or island structures is under discussion. Studies have shown recently that the ratio of archipelago and island structures can be determined by FT-ICR mass spectrometry.

Two asphaltenes with a low heptane to crude oil ratio (HCOR) after short and long precipitation times have been studied by CID fragmentation using ultra high resolution mass spectrometry. Compounds with low double bond equivalents (DBE) fragmented at low fragmentation energies whereas high collision energies were needed for fragmentation of compounds at high DBE. The results of the fragmentation behavior of different compound classes will be discussed.

Poster: 5

Direct infusion ultra-high resolution mass spectrometry (UHRMS) of Saturate fractions from different crude oils

Zahra Farmani, Wolfgang Schrader

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SARA (Saturates, Aromatics, Resins, Asphaltenes) fractionation is a common simplification technique used for decades in petrochemical analysis. Of these fractions, the saturate fraction is kind of forgotten fraction, because only very few reports in the literature dealt with it. Of the very few available studies, almost all have been performed using gas chromatographic (GC) techniques. Therefore, the results of such studies only show the presence of GC compatible compounds which would result in discrimination effects on any present non-volatile, high molecular weight and polar compounds. In this study, for the first time saturate fractions of different crude oils from different origins are analyzed using direct infusion ultra-high resolution mass spectrometry (UHRMS). Electrospray (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are used in positive mode. The observed results show the presence of different heteroatom containing classes, with different chemical identities (i.e. presence of thiophenes, mercaptans and cyclic-sulfides in case of S containing compounds). These results show the high affinity of some specific compounds towards different ionization techniques. Finally, the saturate fraction is shown to include much more than only volatile, saturated and aliphatic compounds. The detected compounds in this fraction present a very wide variety, not only in terms of the number of their carbon atoms per molecule and aromaticity, but also in their functional groups and structural arrangements.

Poster: 6

Environmental and geochemical applications of GC-APLI-MS for sensitive und selective analysis of polycyclic aromatic compounds

Sigrid Richter-Brockmann[1], Jan B. Thiäner[1], Henner Hollert[2], Albrecht Seidel[3], Arne Leider[4], Christine Achten[1]

[1]University of Münster, Germany; [2]RWTH Aachen University; [3]Biochemical Institute for Environmental Carcinogens Prof. Dr. Gernot Grimmer Foundation; [4]University of Bremen; achten@uni-muenster.de

A method using hyphenation of gas chromatography with atmospheric pressure laser ionization and time-of-flight mass spectrometry (GC-APLI-MS) was developed and validated for selective and highly sensitive analysis of polycyclic aromatic compounds (PAC) in the fg range. Laser ionization (248 nm, resonance enhanced multi photon ionization, 1+1 REMPI) was performed using a KrF excimer laser (ATLEX SI laser, ATL Lasertechnik, Germany) [Stader et al., 2013; Große Brinkhaus et al., 2017].

Different applications in the fields of environmental, human health and geochemical research have been developed and are presented: (1) Occurrence of polycyclic aromatic hydrocarbons (PAH) including non-EPA-PAH in samples of soils, sediments, soots, plant materials, dusts and particulate matter samples whereby several reference materials were used, (2) Identification of PAH in sediment fractions revealing toxic effects, (3) Analysis of 3-hydroxybenzo[*a*]pyrene and benzo[*a*]pyrene-tetraol in human urine and (4) Identification of aromatic steroids in sedimentary rocks from the early Proterozoic Eon.

The variety of successful applications in different fields of research show that the use of the less common

laser ionization coupled to GC presents a specific, extremely sensitive (approx. 1000 times more sensitive than common GC-MS) and highly promising tool for identification and quantification of any polycyclic aromatic compound in any sample matrix of interest.
Stader et al., Anal Bioanal Chem (2013) 405:7041.
Große Brinkhaus, S. et al., Anal Bioanal Chem (2017) 409:2801.

Poster: 7

Generating molecular insights into biofuel/fossil fuel blends via high resolution mass spectrometry

David Hamacher, Wolfgang Schrader
Max-Planck-Institut für Kohlenforschung, Germany; hamacher@kofo.mpg.de

In order to move away from the commonly used fossil fuels in heating and transportation, nowadays fuels are substituted with so-called biofuels, hydrocarbon mixtures which are generated through sustainable methods. Some of these environmentally friendly fuels are biodiesels, made from crops such as soy or rape seeds. These biodiesels are consisting mostly of fatty acid methyl esters (FAME) and especially FAMEs with at least one unsaturated carbon bond. These FAMEs cause various problems while stored. They corrode existent pump lines and due to the formation of precipitates through degradation they cause blockages of filter systems and jets. To understand the process of these formations an exact molecular insight into the biodiesels as well as into the blends with fossil resources is needed. These insights can be generated by high resolution mass spectrometry, but considering the huge polarity variance between FAMEs and the majority of the substances present in fossil fuels, the samples cannot be measured with just one ionization method, thus a more varied approach is needed. We will show the results from fuel blends studied by ultrahigh resolution MS using different ionization methods and indicate their advantages and disadvantages. The most promising methods are a combination of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) since they cover a wide variety of the polarity spectrum.

Poster: 8

Enhanced confidence in routine monitoring of river water quality by passive sampling with GCxGC-TOF MS with Tandem Ionisation

Aaron Parker[1], Laura McGregor[1], Anthony Gravell[2], Melanie Schumacher[2], Graham Mills[3]
[1]SepSolve Analytical Ltd, UK; [2]Natural Resources Wales, UK; [3]University of Portsmouth, UK; aparker@sepsolve.com

Routine monitoring of water quality is now a requirement of environmental legislation, e.g. the EU's Water Framework Directive. The cause of a poor water quality status is often unknown and extensive investigative monitoring is needed to determine what chemical maybe responsible. Passive sampling devices (e.g. silicone rubber, semi-permeable membrane devices (SPMD and LDPE) are often used for this purpose.
Silicone rubber samplers were deployed for several weeks in a river course, whose WFD status is classified as poor, to sequester large volumes of water and provide a concentrated, representative extract for analysis by GC×GC-TOF-MS. Samples from eight monitoring points along a river in South Wales will be compared.
While standards can be analysed for target compounds, the identification of knowns unknowns often relies upon spectral matching using commercial libraries. Conventional electron ionisation (EI) is a hard ionisation technique, often resulting in extensive fragmentation, weak molecular ions and /or similar spectra – reducing confidence in identification. Here, we use Tandem Ionisation (TI) to generate both hard and soft EI from a single analysis. Complementary soft EI spectra (10-20 eV) provide confirmatory identification of challenging compounds.
Here, we show the suitability of passive sampling with GC×GC-TI-TOF-MS for environmental investigations into emerging contaminants.

Poster: 9

Human poisoning with the pesticide oxydemeton-*S*-methyl proven by detection of novel cysteine- and albumin-adduct biomarkers with HPLC/ESI-MSMS

Harald John[1,2], Markus Siegert[1,2], Florian Eyer[3], Stefanie Ickert[1], Franz Worek[1], Horst Thiermann[1], Andreas Kranawetvogl[1]
[1]Institut für Pharmakologie und Toxikologie der Bundeswehr; [2]Humboldt-Universität zu Berlin; [3]Technische Universität München; stefanieickert@bundeswehr.org

Organophosphorus pesticides can cause a cholinergic crisis in humans. We report on the µLC/ESI-MSMS detection of five different biomarkers in human serum samples after poisoning with Metasystox®. The corresponding active ingredient oxydemeton-*S*-methyl (ODM) and its oxidized biotransformation product demeton-*S*-methyl sulfone carry either the leaving group 2-(ethylsulfinyl)ethanethiol or 2-(ethylsulfonyl)ethanethiol. Both pesticides form various adducts with cysteine- and tyrosine-containing proteins especially with human serum albumin (HSA). Besides free ODM, the dimer of its leaving group and phosphorylated tyrosine we also detected adducts of the thiol-containing leaving groups. These leaving

groups reacted with cysteine residues forming simple cysteine- as well as HSA-derived cysteine-proline adducts. Therefore, we herein present a toolbox of novel methods for verification of ODM-poisoning.

Poster: 10

Inorganic and organic compound characterisation and quantification from an aerosol filter sample by high resolution imaging MS and ICP-MS

Christof Barth, Klaus-Peter Hinz, Bernhard Spengler

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Polyaromatic hydrocarbons and heavy metals are ubiquitously present in urban aerosols. Both substance classes are responsible for adverse health effects after intense aerosol exposure, as often found in strongly polluted megacities. Detection and quantification of such compounds is complicated if only small amounts of particulate matter are available and since preparation is often complex and time consuming. Here we present a combined method based on direct introduction of aerosol particles into a ICP-MS and a high-resolution imaging AP-LDI MS. The method allows for quantification of inorganic and PAH compounds as well as for statistical characterisation of inorganic and organic particle populations. Sample pre-treatment is reduced to a minimum and very low amounts of particles are necessary to create a comprehensive picture of a particle filter sample.

Poster: 11

Mass spectrometry-based identification of body fluids for forensic purpose

Sascha Roocke[1], Katalin Barkovits[1], Jennifer Stepien[1], Stephan Kuhlmann[2], Annette Dorn[3], Katrin Marcus[1]

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In many forensic investigations, e.g. allegations of sexual violence or homicides, biological samples are used to identify the person involved by DNA analysis, but also the origin of biological samples such as blood, sperm, saliva and vaginal fluid is important for the clarification of criminal offences. Several detection methods are available for blood, sperm and saliva, but especially for vaginal fluid, there is yet no existing method for its unequivocal identification. Moreover, the currently utilized detection methods show a low specificity as well as sensitivity and must be performed separately.

Here we show a liquid-chromatography-mass spectrometry (LC-MS) approach to identify the most relevant body fluids for forensic investigations. For that, different sample preparation approaches such as in-gel, in-solution and FASP-assisted digestion were tested for MS-based analysis of forensic samples. For the identification of specific proteins for each body fluid, a data-dependent acquisition (DDA) was initially used. With this approach, blood, saliva and semen could be unambiguously identified. In parallel, data-independent acquisition (DIA) was utilized for the identification of specific peptides. With this strategy, both the number of identified peptides and the number of specific proteins for each body fluid was increased compared to DDA. Body fluid-specific peptides are currently being used to develop a targeted LC-MS/MS method using parallel reaction monitoring for rapid, robust and sensitive analysis of body fluids for forensic purposes.

Poster: 12

Single-particle Analysis from a Major Fire Incident of Discarded Metal in Rostock, Germany

Hendryk Czech[1,2], Johannes Passig[1,2], Julian Schade[1], Robert Irsig[1,3], Ralf Zimmermann[1,2]

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Short-term exposure to high levels of particulate matter (PM), especially from combustion processes, has been found to increase the number of cardiovascular diseases and respiratory ailments. Apart from scheduled events, such as New Year's Celebration or Bonfire Night, natural events such as sand storms, wildfires and volcano eruptions or incident fires are inevitable and may promptly affect public health without protective measure.

In this study, we investigated PM emissions from a major incident fire of discarded metals on 4th July 2018 in Rostock (Germany) by single-particle mass time-of-flight spectrometer (SPMS) using laser desorption ionisation (LDI) at 248 nm. Measurements from 26th June 2018 were selected as reference for urban PM to separate background exposure from additional PM released from discarded metal burning.

In more than 50% of the ~75,000 detected particles, lead, which was not found in the urban background, and substantial intensities of zinc, iron and possibly copper and aluminium were detected. In addition to metal cations, the composition of complex ions, such as $[ZnCl_3]^-$ in negative LDI, were derived from theoretical isotopic pattern.

A local monitoring station close to the city centre (“Holbeinplatz”) determined a daily PM10 mean concentration of 35 µg m^{-3} which is clearly below the limit of 50 µg m^{-3} from EU legislation. Since all particles of high lead and zinc content appear in the size range which can penetrate deep into the lung, our results emphasise doubts on PM mass concentration as suitable metric to assess air pollution and current EU limits.

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A shadowgraphy technique for visualizing the plume development in MALDI/MALDI-2 at high spatio-temporal resolution

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To advance MALDI-2, a powerful technique in which a Q-switched UV-laser interacts with the MALDI particle plume and initiates numerous secondary ionization processes, a deep understanding of the expansion dynamics is crucial. Here, we developed a shadowgraphy technique to visualize these dynamics at the example of the solid state matrix DHB. By precise alignment of illumination and detection modules, we achieved a spatio-temporal resolution in the low nanosecond and nanometer ranges, respectively, across a ~1 mm^2-wide image plane. Following removal of the background via a differential data acquisition protocol (N_2 MALDI-LASER on/off), this high precision critically supported revealing fine features of the plume development under elevated MALDI-2 pressure (a few mbar of N_2) and at ambient pressure conditions.

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Design of a liquid-microjunction surface sampling probe for ambient mass spectrometry analysis of consumer goods

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We present a home-built liquid-microjunction surface sampling probe (LMJ-SSP) with a parallel capillary design. In combination with subsequent electrospray ionization, the LMJ-SSP provides rapid and simple analysis of a wide variety of surfaces and analytes.

The combination of liquid extraction of analytes from various types of solid samples and ESI for ionization opens a broad field of applications. Examples are given for different pesticides, plasticizers and drugs which were analyzed from representative consumer goods, such as fruits, toys and pills. The method provides for direct sampling without any previous sample preparation steps. All measurements were performed on a high-mass-resolution Exactive Orbitrap instrument or on a portable mass spectrometer.

Poster: 15

Extended application range for a GC×GC high-resolution time-of-flight mass spectrometer platform by hyphenation to thermal analysis

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In recent years, comprehensive gas chromatography coupled to high-resolution time-of-flight mass spectrometry (GC×GC-HR-TOF-MS) has become a powerful technique for the detailed analysis of complex samples. However, matrices that contain substential amounts of non-volatile constituents cannot entirely be targeted by gas chromatography alone. In order to extend the application range of a HR-TOF-MS-platform we used thermogravimetry (TGA-HR-TOF-MS) and direct inlet (DIP-HR-TOF-MS) as additional sample introduction techniques for thermal analysis under different pressure conditions and atmospheres. Since all three techniques are hyphenated to the same vacuum ionization ion-source and multireflectron TOF mass analyzer, direct comparison of acquired mass-spectral information and efficient combination of derived datasets was possible. In this study, we comprehensively investigated heavy fuel oil samples by comparing the results of GC×GC-, TGA- and DIP-HR-TOF-MS. Since each of the deployed techniques showed unique advantages and possibilities, complementarity of the combined approach is demonstrated. Moreover, the applied data-processing strategy enabled effective usage of exact-mass-information and alignment of data-matrices from different experiments, which was crucial obtain homogeneous datasets. Beside standard EI also chemical ionization (CI) and single photon ionization (SPI) were established on the instrument for softer ionization.

Summarized, we present a versatile high-resolution mass-spectrometer-patform, combining GC×GC-, TGA- and DIP-inlet and three different ionization techniques. Furthermore, the performance of each technique is shown and compared for the analysis of complex petroleum matrices, within this work.

Poster: 16

Improvements and applications of the LAMPAS 3 laser mass spectrometer for on-line single particle analysis

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On-line analysis with mobile mass spectrometric systems is well-proven to determine chemical and physical properties of aerosol particle ensembles or individual particles. After impact-free transfer of particles into the instrument, particle detection and particle sizing, a pulsed UV laser evaporates and ionizes the detected particles. Simultaneous analysis of the generated positively and negatively charged ions is performed by two time-of-flight mass analysers with integrated ion reflectors. Several improvements of the LAMPAS 3 system [1] were achieved, such as the optimisation of optical and electrical settings as well as the integration of a delayed ion extraction assembly. Results of these improvements and the performance of the LAMPAS 3 system are presented on the basis of various field and laboratory measurements.

[1] K.-P. Hinz, E. Gelhausen, K.-C. Schäfer, Z. Takats, B. Spengler: Characterization of surgical aerosols by the compact single-particle mass spectrometer LAMPAS 3. *Anal Bioanal Chem* (2011) 401:3165–3172.

Poster: 17

LILBID-MS based method for assessing DNA binding

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Recent work in native mass spectrometry has sought to extend its application from determining only the stoichiometries of noncovalent complexes to also quantifying their binding affinities. In this study, we used two different LILBID-MS based methods to assess the binding strengths of a series of dsDNAs. For both methods, energy is added to the system to induce dissociation, either by increasing the temperature of the sample before transfer into the gas phase or by increasing the amount of laser energy transferred to the sample in the ion source. The ensuing dissociation was then monitored with LILBID-MS. Melting curves and temperatures measured with LILBID-MS were compared to analogous results from the standard method - UV absorption-based melting curves. Percent single-stranded DNA calculated from LILBID dissociation curves correlated strongly with solution state melting temperatures and showed good reproducibility, indicating that this method can be used to predict DNA melting temperatures. Thus, LILBID-based laser dissociation curves show promise as a native MS method for determining quantitative binding affinities, requiring significantly less sample consumption and measurement time than melting- and titration-based MS methods.

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Single-photon ionization of head-space sampled solid and liquid food products and of breath in a dual-ion-funnel MALDI/ESI-Injector coupled to Orbitrap-MS

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Single-photon ionization (SPI) at atmospheric pressure is an established method for analyzing small molecules. However, ion generation in air bears risks for unwanted side reactions. Here, we integrated three RF-Krypton lamps into a Spectroglyph dual-ion funnel injector, operated at ~12 mbar of N_2. Lamps were driven at 13.56 MHz with custom-made electronics that also enabled the option to pulse light emission in bursts down to 80 µs. Vapor from (heated) solid and liquid samples and from aerosols were introduced via thin tubing. Optionally, dopants were added to affect the ionization pathways. Using a Q-Exactive Plus Orbitrap as a mass analyzer (R = 280,000 @*m/z* 200) characteristic singly-charged ions of a broad polarity range were readily obtained from numerous samples without sample preparation. As examplary testing systems, twelve coffee roasts and selected whiskeys/spirits were separated by PCA and breath analysis tracked beverage consumption.

Poster: 19

What determines the postionisation efficiency in MALDI-2: A combined soft-/hardware-based set-up to characterise the role of relevant input parameters

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MALDI with laser-induced post-ionisation (PI), also named MALDI-2, was recently introduced to increase ion yields for numerous classes of lipids, metabolites, and carbohydrates. The MALDI-2 efficiency is highly dependent on a set of parameters, most importantly pulse energies of the two lasers, PI laser wavelength, delay between the laser pulses and buffer gas pressure in the ion source. Here we present a semi-automatic protocol to systematically scan and monitor these five input parameters and to link them to the corresponding mass spectra. We investigated different combinations of matrices (DHB, CHCA, DHAP) and phospholipids (e.g., PC, PE) and identified optimal experimental parameters providing the highest PI efficiency. Furthermore first promising results for MALDI-2 of tryptic peptides are presented.

Poster: 20

A Multi-Function Cyclic Ion Mobility – MassSpectrometry System

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Improvements in the performance and availability of commercial instrumentation have made ion mobility – mass spectrometry (IM-MS) a popular approach for the structural analysis of ionic species as well as for separation of complex mixtures. Here, we present a new research instrument enabling complex experiments which extend the current scope of ion mobility technology. The instrument is a cyclic ion mobility-enabled quadrupole time-of-flight (Q-cIM-oaToF) mass spectrometer. The cIM region consists of a closed loop, T-wave IM separator positioned orthogonally to the axis of the incoming ion beam. A key part of this geometry is the interface between the ion optical axis and the cIM, where a planar array of electrodes provides control over the T-wave direction and subsequent ion motion. On either side of the array, there are RF/DC ion guides used for injection, ejection, storage and activation of ions. In addition to single and multi-pass separations, the instrument design and control software enable a range of 'multi-function' experiments such as: mobility selection, activation, storage, IMS^n and importantly, custom combinations of the above. The utility of the multi-function capabilities will be demonstrated through analysis of a range of isobaric species.

Poster: 21

Advances in Orbitrap™ instrumentation for native top-down analysis of non-covalent protein complexes

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Native top-down studies of intact protein complexes have been reported since the early 1990's, but their characterization using MS3 have only recently been reported and most work has been done on homomeric assemblies. In this work we explore new ways for extending native top-down performance to allow interrogation of heteromeric protein assemblies like proteasome by top-down pseudo-MS^3.
Experiments were performed using a modified Thermo Scientific™ Q Exactive™ Plus MS in which the transmission of high m/z ions and the ability to perform pseudo-MS^3 scans for native top-down analysis were improved by implementing several hardware and software modifications (see the Results section). First, implementation of 'in-source trapping' capability addressed the insufficient or poorly controllable desolvation issue and allowed significantly improved fragmentation into subunits and stripped complexes in the inject flatapole region. Second, the reduction of RF frequencies on all RF guides and the mass filter was aimed at increasing the transmission of high m/z ions. Third, the adjustment of the voltage ramp rate on the central Orbitrap electrode facilitated successfully transmitting the high m/z ions from the C-trap into the Orbitrap analyzer.
Pyruvate Kinase, GroEL, rabbit 20S proteasome, LmrP membrane protein and AmtB membrane protein complex were used as model systems.
The improvements in performance afforded by these modifications were demonstrated in a series of pseudo-MS^3 experiments performed for several homomeric and heteromeric intact soluble protein complexes and membrane protein complexes.

Poster: 22

Automated aroma profiling of alcoholic beverages by GCxGC-TOF MS

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The aroma profiles of alcoholic beverages are composed of a broad range of chemical classes that are important to confidently identify for quality control and authentication purposes, as well as in the

engineering of new aromas.
Traditionally, solid-phase micro-extraction (SPME) has been used to sample volatile organic compounds (VOCs) from foods and beverages, and although fast and simple, it can suffer from limited sensitivity and reproducibility. In addition, immersive sampling is often avoided because it can reduce the fiber lifetime, or cause matrix interference (resulting from capillary effects).
High-capacity sorptive extraction can tackle these issues, by providing a larger volume of PDMS stationary phase (65 µL compared to ~0.5 µL for SPME) that results in higher sample loadings. Used in conjunction with secondary refocusing, this approach offers excellent sensitivity, as well as the ability to re-collect a portion of the sample for repeat analysis in a fully automated workflow.
Nevertheless, the aroma profiles are often highly complex, with important compounds, such as trace-level off-odours, frequently masked by higher-loading components. Comprehensive two-dimensional gas chromatography (GCxGC) coupled with time-of-flight mass spectrometry (TOF MS) is therefore an excellent choice, with the enhanced separation capacity of GCxGC complementing the ability of TOF MS to provide confident identification of targets and unknowns.
In this study, we demonstrate the value of high-capacity sorptive extraction with GCxGC-TOF MS to collect comprehensive VOC profiles from a selection of alcoholic beverages with fully automated workflows and novel data-processing techniques.

Poster: 23

Comparison of different atmospheric pressure photo ionisation techniques with atmospheric pressure chemical ionisation for gas phase ionisation high resolution FT-ICRMS

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The ionisation in atmospheric pressure sources is often based on complex reaction pathways. In contrast to liquid injection, where solvent molecules also participate in the ionisation process, evolved gas analysis (EGA) enables a solvent-free sample introduction. In this study, we investigate the gas-phase ionisation behaviour of complex petroleum-derived samples with EGA coupled to a 7 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) using three different ionisation techniques: Atmospheric pressure photo ionisation (APPI) with a Kr-lamp as well as a Xe-lamp and atmospheric pressure chemical ionisation (APCI) at moderate conditions to avoid artefacts. The sample material was introduced via a coupled thermo balance heated from 20 to 600 °C with 10 K/min. High resolution FT-ICR MS enables an in-depth characterization of the occurring effects.
The investigation of the elemental compositions reveals that APCI produces protonated ions for each compound class and that it can also ionize low double bond equivalent (dbe) species more efficient than the APPI-techniques. APPI-Kr shows radical cations and protonated species, especially for low dbe species. APPI-Xe mostly shows radical cations and high dbe species are pronounced in ionisation. For APPI-Kr and APPI-Xe, a clear shift of the distribution between protonated and radical cations is observed for heavier petroleum samples. Although the lower energy flux Xe-APPI will result in a lower ionisation efficiency and higher limit-of-detection, it might be useful for investigations where sensitivity is not the most important aspect.

Poster: 24

Development of a membrane-introduction photoionization mass spectrometer for real-time analysis of aromatic and polycyclic aromatic hyrdocarbons in aquatic systems

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Fast and sensitive analysis of aromatic and polycyclic aromatic hydrocarbons (PAH) is of high importance due to their impact on human health and the environment. However, low concentrations of these compounds in marine environments complicate their detection. A promising approach, regarding to direct determination of aromatic compounds in water, is resonance-enhanced multiphoton ionization (REMPI) coupled to membrane-introduction mass spectrometry (MIMS). With REMPI, the unique ionization scheme provides a selective and sensitive detection of aromatic systems. In MIMS, dissolved analytes are extracted from the water phase into the MS by using semipermeable membranes.
In this work, we present a REMPI-MIMS system with external inlets for sheet and hollow fiber membranes and its utilization for investigation of different simulated and real world water samples.

By using a sheet membrane assembly, direct detection of aromatics (e.g. toluene, xylene) and small PAHs (e.g. naphthalene, phenanthrene) with concentrations in the lower µg/L-range are easily accessible within minutes without any sample preparation. The response times ($t_{10-90\%}$) can vary from few seconds for toluene to approximately 10 minutes for phenanthrene.
For the hollow fiber membrane setup, the trap-and-release technique can be utilized for direct determination of the selected aromatics and PAHs in water samples down to µg/L-range in less than 5 minutes. Here, the pervaporating compounds are trapped inside of the membrane tube for a certain time and are released by a fast heating of the membrane into the MS.
For both inlet designs, the applicability for the analysis of real water samples can be demonstrated.

Poster: 25

Electrodynamic Droplet Levitation for the Purpose of Time-Resolved Mass Spectrometry

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To understand biological processes it is relevant to analyze the speed of a reaction in addition to identifying the molecules involved. A method delivering both types of information would be of high interest. Laser-induced liquid-bead ion desorption (LILBID) mass spectrometry (MS) is well-suited to perform time-resolved (TR) native MS experiments on a fast time scale. LILBID employs well defined droplets which are irradiated by IR laser pulses to generate gas phase ions. These droplets can be used as reaction vessels if they are levitated in an electrodynamic Paul-trap. This new set-up allows sample manipulation and MS analysis on precise and fast reaction-times. Here we show the construction and characterization of a setup for TR-LILBID-MS.

Poster: 26

Evaluation of the Phase-Constrained Spectrum Deconvolution Method (Phi-SDM) for multiplex TMT application

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The primary goal of most proteomics research is to identify proteins, which abundances are affected by changes in their environment. In quantitative MS-based proteomics, these changes can be effectively monitored by measuring the relative abundance using isobaric mass tagging such as TMT™ labels. This technique allows quantitative analysis of up to 11 different conditions in a single high-resolution LC-MS experiment. However, using high resolution in MS/MS scans tends to reduce the frequency of acquisition. In order to bypass this issue, the **Ph**ase-Constrained **S**pectrum **D**econvolution **M**ethod (Phi-SDM), a new interference-free, super-resolving (*i.e.* beyond Fourier uncertainty) FTMS signal processing approach, is being evaluated in TMT11plex™ quantification experiments on a research grade Thermo Scientific™ Q Exactive™ HF-X Orbitrap™ mass spectrometer.
FSDM offers the possibility to deliver higher resolution mass spectra on shorter transient length results in substantially higher LCMS scan frequency compared to traditional FT data analysis. Selectively applying Phi-SDM to the TMT reporter ion region (between 126-131 Da) may be utilized to produce a high mass resolution segment within a lower resolution spectrum. In these experiments, standard 15,000 resolution settings with windowed application of Phi-SDM in the TMT region is used. Compared to the 45,000 resolution setting for accurate TMT10/11plex quantification experiments, this approach provides an increase in acquisition speed of more than 50%, preserving more than enough resolution to baseline resolve all the reporter ions in the TMT11plex kit. Additionally, approximately 40 % more MS2 spectra were acquired and the number of identified and quantified proteins increased by about 20 %.

Poster: 27

High Resolution Mass Spectrometry of Acoustically-Levitated Droplets

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Acoustic levitators generate standing waves between a transducer and a concave reflector separated by multiple numbers of half wavelengths of the acoustic waves. The result of acoustic levitation is that a small volume (5 nL-10 µL) of sample can be levitated in a contact-free manner. Until now, levitation in analytical chemistry has primarily been associated with optical techniques such as Raman, X-Ray or UV/Vis spectroscopy. Less common are combinations of acoustic levitation with mass spectrometry. One reason for this is that the acoustic field surrounding the droplet effectively shields the sample from reactive species, thus making it inaccessible to most ambient ionization probes. Any effective interrogation of acoustically-

levitated droplets therefore requires the physical removal of some of the sample from the confinement region of the acoustic trap before anaylsis.
Our direct sampling technique is based on irradiation of the droplet with an Er:YAG laser (λ = 2.94 µm) for producing a spray outside the pressure minimum of the acoustic trap. Subsequent ionization is performed by an APCI source and mass analysis achieved using Orbitrap Q-Exactive mass spectrometer. This setup provides high resolution mass spectra directly from the levitated droplets. The laser spray only consumes a minor fraction of the sample droplets, while the entire sampling, interrogation and detection process occurs fully automated on a fast timescale. Thus, the presented concept is ideally suited for online monitoring of reaction kinetics inside wall-less microreactors.

Poster: 28
HILIC, Polar, and Shape Selectivity of a FluoroPhenyl Phase
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The FluoroPhenyl stationary phase has long been marketed as a phase that offers alternative, or orthogonal, selectivity to a C18. The FluoroPhenyl phase offers unique selectivity by incorporating strongly electronegative fluorine atoms on a phenyl ring (Figure 1). In addition to the traditional reversed-phase dispersive interactions, this phase also exhibits shape selective, polar, cation-exchange and even HILIC retention mechanisms which aid in selectivity of specific analytes.
In this presentation we aim to demonstrate the useful and alternate retention of the FluoroPhenyl stationary phase. We chose several relevant target analytes which we plan to use to exemplify the unique retention characteristics of the FluoroPhenyl phase when used in either HILIC or reversed-phase mode. All of these analytes have been pursued due to either poor retention, poor resolution, or both on a traditional C18 phase.

Poster: 29
Ion isolation, stability boundaries, and space charge effects in the omnitrap platform driven by rectangular RF waveforms
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The omnitrap platform is a segmented linear quadrupole ion trap driven by two anti-phase rectangular RF waveforms and fast switching DC potentials for lossless transfer of ions between trapping regions, facilitating a diverse set of ion activation steps applied in tandem. The extensive functionality of the omnitrap is enabled by the ability to perform intermediate ion isolation steps in a dedicated trapping region by precise control of the RF and DC parameters applied to the poles of the trap. A comparison of the different options available in the omnitrap platform to perform ion isolation is presented, including (a) the superposition of multiple-frequency sweep waveforms applied in dipolar form, (b) controlling the duty cycle of the rectangular RF drive and (c) the application of a static resolving DC. The effect of stretching the poles of the linear ion trap in one direction only is investigated experimentally and by ion optical simulations and shown to narrow down the window of ion isolation during the application of a resolving DC. The boundaries of the stability diagram are also investigated experimentally by scanning the frequency of the RF drive and by applying a resolving DC to displace ions across the a-q stability space. Reduced trapping efficiency is observed at specific high q values, associated with the method employed to perform the frequency jumps and also with the number of ions injected in the trap.

Poster: 30
Rapid Profiling and Quantification of 17 Bile Acids in Human Plasma by LC-MS/MS
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Bile acids are a group of major catabolic products of cholesterol. They are important biomarkers for signaling potential harmful side effects for new drug development. There are two main types of bile acids according to their functional groups; the first is free bile acids, or unconjugated bile acids, and the second is the conjugated bile acids. Quantitation of bile acids in matrices proves to be very challenging due to a number of factors, including, the similarity of structures, varying polarities and stereochemistries, the presence of isomers, limited fragmentation for unconjugated bile acids in mass spectrometer, high endogenous levels in matrices, and matrix effects caused by phospholipids or triglycerides.

A rapid, robust, selective and reliable LC-MS/MS method was established and well validated. Hydrophilicity, and therefore retention on a reversed phase C18 column, is influenced by both the bile acids nucleus and side chains structures. Therefore, the tri-hydroxy bile acids (e.g., CA) elute earlier than di-hydroxy bile acids (e.g., DCA and CDCA), which in turn elute earlier than the mono-hydroxy bile acids (e.g., LCA). However, the retention time is also determined by the position a stereochemistry of hydroxyl groups, where the UDCA (di-hydroxy) elutes earlier than CA (tri-hydroxy). One of the significant improvements was baseline separation of all 17 bile acids in 6 minutes. Excellent linear correlation (R^2 0.9979-0.9999), accuracy (92.6%-109.9%) and precision (1.0%-11.8%) were observed for all compounds. Matrix effect caused by phospholipids was greatly reduced by column forward flushing. The column pressure maintained the same after 100-time injections.

Poster: 31

Using Free, High-Performance, Computer Modeling Software to Simulate Gas Chromatographic Separations

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Our recently introduced modeling software is a selectivity tool that relies on a pre-loaded library of thermodynamic retention indices. This makes it possible to predict retention times and optimize chromatographic methods without the need to analyze compound sets under many different conditions. The program allows the user to select the stationary phase and simultaneously adjust: film thickness, temperature, column length, column internal diameter and flow. Users can enter each compound or cut/paste large lists of compounds into the program.
Since its introduction there have been thousands of searches across a broad range of compound classes. The program outputs: compound retention time, resolution and peak width along with the column conditions and dimensions. A model chromatogram is provided to illustrate retention, peak width and resolution. Users have the option to view compound mass spectral data with the added benefit of overlaying mass spectra for coeluting analytes. Specific searches can be saved and accessed at a later date. Examples of these features will be presented with a focus on challenging separations.
The program allows user to simulate GC separations using a laptop. It's the most easy and cost-effective way to optimize separations in the shortest time. It only needs a registration. You can access it at: https://www.restek.com/proezgc

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Poster: 32

Vacuum Photoionisation ToF-MS as technique to analyze complex gas mixtures on-line and in real time

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Within the last years e-cigarettes and other new smoking/vaping products became more and more commonly used. There are two general ionization techniques that are covered by vacuum Photo-Ionization Time-of-Flight Mass-Spectrometry (PI-TOFMS). On the one hand there is SPI (Single-Photon-Ionization) ionizing a wide range of organic molecules and on the other hand there is REMPI (Resonance-Enhanced-Multi-Photon-Ionization) focusing primarily on aromatic structures. Especially the complementary use of SPI and REMPI can access new information.
SPI is using varying ionization light sources based on lamps or lasers. The respective wavelengths /ionization energy limit the range of ionizable organic compounds being roughly in a range of 7 to 11 eV (177 nm to 112 nm). Because most matrix gases such as oxygen, nitrogen, carbon dioxide and especially water vapour have higher ionization energies of 12 eV and more, they will be suppressed efficiently. Depending on the specifically used REMPI method at least two photons are used for ionization. This ionization mechanism requires a stable excitable intermediate state that is primarily present and accessible in aromatic structures.
Soft photoionization can be applied in various research fields and applications dealing with complex gas mixtures that need to be observed in real time. Especially a high temporal resolution enables the investigation of fast and dynamic processes.
The PI-MS approach can be also transferred to other application needing a temporal and spatial analytical resolution.

Poster: 33

A matter of composition- ion suppression effects among phospholipids in MALDI mass spectrometry imaging investigated by use of artificial tissues

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A major hindrance for quantitative (Q)MALDI-MS imaging applications are ion suppression effects. To obtain a deeper insight into these phenomena for phospholipids (PL) we here developed artificial tissues (AT) which mimic essential characteristics of biological material while containing controlled concentrations of selected PLs. Molecular signal intensities of one PL class (e.g., phosphatidylcholines) were compared to those of a second (e.g., phosphatidylethanolamines) in dependence of the individual PL concentrations. Our study reveals an intricate interplay of suppression- and ionization boost effects between the different PL classes. Additionally these effects are influenced, among others, by the choice of MALDI matrix, laser wavelength, fluence and focal spot size. Different relationships are, again, found, if the classical MALDI analysis is supplemented by laser-postionization (MALDI-2).

Poster: 34

Adduct Suppression at Oligosaccharides and SORI-CID Fragmentation

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Because of their importance in biological processes, the analysis of oligosaccharides is an interesting field for mass spectrometry and fragmentation experiments. It is known that alkaline adduct-ions influence the fragmentation behavior [1,2]. The experiments were performed on a 9.4 T APEX-IV-Qe FT-ICR mass spectrometer (Bruker-Daltonik) equipped with an Apollo-I ESI-source. Argon was used as collision-gas for the SORI-CID experiments. To counteract the cation induced signal suppression, ammonium-acetate was used. Thereby, the intensity of the protonated molecular ion could be highly increased [3]. Also, the following collision induced fragmentation experiments were performed with different SORI parameters [4].. But the intensity of the fragments could not be highly increased by varying these parameters. However, different fragmentation-patterns could be observed for adducted and non-adducted molecules.

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Poster: 35

Effects of physical and chemical interactions in ion mobility spectrometry (IMS) depending on the reduced field strengths

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The chemical and physical interactions of ions and neutrals in mass spectrometry (particularly API-MS) are also observed in ion mobility spectrometry (IMS). As a result, in IMS molecular analyte-ions are generally participating in complex chemical reaction systems. In our work, details of often encountered cluster reaction systems are investigated with a HiKE-IMS (**Hi**gh **K**inetic **E**nergy **IMS**).

The HiKE-IMS consists of a low-pressure reaction drift tube for generating ions coupled to a second low-pressure drift tube for ion separation based on ion mobility. Thereby, the HiKE-IMS can generate high reduced field strengths (>120 Td) in the drift tubes. At these conditions, the effective ion temperature is significantly increased and can reach values in the range of 1000 K.

Therefore, this setup allows to directly observe cluster transitions in depence of the reduced field strength / effective ion temperature and additional chemical and physical parameters with high resolution. Such experiments provide information about complex dynamic reaction systems prevailing in the drift tube. This information can also be transferred to reaction systems active in the transfer stages and ion sources of mass spectrometers.

Poster: 36

Gas phase reactions of heptamethine cyanine dyes using femtosecond-laser-pulse induced photodissociation and collision-induced dissociation

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Heptamethine cyanine dyes with indole moieties are widely used as a fluorescent dye in the NIR region, e.g. as fluorophores for in vivo imaging.
The trapped ions of cyanine dyes were analysed on an Apex III FT-ICR mass spectrometer (7.05 T) (Bruker Daltonik, Germany) by means of femtosecond-laser-pulses (NIR region) (Ti-Light, Quantronix, USA) and collisions with argon. The ionization was performed via electrospray ionization from different solvent mixtures. In previous experiments, indocyanine green showed different stability for protonated ions and sodium ion adducts. The incorporation of a rigid chlorocyclohexenyl ring in the polymethine chain can decrease the unfavourable photodegradation of the long heptamethine chain [1]. The molecular ions and different metal ion adducts were compared with respect to their stability and their fragmentation products.
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Poster: 37

Host-guest chemistry of azafullerene derivatives

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We are investigating triaryldihydro and tetraarylmonohydro azafullerenes, in which the pyrrole unit is formally isolated from the rest of the π-system by the addition of the aryl-ligands and hydrogen atoms to the former double bonds surrounding it.[1,2] The aryl addends form a partial cavity into which another fullerene can settle. In the gas phase both homodimers $M(M - H)^-$ and heterodimers with other fullerenes, e.g. $C_{60}(M - H)^-$ can be observed.
These dimers were investigated using energy-dependent collision-induced dissociation to determine their relative bond strengths. It was found that different isomers of the azafullerenes show a surprisingly large difference in binding strengths.
The MS/MS spectra also showed that charge transfer between the deprotonated azafullerene and C_{60}/C_{70} is possible. The extent of electron transfer varies considerably amongst the different dimers. As expected, charge transfer is more pronounced towards C_{70} than to the less electron affine C_{60}.
Furthermore, it was found that enantiopure triaryldihydro dimers can generate covalently bound dimers of the form $(M - H)(M - 2H)^-$ by each formally losing a hydrogen radical and forming a single bond between the remaining fullerene radicals.[3] Breakdown graphs measured for these singly bonded azafullerene dimers show that their bond strength is comparable to that of the heterodimers $C_{60}(M - H)^-$ and $C_{70}(M - H)^-$.
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Poster: 38

Ion-solvent interactions in nanoESI-MS: Comparison of different ion transfer settings and analyzer systems

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The addition of non-volatile solvents (supercharging reagents) to the liquid phase in ESI can increase the mean charge state of the generated ions. In addition to this conventional technique, a different approach to supercharging is studied: By exposing electrospray droplets to solvent vapor, charge conservation and charge depletion are observed in dependence on the added solvent. The ability to form stable ion-solvent-clusters seems to be crucial to prevent proton transfer reactions resulting in charge depletion. The transfer and analyzer system of the MS determines if generated ion-solvent-clusters, which are ubiquitous in API-sources, are observable in the analysis. We investigate the influence of varying ion activation conditions on the observed mean charge state in detail to elucidate the underlying mechanisms.

Poster: 39

Kinetic energy distribution measurements for ion dynamics studies

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Experimental evaluations of mass resolved kinetic energy distributions in Bruker ion trap mass spectrometers have shown systematic deviations from absolute ion current measurements with a faraday

cup-electrometer setup. Experiments and ion trajectory simulations using SIMION 8.1 demonstrate that this difference is attributed to the kinetic energy dependent ion acceptance of the ion trap and the rf phase dependent fringe field between the terminal transfer lens and the trap entrance hole. Even though the absolute kinetic energy distribution cannot be evaluated without measuring absolute ion currents, relative changes of the distribution are very well detectable with this method. Preliminary results are presented, which suggest that kinetic energy distribution measurements can be a valuable tool for probing ESI droplet and ion release dynamics.

Poster: 40

Mechanistic studies of L-proline-catalyzed Diels-Alder reactions of unsaturated aldehydes with ESI-MS

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Organocatalysis is an ever growing field of high current interest. Based on our mechanistic investigations of L-proline-catalyzed Aldol- and Aza-Diels-Alder reactions [1, 2], we now set out to study the mechanism of the L-proline catalyzed Diels-Alder reaction of hexenal published by Griesbeck et al.[3].

In the proposed catalytic cycle, two intermediates are postulated: The first step is a condensation of proline and the aldehyde leading to α,β-unsaturatated enamine (intermediate I). This molecule can react as a diene in a Diels-Alder reaction with another equivalent of the aldehyde which acts as the dienophile to form cyclic intermediate II. The second intermediate then can eliminate L-proline to yield the product and regain the catalyst.

While NMR of the reacting solution is a very challenging task due to extremely complex spectra with numerous signal superpositions, ESI-MS of the reacting solution gives highly elucidating results. Both postulated intermediates can be detected and characterized by accurate mass determinations and CID. Further, their temporal progress can be monitored directly. However, neither the reactant nor the product could be detected with ESI MS. We currently are working on the combination with other methods such as IR and APCI MS.

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Poster: 41

Performance of Cassinian ion traps in dependence of their trap length

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The Cassinian ion trap [1] is a variation of the Kingdon trap. In this work [2] we have calculated Cassinian traps of different lengths. After calculating the corresponding ion motion in all traps we compared the results with experimental data.

All electric fields were calculated solving the Laplace Equation using a commercial Fenite-Element-Method tool (COMSOL v.4.3b). Induced signals were determined using Shockley-Ramo theorem. Experimental data were recorded on the Bruker Twin-Trap experiment [3].

Calculating the induced signal along the longest axis of the trap we got an oscillating signal. The upper envelope of this oscillation shows an exponential decay in a logarithmic plot. This way we can correlate the intensity of the induced Signal and the truncation of the Trap.

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Poster: 42

The strong lever of phenetole: In-depth investigation of the vibronic structure in the first excited state and ionic ground state

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We investigated the effect of electronic excitation and ionization upon the vibronic structure of phenetole by utilizing resonance enhanced multi photon ionization (REMPI) and mass analyzed threshold ionization

(MATI) spectroscopy with a time of flight mass spectrometer. [1]
The aromatic moiety of phenetole is similar to the one of the amino acid tyrosine, which is responsible for UV absorption in biochemical compounds. [2,3]
Both the S_1 excitation and adiabatic ionization energy of phenetole were determined with high accuracy. In addition, we assigned all significant signals to vibrations with the aid of Franck-Condon simulations on the (TD)DFT level of theory. The in-plane bending vibration of the side chain had a remarkably high activity in the first excited state, which was even more pronounced in the ionic ground state. Besides the vibronic analysis, we also observed a strong Duschinsky rotation effect for several $D_0 \leftarrow S_1$ transitions.
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Poster: 43

Walk-on-sphere rearrangement and retro-Bingel reaction of gas-phase fullerene malonate ions

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$C_{60}L_X$ (L=C(COOEt)$_2$; x=4-6) are investigated by APCI with a QIT and a Q-TOF mass spectrometer to study their chemical stability as radical ions in the gas phase.
The samples were synthesized by a published procedure.[1] $C_{60}L_6$ and $C_{60}L_5$ are isomerically pure (determined by ^{13}C NMR). $C_{60}L_6$ has an octahedral addition pattern with T_H symmetry. $C_{60}L_5$ has a quadratic pyramidal addition pattern with C_{2V} symmetry. $C_{60}L_4$ consists of a mix of isomers with varying symmetries.
For all examined fullerene adducts, radical anion ($C_{60}L_X^{\bullet -}$) and radical cation ($C_{60}L_X^{\bullet +}$) formation is observed applying APCI. Fragmentation experiments with a QIT-MS show the repeated loss of L_2 as the main fragmentation pathway for $C_{60}L_X^{\bullet -}$ and $C_{60}L_X^{\bullet +}$(x=4-6). Energy-dependent fragmentation experiments using a QTOF-MS show a decreasing energy-demand for the fragmentation when the number of malonate moieties increases. Furthermore, dissociation of the radical anions is less energy demanding than of the radical cations, this observation is in agreement with experiments by Kessinger et al. which show isomerization of $C_{60}L_2$ in solution under electrochemically reducing conditions by a walk-on-sphere rearrangement.[2]
Both $C_{60}L_X^{\bullet -}$ and $C_{60}L_X^{\bullet +}$ (x=4-6) show a very selective fragmentation with the loss of L_2. This fragmentation can only occur when two diethyl malonates “walk” on the surface towards each other. The walk-on-sphere rearrangement is followed by the formation of a malonate dimer (L_2) which is then released from the fullerene.
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Poster: 44

Following the conformational diversity of the PYP photoreceptor with native Ion-Mobilty MS

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Ion mobility mass spectrometry (IM-MS) is a versatile tool to investigate structural changes of intact proteins not only in the gas phase. Upon excitation with blue light the Photoactive Yellow Protein (PYP) enters a reversible photocycle including fundamental structural rearrangements in the time range from femtoseconds to seconds. When a photon is absorbed by the proteins chromophore the protein leaves its dark state (pG) and subsequently enters several intermediate states before it recovers back into the initial state. In the case of PYP the intermediate state with the largest structural changes is the so called signaling state (pB) which is reached after 1.5 ms. Several mutants show different behaviors in their recovery times from the pB back to the pG state which we track by time resolved IM-MS.

Poster: 45

Linewidth Pressure Measurement (LIPS) by means of protonated aminoacids in ICR-traps

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In mass spectrometry accurate pressure measurements are fundamental to obtain conclusions about equilibria, reaction rates, collision cross sections, collision and dissociation energies. In this work Linewidth

Pressure Measurement (LIPS) [1] was applied to two different Fourier-Transform Ion Cyclotron Resonance (FT-ICR) devices based on known Cross-sectional Areas by Fourier Transform Ion Cyclotron Resonance (CRAFTI) [2] of six different amino acids [3]. LIPS was compared with the pressure measurement of the installed ionization vacuum gauges. Deviations of up to a factor of 150 to lower pressures were observed in the case of ionization vacuum gauges. In addition a linear relationship between the opening time of the pulsed gas inlet valve (0,5 to 5,0 ms) and the resulting LIPS pressure inside the ICR-cell could be shown.

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Poster: 46

Trapped Ion Mobility Spectrometry as post-ionization separation technique for biomedical samples

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Ion mobility spectrometry (IMS) is an emerging separation technique in various analytical laboratories. The integration with mass spectrometry (MS) instruments allows gas-phase separation of ions based on their mass-to-charge ratio, size and shape. While IMS is often used as an additional post-chromatographic separation dimension, e.g. in LC-IMS-MS systems, it has high potential as stand-alone separation technique. The differentiation between isobaric and isomeric species is one of the major challenges in analytical chemistry that can be addressed by IMS. While chromatographic separation techniques require comparable long analysis times, IMS enables fast separation on a millisecond timescale.

The unambiguous differentiation between isobaric species is important for several applications in the life sciences. 5-Aminoluvenic acid (5-ALA) is applied in fluorescence-guided surgery of glioblastoma as a prodrug for the biosynthesis of the fluorescent protoporphyrin IX. The interpretation of the accumulation and transformation of 5-ALA is limited by the presence of several isobaric interferences in biological tissue. On the molar mass of 131 $g \cdot mol^{-1}$, these include creatine as well as the amino acids leucine, isoleucine and 4-hydroxyproline. The latter is a direct isomer to 5-ALA. Trapped IMS (TIMS) was applied for a post-ionization separation of the isobars. Direct sample introduction into an ESI-TIMS-QTOF-MS instrument enables the fast differentiation between 5-ALA and its isobaric interferences.

Poster: 47

Investigation of Non-Covalent Clusters of Anisole and various Aniline-Derivatives via REMPI-Spectroscopy

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In previous works on non-covalent intermolecular interactions, anisole-clusters showed only a single electronic ground state in Resonance-enhanced multiphoton ionization (REMPI) spectra [1]. This differs from the behaviour observed in similar benzene-phenol-clusters [2].

To further investigate whether this is universally valid, additional experiments were carried out introducing new clustering partners. Aniline-derivatives were chosen to introduce amino-groups, another feature present in structures like DNA base pairs.

So far, findings are in good agreement with previous results showing only a single electronic origin, with different functional groups affecting the spectra further. The spectra share some characteristics with those of the anisole-dimer, although the red-shift relative to the anisole-monomer is even more pronounced.

Quantum-chemical calculations are pending to gain deeper insight into the structure of these newly formed clusters.

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Poster: 48

Aufarbeitungstrategien von Krebstieren in Vorbereitung der LC-MS-Messung

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Krustentiere stellen eine begehrte und zukunftsrelevante Nahrungsquelle dar. Aufgrund ihrer Nährwerte werden sie global in erheblichem Umfang konsumiert. Das Bedürfnis nach einer gesunden und

abwechslungsreichen Ernährung führt zu einem vermehrten Interesse an hochwertigen Fischereierzeugnissen. Der Nährwert von Krustentieren zeichnet sich durch einen hohen Proteingehalt in Kombination mit geringen Fett- und Kohlenhydratanteilen aus. Durch den erhöhten Bedarf an einer Vielfalt von Nahrungsmitteln und der voranschreitenden Globalisierung des Handels, ist das Risiko für Verfälschungen innerhalb der Lebensmittelkette gestiegen. Die morphologische Bestimmung zur Speziesidentifizierung von Krustentieren ist aufgrund der phänotypischen Ähnlichkeiten kompliziert. Darüber hinaus wird in prozessierten Lebensmitteln meist das Außenskelett entfernt. Schnelle und verlässliche analytische Methoden sind daher notwendig, um die Kennzeichnung von Krebstieren sicher überprüfen zu können. Die Differenzierung von nah verwandten Spezies stellt dabei eine besondere Herausforderung dar.
In Vorbereitung zur LC-MS-Analyse wurden nun unterschiedliche Aufarbeitungs-strategien für Krebstiere entwickelt und verglichen. Die effiziente Probenextraktion ist bereits ein wichtiger Schritt für die Entwicklung einer empfindlichen und reproduzierbaren Analysemethode. Hierbei wurden zunächst verschiedene Möglichkeiten der Einwaage in Form von reinem Muskelfleisch sowie gefriergetrocknetem Material getestet. Weiterhin wurden drei verschiedene wässrige Pufferlösungen sowie drei verschiedene Geräte zur Probenhomogenisierung eingesetzt und hinsichtlich der Proteinextraktion evaluiert. Die extrahierten Proteinanteile wurden mit einem Nanophotometer vermessen. Die erhaltenen Ergebnisse wurden darüber hinaus mit einer *sodium dodecyl sulfat* Polyacrylamid-Gelelektrophorese (SDS-PAGE) von einer Hummerprobe auf ihre Gültigkeit überprüft. Letztlich wurden die verschiedenen Aufarbeitungsstrategien mittels LC-MS gemessen und die Unterschiede kritisch diskutiert.

Poster: 49

Coupling of pectin-derived oligosaccharides with phosphatidylethanolamine yields side products from Amadori rearrangements

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Citrus pectins composed of poly(α1-4)GalUA were reduced and hydrolyzed yielding (α1-4)Gal oligosaccharides ((α1-4)Gal_n). Glycans were coupled with phosphatidylethanolamine (PE) by reductive amination. Besides the desired (α1-4)Gal_n-PE products a second series of Gal_n derivatives was observed which exhibited a −2 u lower molecular weight than the corresponding (α1-4)Gal_n-PEs and were assigned as Amadori rearrangement products termed (α1-4)Gal_n=PEs. Their structures were verified by nanoESI MS und CID experiments. Especially fragmentations of the galactose representing the former reducing end were diagnostic. Interestingly, similar rearrangement products have never been observed upon coupling of glucose-terminated glycans like lactose, sialyllactose, or globotriaose with PE. Schiff bases of galactoses at the reducing end of oligosaccharides seem to be more prone to Amadori rearrangements than to reduction.

Poster: 50

Entwicklung innovativer Analysenverfahren zum Nachweis von Molkenproteinen und Etablierung von Biomarkern als Qualitätsparameter bei Molkenprotein-angereichertem Schnittkäse

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Nachhaltiges Wirtschaften sowie eine ressourceneffiziente Produktion von Lebensmitteln nehmen einen immer weiter wachsenden Stellenwert ein und stehen somit im Fokus von vielen Unternehmen. Zur Effizienzsteigerung in der Käseherstellung wird angestrebt, den Molkenproteinanteil (MPA) im Käse durch z.B. intensivere thermische Behandlung der Käsereimilch zu erhöhen und die Molkenproteine - auch unter dem Gesichtspunkt der ernährungsphysiologischen Qualität - auf diesem Wege innovativ für die menschliche Ernährung verfügbar zu machen. Für eine entsprechende Kennzeichnung der Produkte für den Verbraucher fehlen bislang geeignete Analysenmethoden, die einen Nachweis des erhöhten MPA in Käse erlauben.
Basierend auf Shotgun-Proteomics-Strategien („Bottom-up“), sollen innovative Analysenmethoden entwickelt werden, die es ermöglichen, den MPA anhand spezifischer Peptidmarker mittels LC-MS zu quantifizieren. Nach der Entwicklung und Optimierung eines geeigneten Verfahrens zur Probenaufarbeitung werden hierbei die Milchproteine und Polypeptide aus der Käsematrix einem enzymatischen Verdau unterzogen und die erhaltenen Peptidprofile nach flüssigchromatographischer Auftrennung mittels nano-HPLC massenspektrometrisch detektiert und analysiert. Durch online-Datenbank-gestützte Auswertung mittels Proteome Discoverer soll versucht werden, spezifische Peptide als Marker für einzelne Molkenproteine zu identifizieren und deren Eignung zur Quantifizierung des MPA in gereiftem Käse zu prüfen.
Zur Charakterisierung und qualitativen Bewertung Molkenprotein-angereicherter Käse erfolgt ebenfalls eine

direkte Analyse der Peptide aus der Käsereifung mittels LC-MS. Hierzu werden die Käseproben entsprechend ohne enzymatischen Verdau aufgearbeitet und die erhaltene Peptidmuster genutzt, um Biomarker für die sensorische Qualität zu identifizieren.

Poster: 51

From cigarettes to joints - Puff resolved online investigation of conventional and new smoking products using Photoionization Mass Spectrometry

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Within the last years e-cigarettes and other new smoking/vaping products became more and more commonly used. With the increased (legal) availability of marijuana/cannabis and THC containing smoking products not only for medical purposes, the interest in understanding the release processes of the active smoke constituents beside potentially harmful compounds is increasing as well.

The nicotine release profile is the most important marker for cigarettes and new smoking devices. The general target is to mimic the nicotine and flavor release of a cigarette while the concentration of HPHCs is significantly reduced. Typically, the nicotine release in a conventional cigarette is increasing from puff to puff. Most e-cigarettes have a rather constant release. The nicotine release profiles of the tobacco heating products are quite more complex and also related to conditioning of the used tobacco sticks. E-cigarettes as well as the new tobacco heating products reduce the concentration of most HPHCs by several orders of magnitude.

Dealing with unconventional smoking products, such as joints, the puff-by-puff nicotine release becomes less important. The active compounds here are delta-9-tetrahydrocannabinol (Δ9-THC) and cannabinol. Due to their different physiologic and psychologic effect the respective release profiles in joints, containing different types and parts of the marijuana plant are of special interest. The Cannabinol concentration in the used hashish samples is about 10 times higher than in weed or marijuana samples. Respectively, either the plant parts used for producing the hashish contain more cannabinol or the extraction process has a higher selectivity on this compound.

Poster: 52

LC-MS analysis of two subcellular fractions of brown algal phlorotannins

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Phlorotannins are specific brown algal phenolic metabolites, the polymers of phloroglucinol with different degree of polymerization (DP) and molecular sizes from 126 to 650 kDa. Depending on the structure of the molecules, six major classes of phlorotannins have been defined. There are two phlorotannin fractions in the algal cells, one located in unique organelles, physodes, the other – in the cell walls (CW). Here we study polyphenol profiles of three brown algal species to compare the molecular composition of two subcellular phlorotannin fractions.

Samples of algal tissue were ground and extracted with 70% acetone (physode fraction) and then with 1 M NaOH (CW-fraction). Chromatography was performed on Agilent 1100 HPLC system with a reversed-phase column Gemini C18. MS data were acquired on Bruker Esquire 3000 Plus ESI ion trap mass spectrometer in negative mode.

As phlorotannins with DP>10 don't form individual chromatographic peaks, the analysis was based on MS data. Each group of molecules with a similar structure but different DP, was observed as a specific *m/z* series with increments corresponding to multiples of the phloroglucinol moiety. MS-analysis revealed in the physode fraction several *m/z* series indicating the presence of different classes of phlorotannins with DP 3-38. The major series corresponded to fucol or fucophlorethol molecules. In the CW fraction only one phlorotannin-type *m/z* series (presumably, carmalols with DP 4-12) was detected. This series was also found in the physode fraction, though it was not dominant there.

The study was supported by the Russian Foundation for Basic Research (project 17-04-01331).

Poster: 53

Mass Spectrometric Characterization of the Zein Protein Composition in Maize Flour by SDS-PAGE and 2D Gel Electrophoresis

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Using 95% ethanol, a-zein was isolated from maize in an environmental-friendly process. High purity of a-zein was determined by 2D gel electrophoresis followed by MALDI-ToF-MS peptide mass fingerprinting after in-gel chymotryptic digestion. Being a natural product, encoded by multiple gene copies, the polymorphic a-zein revealed ten spots in two rows, one at 25 kDa and one at 20 kDa. MALDI-ToF-MS peptide mapping proved the presence of only a-zein. By contrast, when extracted with 65% ethanol, other zein proteins were detectable as well. Due to polyploidy and genetic polymorphism of the plant, the application of high resolution separation methods in conjunction with precise analytical methods, such as MALDI-ToF-MS, is required to determine homogeneity of zein protein devoted for industrial use.

Poster: 54

Mass spectrometric structural characterization of mucin-derived *O*-glycans obtained by HILIC solid phase enrichment

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Mucins are glycoproteins densely decorated with *O*-glycans serving both as protection of the intestine and nutritional source for commensal and pathogenic bacteria. To this day, there is a substantial lack of knowledge of the precise structures of the glycans from mucinous glycoproteins.
To unravel the structural heterogeneity of *O*-glycans derived from mammalian mucins, we utilized the following novel approach involving three major steps: (i) chemical liberation of *O*-glycans from the protein backbone by use of β-elimination reactions; (ii) selective enrichment of released carbohydrates by use of a novel zwitterionic hydrophilic interaction liquid chromatography (i-HILIC) stationary phase; and (iii) structural characterization using MS^1/MS^2-analysis.
Preliminary data suggest that i-HILIC provides a similar selectivity for *O*-glycans as observed for the ZIC-HILIC material.

Poster: 55

Qualitative and quantitative analysis of sulfur compounds in heavy crude oil and its fractions

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Removal of sulfur is a crucial upstream process during crude oil refining. The process typically involves hydrogenation at elevated temperatures using a heterogeneous catalyst. Depending on the chemical nature of the sulfur containing compounds within a given oil, desulfurization is more or less effective. While sulfidic compounds are desulfurized relatively easily, more condensed systems like thiophenes, benzothiophenes, dibenzothiophenes and 4/6-alkylated-dibenzothiophenes are increasingly resistive towards the reaction at the catalyst surface. For an economically as well as ecologically optimized process it is necessary to gain as detailed information on the amount of sulfur containing species in the sample and their distribution into the different chemical groups as possible.
The method presented here combines a liquid chromatographic separation of crude oil constituents into the three groups of thiophenes (eluting together with sulfur-free hydrocarbons), condensed thiophenes (benzothiophenes and above) and sulfides with direct mass spectrometric detection.
Mass spectrometry is employed both, qualitatively and quantitatively, using two different setups. For qualitative MS an FT-Orbitrap mass spectrometer is used. This is done to verify the successful separation of compounds. For sulfur selective detection with uniform response (irrelevant of the chemical nature of the compound) the LC was coupled to an ICP-MS/MS system that allows sensitive and interference-free detection of sulfur in mass-shift mode.
We present the only quantitative application of the method on a heavy crude oil and its individual SARA fractions that is reported by now. Using such a difficult sample, the capabilities, as well as the limitations of the method are evaluated.

Poster: 56

Routine LC-MS/MS method optimization for clinically relevant metabolites of the kynurenine pathway

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Several metabolites of the kynurenine (KYN) pathway are becoming increasingly relevant in clinical routine applications for fast and reliable diagnosis of inflammatory bowel disease (IBD). However, the established analytical methods using LC-ESI-MS/MS are typically based on solvent combinations of methanol, water

and formic acid (FA).
Some of the metabolites of interest however, in particular kynurenic acid (KYNA) and xanthurenic acid (XANA), show strong matrix-dependent chromatographic interferences. This results in poor peak shape, low reproducibility, retention time shifts as well as in sensitivity losses. Therefore, this study describes an optimized LC-ESI-MS/MS approach with an alternative solvent system using very low concentrations of trifluoroacetic acid (TFA) as a modifier. However, TFA is a challenge for LC-ESI-MS/MS systems. Plastic seals, capillaries, connectors or even stainless steel components, such as the electrospray nebulizer needle, may corrode or degrade. Therefore, this study evaluated the potential for decreasing the TFA modifier content, while at the same time, preserving good chromatographic behavior.
In comparison to methods based on weaker acids, an addition of 0.01% of TFA led to significant improvements in the peak shape of KYNA and XANA in human urine and plasma samples. Higher signal intensities for lower metabolite concentrations, sharper peaks and stable retention behavior were observed when using this level of TFA in the solvent system.
Within further method optimization, the concentration of TFA in the solvent system was reduced stepwise until chromatographic quality significantly decreased. This resulted in an optimal range for TFA between 0.01 – 0.005 %, which was adapted into the method.

Poster: 57

LipidXplorer Web: An online, rapid identification and quantification tool for bottom-up and top-down shotgun Lipidomics, based on customizable queries

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LipidXplorer-Web, the web-version of popular LipidXplorer shotgun lipidomics software, maintains the core functionality and key features of LipidXplorer. However, there is no need to install and setup the software on a local desktop computer and the most up-to-date version is always available for the users. In the web-version lipid identification routine is vastly simplified and streamlined it also allows users to share best practices and experiences and find default setting and best practices that are known to work. Local installation of the PeakStrainer software can be used to reduce size of shotgun spectra datasets by peak intensity-independent repetition rate filtering that is particularly efficient for high and ultra-high resolution Orbitrap spectra. Lipid identifications rely upon a curated collection of MFQL (Molecular Fragmentation Query Language) files describing the identification routine for each lipid class using most common forms of molecular ions and fragments of lipids and users are only required to specify key instrument-dependent settings, while other parameters will be extracted from raw data. In this way, LipidXplorer-Web is made available for rapid trial experiments performed by newcomers to the lipidomics field; it is not bound to an instrument platform or pre-defined database of lipid structures. LipidXplorer-Web read multiple types of inputs, including public and proprietary formats. The output from PeakStrainer can also be used as an input to locally installed LipidXplorer or to LipidXplorer-Web and provides new and technically simplified way of doing shotgun lipidomics.

Poster: 58

Applying Trapped Ion Mobility Separation (TIMS) in combination with Parallel Accumulation Serial Fragmentation (PASEF) for analysis of lipidomics samples

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For a deeper understanding of biochemistry, the analysis of lipids is important. Untargeted lipidomics workflows profile changes in the lipidome to discover relevant lipids as potential biomarkers. We present an improved lipidomics identification workflow that is based a combination of IMS-LC/MS together with a fast data-dependent MS/MS fragmentation (PASEF). Serum samples were extracted, LC/MS/MS was performed in PASEF mode (Parallel Accumulation Serial Accumulation) on a timsTOF PRO / Elute UHPLC (Bruker). The resulting data sets were processed in MetaboScape 4.0 software.The targeted approach allows TIMS separation of isobaric lipids with resolution of 180. The novel 4-dimensional feature finder (T-ReX 4D) in MetaboScape enables thorough and reproducible extraction of all relevant features including collisional cross sections and corresponding MSMS information.

Poster: 59

A closer look at the porcine lung: the lipidome and changes due to inter-individual variation

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Alterations in the human lung lipidome mirrored pathologies in lung tissues but also gave first insights into the lipid metabolic system and its dependence to clinical parameters like age, sex and weight. All data was acquired from a cohort of lung cancer patients and consisted of tumor-free tissue samples and cancer biopsies. However, tissue sampling was biased towards older individuals ([41-86], 67±10 years), particularly smokers with a higher portion of males.
In view of the ethical requirements it is not possible to collect tissue samples from healthy individuals, the use of animal model is required. The pig as an animal model is already in use to study lung diseases like cystic fibrosis, lung inflammation and COPD as well as of its close resemblance to human physiology and anatomy. Not much is known about the porcine lung lipidome in general and the differences to the human system in particular.
In this study, we analyzed the porcine lung lipidome by using a shotgun lipidomics approach and assessed the histopathological quality of the lung tissue by scoring for histological features such as inflammation, fibrosis, emphysema and hemorrhaging. The cohort consists of 66 female, male and neutered male pigs, of differing breeds like the “Angeln Saddleback”, “German Large White”, “German Landrace” or crossbreeds like “Pietrain x German Large”.
We quantified approximately 400 lipids from 14 lipid classes and will discuss the outcome of the lipidome comparisons using correlation analyses as well as the LUX score approach to measure lipidome homology.

Poster: 60

A Web-Tool to Compute Lipidomes Homologies based on Template SMILES

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Lipids are involved in a manifold of biological processes in all organisms. Recently, technological advances enabled to quantify large portions of the lipidome of different cell types and tissues from model organisms and humans. To gain insight into the correlation between molecular distribution of alipidome and its functional impact, we developed the LUX score as measure of homology [Marella et al. 2015]. To calculate the LUX score, lipidomes are represented in chemical space models utilizing the Simplified Molecular-Input Line-Entry System (SMILES). Unfortunately, SMILES are not unique by nature because valid SMILES strings can be written starting from any position in a chemical structure.
Consequently, we propose an approach to generate Template SMILES following a dedicated database model that contains building blocks and their connectivity.
SMILES of different lipid classes were aligned and the most conserved structural position is determined and further on considered as a reference point for related lipids. Furthermore, the connectivity and order how side chains are combined into the SMILE string is fixed by a template.
The new Web-Tool includes now this SMILES generator, which corrects some errors in the generation of Template SMILES. Analyses of model data sets of yeast and drosophila showed improved clustering behavior. We applied this approach for the first cross species analysis of human, mice and pig lung tissues lipidomes.
Additionally, soon the web based version will offer an online input data validator and the lipidomes homology will be performed based on different multivariate scales.

Poster: 61

Absolute quantification of phospholipids using nano-ESI and short acyl chain analogues

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Lipid quantification is often achieved by spiking lipid extracts with standard lipids. Challenging, however, is the non linear instrument responses depending on fatty acyl chain length and saturation. Usually, conventional ESI is employed requiring high sample amounts. Here, we evaluate the use of nano-ESI for lipid quantification employing deuterated or short- and odd-fatty acyl chain analogues.
While saturation did not affect ionisation in our instrumental set-up, short-chain lipids showed higher intensities when compared with their long-chain analogues. To compensate for these differences, we generated a calibration curve over a range of lipids with increasing chain length. Evaluation was performed using the various lipid standards. Both approaches delivered comparable quantities and are therefore applicable for accurate lipid quantification.

Poster: 62

Double-bond resolved shotgun MS investigations of the *Schistosoma mansoni* lipidome

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The lipid metabolisms of *Schistosoma mansoni* flatworm parasites and vertebrates differ significantly. Although *S. mansoni* is unable to biosynthesize phospholipids, previous identification of PC(16:0/18:1(Δ5)) showcases the unique metabolic processes that take place in this parasitic organism. To study lipid metabolism of *S. mansoni* in more detail, the lipidome of male and female trematodes as well as the hamster host were investigated employing Paternò-Büchi shotgun ESI-MS/MS. Mass spectrometric signal intensities of different phospholipid classes were used to relatively quantify fatty acid compositions and double-bond positions and deduce lipid isomer abundance variations in male/female and host organisms. The results show that *S. mansoni* either transforms or enriches host lipids to increase the abundance of saturated lipids and lipids containing Δ5-double bonds.

Poster: 63

LipidCreator: A workbench to probe the lipidomic landscape

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We developed LipidCreator, the first software fully supporting targeted lipidomics assay development. LipidCreator provides a comprehensive framework to compute MS/MS fragment masses of over 60 lipid classes. LipidCreator provides all functionalities to define fragments, manage stable isotope labeling, optimize collision energy and generate in-silico spectral libraries. LipidCreator proved to be an efficient tool for investigating phospholipid derived mediators to dissect lipid-signaling pathways of human platelets during activation.

Poster: 64

Long-chain n-3 PUFAs are incorporated into a series of phospholipids of the muscle in pigs fed microalgae supplemented diet

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To date, no studies have been conducted in pigs to better understand the influence of dietary polyunsaturated fatty acids (PUFA) on muscle lipid metabolism. We performed a pig diet experiment (German Landrace sows) with microalgae (*Schizochytrium sp.*) supplementation. The animals were allocated to a control group (n=15) and a microalgae group (n = 16). The lipidomics approach describes the effects of a DHA-rich diet on incorporation in lipid classes of *longissimus dorsi* muscle. Shotgun lipidomics approach was applied to pig muscle lipidom using a Q Exactive Plus (Thermo, Bremen, Germany) coupled with Triversa Nanomate (Advion, Ithaka, USA).

We quantified roughly 340 lipids from seventeen lipid/phospholipid classes including PC, PC-O, PE, PE-O, PS, PI, PG, CE, SM, LPC, LPE, LPI, LPG, PA, Cer, DAG, and TAG. Primarily results showed, that the micro algae intervention was reflected in the lipids of pig muscle compared to the control group. The most abundant lipids in PC and PE of pig muscle in both diet groups were PC (16:0/18:2), PC (16:0/18:1) and PE (18:0/18:2), respectively. In case of neutral lipids, predominantly TAG 52:0, TAG 52:1 and DAG (16:1/18:1) were identified. The incorporation of microalgae-based DHA and EPA could be shown in almost all phospholipid classes. In PC and PE classes, DHA and EPA were incorporated predominantly in PE (18:0/22:6), PE (16:0/22:6), and PC (16:0/20:5), PC (18:0/22:6), respectively. In addition, *n*-3 dietary PUFA were integrated into a series of alkenyl-acyl lipid metabolites (Plasmalogens) of PC and PE (PC O-18:1/20:4, PC O-16:1/22:6, PE O-18:1/22:6).

Poster: 65

Oxidative modification of skin lipids by cold atmospheric plasma (CAP) - a standardizable approach using LESA and LC/MSMS

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An emerging source for the spatially controlled delivery of reactive species are cold physical plasmas. A clinical potential has been identified in the control of inflammatory processes, such as chronic wounds, yet the biochemical mechanisms beyond are underexplored. Lipids are important interfaces between interior and exterior that potentially orchestrate and modulate the biological responses observed after cold plasma treatment.

Stratum corneum-derived lipids from 22 participants were collected on glass slides and treated with a cold plasma device (kINPen®). Lipids were extracted and measured either by a direct infusion-liquid extraction surface analysis (DI-LESA) approach or reversed phase LC/MSMS. Major *stratum corneum* lipid classes were identified along with oxidation products related to the treatment, indicating a potential role in plasma – tissue interaction.

Poster: 66

Robust and Sensitive LC-MS/MS Based Plasma Lipid Profiling on a Thermo Scientific™ Q Exactive™ HF-X Mass Spectrometer

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Lipids play an important role in biology. Characterization and quantitation of lipid species requires robust and reproducible analytical workflow that enables identification and quantification of multiple lipid classes. Accurate detection and quantification of lipids can be compromised by a number of factors including their extraction from biological samples, chromatographic separation, ionization conditions and detection by mass spectrometry. The Thermo Scientific™ Q Exactive™ HF-X is a new Orbitrap™ based mass spectrometer with increased scan speed and sensitivity, both of which should benefit large scale lipid profiling experiments. In this study, we optimized a complete workflow including lipid extraction from plasma and mass spectrometry parameters to achieve high quantitative performances, which could be applicable to large cohort studies.

Different plasma extraction volumes were tested in order to maximize the number of lipid species which could be detected and quantified with good precision. This evaluation was conducted by extracting the ion signals for 3 lipids (high, medium and low abundant lipids) from each lipid extract.

Further optimizations included the identification of conditions that minimize the in-source fragmentation of labile lipids and avoid excessive losses of lipid ions.

In optimized data dependent LC-MS/MS experiments, lipids are quantified based on the peak areas in MS1 and the high quality MS2 spectra generated in view of the higher scan rate are used for assignment of their structural composition. LipidSearch software was used to confidently identify and quantify several hundreds of lipids in an automated way.

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Poster: 67

SIMPLEX: A multi-omics approach for screening of modified hippocampal lipid signaling pathways triggered by lifestyle condition

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Enriched environment (EE) animal studies revealed fundamental changes in the neuronal structure, development, and electro-physiology of hippocampal brain regions whereby EE housed animals also show a better performance in physical and cognitive tests. However, until today, it is hardly possible to correlate these features to differences in the lipidome/proteome composition of the hippocampal region of EE animals. To explore the role of general lipid/protein composition we established an integrative multi-omics approach combining metabolomics, lipidomics and proteomics techniques.

Global proteomics analysis identified 2164 proteins from which were 57 up and 121 down regulated in 27

altered pathways related to synaptic plasticity and signal transduction of the synaptic junction, indicated by minimum log2-fold change with a p-value <0.05. The retrograde endocannabinoid signalling pathway (RESP) was identified as the solely altered lipid messenger pathway, which is involved in regulating pain, addiction, cognition, and neurodegeneration in the brain. The 2-arachidonoyl glycerol (2-AG) hydrolase and fatty acid amide hydrolase responsible for substrate degradation displayed significant changes. Targeted nano-LC high-resolution lipidomics experiments underscored these findings by displaying a significant reduction of the main RESP lipid neurotransmitter 2-AG (from 139.41±27.18 vs. 101.73± 14.41 nM/mg protein, p=0.046, non-EE vs. EE, respectively) whereby anandamide remained unchanged in the synaptosome.
In conclusion, the RESP is down-regulated on protein and lipid messenger level in EE mice, leading potentially to a higher neuronal plasticity. An integrative multi-omics approach is suited to give detailed insights of neuronal metabolic mechanism even under soft or non-invasive lifestyle interventions.

Poster: 68

Simultaneous determination of polar and non-polar lipids in yeast by means of heart-cut two-dimensional liquid chromatography - mass spectrometry

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Lipids are a highly diverse group of biomolecules, which fulfill various functions and are linked to disease development. Due to the different functions, lipid structures are complex and lipid properties differ greatly. By hydrophilic interaction liquid chromatography (HILIC) lipids are separated class-specific according to the polar head groups. However, non-polar lipids, such as triacylglycerols and cholesterol esters, experience insufficient retention. Hence, an on-line heart-cut method was developed that employs the direction of lipids eluting in the void volume of HILIC onto a reversed phase (RP) column. The used set-up enables the mass spectrometric measurement of both chromatographic dimensions, requiring little additional technical effort. The applicability of the developed method is demonstrated by analysis of a *Saccharomyces cerevisiae* total lipid extract. Phospholipid species from different classes and their fatty acid composition can be identified in the HILIC retention time span. Different DAG- and TAG-species are separated by RPLC.

Poster: 69

Structural characterization of complex lipids by ozone-induced dissociation and ultraviolet photodissociation on high-resolution mass spectrometers.

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Lipids play different physiological roles as sources of stored metabolic energy or structural matrices in biological membranes. Alterations to the lipidome have been associated with the onset and progression of numerous diseases such as cancer, diabetes and cardiovascular diseases. Detailed structural characterization of lipids in complex extracts is thus desirable.
Current tandem mass spectrometry approaches identify lipid class, total carbons and double bonds, but not further information. In this study, we investigate the utility of new ion activation techniques, specifically, ozone-induced dissociation (OzID) and ultraviolet photodissociation (UVPD) for more comprehensive lipid structure elucidations. Preliminary experiments were performed incorporating OzID on a Thermo Scientific™ Q Exactive™ HF MS to identify the position of acyl chain position and double-bonds in phospholipids and triacylglycerols. By combining HCD activation with OzID on selected lipid ions on the Q Exactive, the relative position of acyl chains on the glycerol backbone could be determined without compromising in scan time. Additionally, reaction of selected lipid ions with ozone inside the mass spectrometer can produce two characteristic product ions: an aldehyde and a Criegee ion. These ions are diagnostic for the double bond position and can be obtained by OzID increasing the HCD cell fill time and decreasing the collision energy. With this technique, double bond position of the analyzed lipids can be readily obtained. OzID experiments were compared with lipid fragmentation by UVPD on an Orbitrap Fusion Lumos MS equipped with a 213 nm laser.

Poster: 70

Informed unTargeted Metabolomics using LC-MS/MS

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As part of the "Omics" studies, metabolomics gain more and more importance when it comes to the understanding of biological systems. However, untargeted metabolomics offer some challenges, including unambiguous metabolite identification as well as the inevitable bias that comes with the choice of analysis conditions. To address these challenges, we generated a MS^2 library of high resolution and accurate mass spectra and performed a study on LC behavior of numerous metabolites. Based on this data a metabolomics screening method was established and used for Informed unTargeted metabolomics of 530 human serum samples.

We used a library of synthetic standards of > 600 metabolites. A flow injection/t-SIM/dd-MS^2 method was developed to generate the MS^2 library with no need for manual injection. MS^2-spectra in positive and negative mode were acquired automatically for 6 different collision energies using a hybrid quadrupole-Orbitrap mass spectrometer (Q-Exactive). 56 different LC conditions were tested to generate the LC database using a Full Scan/dd-MS^2 method that was also applied for the metabolomics screen. Samples were extracted using a protein precipitation protocol, LC was performed on a ZIC-HILIC column. Data was analysed by using FreeStyle, Library Manager, Tracefinder and Compound Discoverer.

The MS^2 library currently contains about 3500 MS^2 spectra for 440 metabolites, LC data was generated for 295 metabolites. The Informed unTargeted metabolomics study resulted in 114 confirmed metabolites within certain quality parameters. Identification using the inhouse MS^2 library was superior compared to mzCloud for 82 % of metabolites having a matching MS^2 library entry.

Poster: 71

A novel derivatization method for vitamin D metabolites to improve both sensitivity and separation ability of isomers in LC-MS/MS

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Vitamin D_3 and its major metabolites 25-hydroxyvitamin D_3 ($25(OH)D_3$), 1,25-hydroxyvitamin D_3 ($1,25(OH)D_3$), 24,25-hydroxyvitamin D_3 ($24,25(OH)D_3$) and the C3 epimer of $25(OH)D_3$ (3-epi-$25(OH)D_3$) play important roles in the pathology of many diseases. The quantification of these metabolites is challenging however. While serum levels of $25(OH)D_3$ can usually be directly measured by LC-MS, the much lower concentrations of $1,25(OH)D_3$, $24,25(OH)D_3$ and 3-epi-$25(OH)D_3$ require prior chemical derivatization to enhance detection sensitivity. Derivatizations using dienophile reagents are well established but often lead to problems during chromatographic separation of isomers due to diastereomer formation during reaction. Derivatization of the hydroxyl group presents an alternative opportunity. Previous studies have shown varying results for isomer separation for the same derivatization reagents and for increases of sensitivity, making a comparison of derivatization reagents very difficult.

In this study, we compare the detection sensitivity and isomer separation for vitamin D_3, $25(OH)D_3$, 3-epi-$25(OH)D_3$, $1,25(OH)D_3$, $24,25(OH)D_3$, 7-dehydrocholesterol, 25-hydroxy cholesterol, cholesterol and α/β/γ/δ-tocopherol using three dienophile reagents (PTAD, DMEQ-TAD, Amplifex) as well as three chemical reagents for hydroxyl groups (dansyl chloride, 2-picolinic acid, 2-fluoro-1-methylpyridinium p-toluenesulfonate) on a C-18 HPLC column. The comparison of the derivatization reagents showed that isomer separation was improved by using hydroxyl derivatization reagents reagents over the dienophile, while the results for the detection sensitivity depended on the analyte structures, with vitamin D metabolites giving better sensitivity for the dienophile reagents, because of the more selective derivatization.

Poster: 72

A novel method for identification and quantification of sulfated flavonoids in plants byneutral loss scan mass spectrometry.

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Sulfur is present in plants in a large range of essential primary metabolites, as well as in numerous natural products. Many of these secondary metabolites contain sulfur in the oxidized form of organic sulfate. However, except of glucosinolates, very little is known about other classes of such sulfated metabolites, mainly because of lack of specific and quantitative analytical methods. We developed an LC-MS method to analyse sulfated flavonoids, a group of sulfated secondary metabolites prominent, e.g., in plants of the genus Flaveria. The sulfated flavonoids are detected by mass spectrometry (MS) in a negative mode, using a

neutral loss of 80 Da after a collision induced dissociation. We could detect all (mono)sulfated flavonoids described before in Flaveria plus a number of new ones. We showed that sulfated flavonoids represent a substantial sulfur pool in Flaveria, larger than the thiols glutathione and cysteine. The individual species possess different sulfated flavonoids, but there is no correlation between the qualitative pattern and type of photosynthesis. Similar to other sulfur-containing secondary compounds, the concentration of sulfated flavonoids in leaves is reduced by sulfur starvation. The new LC-MS method will enable qualitative and quantitative detection of these secondary metabolites in plants as a pre-requisite to addressing their functions.

Poster: 73

Application of capillary ion chromatography-MS for metabolite profiling of yeast metabolites

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The identification and quantification of metabolites is of great interest in different biological and medical research areas, because of their importance for a functional organism. Considering the characteristics of anionic metabolites involved in the primary metabolism, it can be noted that their analysis is a challenging task. Common LC separation techniques such as reversed phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) hyphenated to MS have shown insufficient retention or selectivity for the small polar or charged analytes.

Therefore, the separation by capillary ion chromatography (Cap-IC) provides an appropriate alternative especially for anionic compounds. The analytes are retained by their chemical affinity to charged quaternary ammonia groups on the surface of the stationary phase and eluted by a potassium hydroxide gradient. To be able to analyze the separated metabolites by MS, a neutralizing step by a suppressor is inserted after the separation column.

Through the application of this system for the analysis of twenty metabolites, which are included in glycolysis, pentose phosphate pathway or the citrate cycle, a separation and quantification was achieved by high-resolution MS applying negative electrospray ionization. The deprotonated molecular ions could be detected and used for the metabolite profiling of *Saccharomyces cerevisiae* extracts, which is also known as bakers yeast and one of the most utilized eukaryotic model organisms. By means of additionally performed data-dependent fragmentation experiments, the differentiation of isomeric structures was carried out.

Poster: 74

Detection of drugs and metabolites in urine by Fast Flow Injection Analysis (FIA) coupled to Magnetic Resonance Mass Spectrometry (MRMS)

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A fast method for detection of drugs and their metabolites in urine using FIA-MRMS is presented. Pooled urine samples were purified and analyzed by FIA-MRMS using a scimaX (Bruker) in ESI. Analysis of data was performed with MetaboScape 3.0. ESI(+) and ESI(-) data were combined for feature analysis.300 drug candidates have been detected in the urine samples using a HMDB urine database with 0.5 ppm mass error tolerance. The detected drugs have been compared with the medication of the patients. Results have shown that drugs and their metabolites can be detected by FIA-MRMS. This method could even be used for quantification when internal drug standards are added. Very accurate mass detection and ultra-high mass resolution are prerequisite for this workflow.

Poster: 75

Formation of α-dicarbonyl compounds and advanced glycation end-products (AGEs) from blood plasma monosaccharides

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Glycation is usually referred to as a non-enzymatic post-translational modification formed by reaction of protein amino and guanidino groups with carbonyl compounds, predominantly reducing sugars and α-dicarbonyl products of their oxidative or non-oxidative degradation. α-Dicarbonyl compounds, glyoxal (GO) and methylglyoxal (MGO), readily react with lysine and arginine residues of proteins yielding advanced glycation end products (AGEs). This heterogeneous group of derivatives is known for their pro-inflammatory

effects in mammals and an essential impact on ageing and pathogenesis of metabolic disorders – diabetes mellitus and Alzheimer disease. Although the pathways of α-dicarbonyl formation are generally known, the relative impact of the major non-glucose minor plasma monosaccharides in their generation and related glycation reactions is still not characterized. Therefore, here we address the relative potential of glucose, fructose and ascorbic acid to form α-dicarbonyls and resulted arginine-derived AGEs using a combination of LC-QqTOF-, LC-IT- and GC-EI-Q-MS approaches. For this, the kinetics of sugar degradation was considered simultaneously with that of GO and MGO formation. These data were interpreted in the context of the results, obtained with *in vitro* human serum albumin (HSA) glycation model. The experiments revealed ascorbic acid as the most reactive carbohydrate, yielding the highest amounts of the both carbonyls. Accordingly, incubations with this compound yielded the highest rates of GO- and MGO-derived hydroimidazolones. Thereby, the kinetics of AGE formation was in agreement with the kinetics of sugar degradation and dicarbonyl generation.
The reported study was funded by RFBR according to the research project № 18- 34-00927.

Poster: 76

High Throughput Targeted Workflows for Metabolomics / Lipidomics Studies

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Background: Here we present a targeted, robust, LC-MRM high-throughput strategy for the identification and quantitation of large numbers of metabolites and lipids in complex samples, providing high quality identification and quantitation and simplifying the downstream informatics.
Methods: Two methods each ~25 mins in duration, have been optimized for the QTRAP® 6500+ system with IonDrive™ Turbo V Source. Method 1 targets a total of 312 polar metabolites across the key metabolic pathways (187 positive mode and 176 negative mode MRM's) using HILIC separation at pH9 using Luna-NH2 columns (Phenomenex). Method 2, using the *Scheduled* MRM™ Algorithm, targets over 1200 lipid molecular species using a XBridge Amide 3.5 µm column (Waters). Data was processed using MultiQuant™ Software 3.0.
Conclusions: For Method 1, the Luna-NH2 HILIC chromatography provided excellent chromatographic separation of polar metabolites, and with method adjustments to loading conditions very good retention time reproducibility for polar metabolites was achieved. Both high flow and microflow chromatography was explored, with microflow providing a significant improvement in sensitivity and therefore metabolite coverage.
Due to the challenge of isomer interference, the chromatography for method 2 was optimized to provide separation between lipid classes, focusing on CE, CER, DCER, HCER, LCER, TAG, DAG, MAG, LPC, PC, LPE, PE, LPG, PG, LPI, PI, LPS and PS. An internal standard strategy was developed to simplify retention time determination and quantification. The target list is customizable and expandable to include new lipid classes or the list can be shortened for a class-specific study.

Poster: 77

MS-based study of the plasma liquid chemistry: effects on thiols

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Cold atmospheric plasma (CAP) has been proposed as medical tool in inflammatory disease control. Their effectiveness is assumed to depend on the deposition of reactive species in bio-fluids or tissues, whose composition and delivery can be controlled spatially. To control and standardize cold plasma discharge liquid chemistry, small molecule tracers are used.
Cysteine was used as model compound and CAP-induced derivatives were identified with high-resolution MS. Quantification was achieved by a HILIC/MS-based MRM strategy. Isotopically labeled gases were included to trace reactive oxygen species trajectories. A strict dependency of the product portfolio on discharge parameters, e.g. direct versus indirect discharge, distance, or feed gas composition was observed supporting biomedical observations.

Poster: 78

Multiomics data integration for the identification of genotype/phenotype-interactions in non-inbred mouse strains

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To identify molecular pathways related to genotype/phenotype interactions in non-inbred mouse strains, we

used mice that were long-term selected for high treadmill performance (DUhTP mice) and unselected controls (DUC mice), both from the same genetic pool. As an environmental factor, we tested the effects of training on metabolism in both mouse strains. We performed RNAseq in muscles, liver, fat and brain in all experimental groups for the genome-wide evaluation of the regulation of molecular signaling pathways. The entitity of coding mRNA transcripts was sequenced by NGS (HiSeq 2500 Illumina). Metabolic profiling was performend by LC-MS/MS using C18 column (Vanquish flex) and the Q Exactive Plus MS System (ThermoFisher Scientific). For bioinformatic pathway analysis we used Ingenuity Pathway Analysis (IPA, Qiagen).
DUC and DUhTP mice underwent three weeks of treadmill training before tissue analysis, NGS, and LC-MS/MS analysis.
NGS sequencing revealed strong line-specific differences in the tested tissue. In particular, lipid metabolism and oxidative phosphorylation were altered in different tissues of DUhTP mice compared to control and under training conditions. The necessary tissue interaction via serum will be described by holistic metabolic analysis.
Because we use a non-inbred model for the assessment of genotype/phenotype/environment interactions, our results could be relevant for a broader genetic background. Our studies will test the relevance of environmental and genetic factors for the control of energy metabolism.

Poster: 79

Personalized nutrition: A metabolomics-based approach for a detailed comprehension of gut bacterial pathways by UPLC-IMS-QToF

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Recent research has revealed that the impact of nutrition on metabolic reactions is highly individual. This interpersonal variability is determined by different gut microbiome composition and function. The aim of this research project is to identify nutrition specific metabolites for deeper characterization of person-specific metabolism.
For this metabolomics-based approach, capillary blood from dried blood spots was extracted and analyzed via UPLC-IMS-QToF. Multivariate data analysis was carried out to identify differences in the metabolic pattern between various diets.
In the future selective marker substances shall be identified using open source databases. Finally, a comprehensive view of the individual metabolic reactions and biotransformation of food during digestion by the intestinal microbiota is to be obtained.

Poster: 80

Probing age-related changes in pea (*Pisum sativum*) root nodule metabolome by mass spectrometry

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Legume crops are the prominent source of dietary protein. Achieving high yields of these crops lies in the focus of the modern agriculture. In this context, success of symbiosis with nitrogen-fixing rhizobia is one of the main factors affecting productivity of legumes. Understanding of the molecular mechanisms, underlying development of this plant-microbial association might essentially impact on improving of existing cultivars. Here we address changes in metabolism of pea nodules accompanying their ageing. In parallel to natural ageing, we considered accelerated ageing, established with pea plants, containing a mutated gene *sym27* (SGEFIX-7), phenotypically manifested with early degradation of bacteroids. The nodules were harvested after 2-7 weeks post-inoculation. Metabolites were isolated from the frozen material by water-methanolic extraction. The extracts were dried, and the residues were analyzed by GC-MS after sequential methoximination and trimethylsylilation procedures. The processing of the acquired data relied on AMDIS, MS-Dial and Xcalibur tools. Identification was based on spectral similarity search using NIST and in-house libraries. The analysis of wild type plants revealed about 90 metabolites, demonstrating age-dependent alterations in relative abundance. Seventy of them (monosaccharides, amino acids) were up-regulated. In contrast, accelerated aging of nodules in SGEFIX-7 plants was associated with dramatic decrease in the

levels of the most of the metabolites (amino acids, di- and monosaccharides and their phosphorylated derivates). These results were in agreement with our data on age-related changes in nodule proteome and indicated suppression of biosynthetic processes by the *sym27* mutation. The research was supported by the Russian Science Foundation (project 17-16-01042).

Poster: 81

Epitope and affinity determination of recombinant Mycobacterium tuberculosis Ag85B antigen towards anti-Ag85 antibodies using proteolytic-affinity mass spectrometry and biosensor analysis

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Tuberculosis is caused by the bacillus Mycobacterium tuberculosis. Multidrug-resistant TB arises when an antibiotic fails to kill all the bacteria, with the surviving bacteria developing resistance to that antibiotic. The only antitubercular vaccine currently in clinical use is Bacillus Calmette-Guérin (BCG), but its efficacy is not achieved with certainty. The aim of this work is to provide a basis for the rational design of a neo-glycoconjugate vaccine against TB. Structural characterization of recombinant antigenic proteins from Mycobacterium tuberculosis (MTB) Ag85B (rAg85B, variants and semi- synthetic glycoconjugates) was initially carried out. Identification of antibody epitope analyses by proteolytic affinity-mass spectrometry and surface plasmon resonance (SPR) biosensor analyses were performed for the identification and characterization of the interaction structures of the antigens with antibodies from different sources. A commercial monoclonal antibody, and polyclonal antibodies from different sources (patients with active TB, vaccinated individuals and a healthy control) were employed to analyze antigen-antibody interactions. These combined approaches provided the identification of different assembled epitope regions on the recombinant MTB antigens; the sequences [9-17], [45-58], [143-150] and [236-292] of rAg85B. The localization of these areas on the same side of protein surface suggests the possible contribution of protein conformation in antibody binding and the consequent presence of an assembled epitope or group of epitopes. Furthermore, the affinity binding constants in the interactions with specific antibodies revealed the importance of protection from excessive glycosylation. The identified epitope peptides should constitute a suitable basis for the design of new specific target vaccines.

Poster: 82

Fast active sampling of volatiles for field experiments

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Active sampling under well-established standard conditions using thermal desorption (TD) tubes is one of the most common methods for volatile analysis by TD-GC/MS. Under field conditions, however, the required exposure time to the source of transient volatiles signals can be a restricting factor (e.g. when sampling fast-changing environmental conditions or wild animals). Therefore, reducing the sampling time is often a desired option. However, using uncommonly higher flow rates or collecting very low sample volumes might affect the sampling efficiency and subsequently impair both, quality and quantity of the volatile profiling. Therefore, we systematically evaluated fast sampling conditions for three common sorbent materials, XAD-2, Tenax TA and silicone tubing, and the respective chromatographic displacement. Recovery of volatiles under two sampling regimes, namely high flow rates versus low sample volumes showed markedly differences among sorbents in terms of sensitivity and reproducibility, different trends for specific compounds, and potential interferences due to sorbent background contribution. Finally, we discuss the advantages and drawbacks of each sorbent material for volatile profiling under the different conditions for fast sampling.

Poster: 83

Mass spectrometry-based investigations of cold atmospheric plasma-induced post-translational modifications (PTM's) in peptides

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PTM's are important regulators of cellular signaling pathways. Using high-resolution mass spectrometry, this study wants to answer the question: which non-enzymatic modifications can be induced by cold atmospheric plasma (CAP) which are of interest for their role in inflammation control. Using model peptides a PTM-library is under construction to forecast CAP derived PTM's in complex samples.

CAP's can act as a rich source for various reactive species and thus also for PTM's, with numerous modifications like oxidations, dioxidations, carbonylations and didehydroxylations detected by LC-ESI-MS. Efficient and robust identification and quantification of the PTM's must still be solved. In future experiments, it is planned to use cell-permeable peptides for *in vitro* investigations.

Poster: 84

Gas Phase Binding Stability Determination of RNAse S by nanoESI MS Analysis out of Solutions with Different Methanol Contents

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ITEM-TWO enables to determine thermodynamic properties like apparent dissociation constants and Gibbs free binding energies of non-covalent complexes in the gas phase. We studied RNAse S stabilities, i.e. non-covalent protein-peptide interactions, upon electrospraying RNAse S dissolved in 200 mM ammonium acetate which contained increasing amounts of methanol (10 – 40 % w/w). NanoESI MS was carried out on a Q-ToF II mass spectrometer using the quadrupole to prevent transmission of ions in the mass range < m/z 1750. Intact RNAse S passed the quadrupole and was dissociated into S-protein and S-peptide upon collision with Argon gas when ramping up the collision cell voltage difference. The stability of the RNAse S complex decreased with increasing methanol content, consistent with in-solution data.

Poster: 85

Influence of the protein charge state on 213 nm top-down UVPD results

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Tandem mass spectrometry (MS/MS) methods are widely used for proteomics. Ultraviolet photodissociation (UVPD) outperforms other MS/MS methods in terms of sequence coverage and structural information. [1] Sequence coverage can however depend on protein charge and conformation.

Here we systematically investigate the influence of protein charge state on UVPD sequence coverage. Protonated ubiquitin, myoglobin and cytochrome c ions were studied using a home-built 213 nm UVPD setup on a FT-ICR mass spectrometer. Charge state ranges were extended using supercharging reagents.[2]

We found that fragment number and sequence coverage increases with the charge state for all proteins. The change of sequence ion number with the charge state will be presented and implications for the structure dependence of UVPD will be discussed.

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Poster: 86

A Biuret-derived reagent with urea-like MS-cleavability for cross-linking of proteins

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The broad applicability of the MS-cleavable urea group for bioconjugation and chemical cross-linking of proteins resulted in the development of several urea-containing cross-linking reagents. Beside the now commercially available cross-linker disuccinimidyl dibutyric urea (DSBU) and its analogues, the urea group has served as scaffold for the diallyl urea (DAU) cross-linker, the first reagent relying on the thiol-ene click reaction. Here, we present the first results on a novel Biuret-derived, MS-cleavable cross-linker and show its efficient fragmentation behavior with model peptides in CID-MS/MS experiments using an ESI-Orbitrap Fusion mass spectrometer. We envision that the central imide-group might be modified with affinity tags, such as biotin, to allow an enrichment of cross-linked species from highly complex mixtures.

Poster: 87

Evaluation of an Isotope-Labeled MS/MS-Cleavable Cross-Linker for Protein Structure Analysis

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To facilitate the automation of cross-linking/MS experiments we introduce a D_{12}-stable isotope labeled version of the disuccinimidly dibutyric urea (DSBU) cross-linker, combining the advantages of MS/MS-cleavable linkers and isotope-labeling. DSBU-D_0/-D_{12} mixtures were used to cross-link bovine serum albumin, the *E. coli* ribosome, and *Drosophila* embryo extracts. Cross-linked proteins were digested and analyzed by nano-HPLC/nano-ESI-Orbitrap-MS/MS (Orbitrap Fusion and Q-Exactive Plus instruments); cross-links were identified by the MeroX software. Interestingly, D_0 and D_{12} species of DSBU-cross-linked peptides exhibit overlapping elution profiles during LC/MS analysis enabling the mass difference-triggered selection of cross-linked products. During HCD-MS/MS experiments, the characteristic fragmentation pattern of DSBU allows an unambiguous assignment of cross-links. Applying this approach allowed identifying more cross-linking sites in proteins compared to standard data-dependent experiments.

Poster: 88

Characterization of specificity determining positions within the HER2-Herceptin epitope using *in-silico* biocomputational methods

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To precisely elucidate their epitope-paratope interactions is essential for antibodies in clinical use. We applied biocomputational methods to identify “specificity determining positions (SDPs)” within HER2´s Herceptin epitope. Starting from a three-dimensional structure of the immune complex, we first determined that 22 amino acid residues of the extracellular HER2 domain were involved in Herceptin binding. Calculation and comparison of accessible surface areas (ASA) defined the epitope residues. Second, relative binding energy changes were computed upon site-specific exchanges of all amino acid residues of the epitope. Only 8 of the 22 wild type amino acid residues of the epitope (Y532, E558, K569, P571, P572, F573, Q602, and P603) caused significant energy deviations when exchanged and, hence, were considered essential for Herceptin binding.

Poster: 89

Application of PASEF MS/MS scans to monoclonal antibody peptide mapping

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Regulatory requirements when developing a biological drug involve the verification of the primary sequence and in-depth characterization of low level heterogeneities. Here we describe how PASEF scans (parallel accumulation and serial fragmentation) are implemented on a modern IMS-QTOF for peptide mapping with high sensitivity, speed and selectivity.The NISTmAb Reference 8671 was run on a timsTOFpro / Elute UHPLC (Bruker) in 10 min gradients (2-40 %B). PASEF MS/MS spectra were acquired with 100ms mobility frames and 12 precursors per frame. Data was searched in Byonic (Protein Metrics).Full mAb sequence coverage was performed with rapid 10min gradients. Highly distributed modifications such aslysine glycation were detected. Separation of chimeric MS/MS spectra was performed using TIMS as a precursor selection tool.

Poster: 90

Structural characterization of IDP interactions of full-length tumor suppressor p53

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The tumor suppressor p53 plays a crucial role in preventing the development of cancer. To date, establishing structure/function relationships between p53 and its various protein binding partners have not been performed for recombinantly expressed wild-type full-length p53. Especially the *C*-terminal domain (CTD) of full-length p53 is still poorly understood due to its high degree of disorder. The CTD adopts a variety of secondary structures when bound to different proteins causing disorder-to-order transitions. We perform interaction studies with S100ß and sirtuins that both bind to the CTD of p53 using a combination of

chemical cross-linking and mass spectrometry (XLMS). Our results will give structural insights into the conformational changes of p53´s CTD upon target binding.

Poster: 91

PASEF for ultra-sensitive shotgun proteomics

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Time and space focusing of ions focused by their collisional cross section in trapped ion mobility spectrometry (TIMS) boosts the sensitivity of QTOF MS. In addition, TIMS enables parallel accumulation–serial fragmentation (PASEF), which couples high sequencing speed (>120 Hz) at 100% duty cycle without sacrificing spectral quality. We show the combined increase in sensitivity and speed can be used for deep proteome coverage using very low sample amounts.
Sensitivity of the timsTOF Pro instrument with PASEF was evaluated on sample concentrations of ~ 3ng and up to 100ng on column, resulting in the identification of 1,650 up to 5,091 protein families. Tests with four different admixtures of HeLa and E.coli (100ng total amount) with various ratios from 3/2, 3/1, 7/1 and 16/1 showed good reproducibility and sensitivity.

Poster: 92

Structural investigations of highly pathogenic negative strand RNA viruses

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Some highly pathogenic representatives of the negative strand RNA viruses, such as Ebola and Marburg virus (EBOV), Lassa virus (LASV) or Crimean-Congo-haemorrhagic-fever virus (CCHFV) entail an increased risk. Native mass spectrometry gives us dynamic insights into the molecular interactions and mechanisms of the viral life cycle. It is of particular interest to elucidate the assembly mechanism of the EBOV nucleocapsid. Another focus is the investigation of interactions within the replication complexes of Arena- and Bunyaviruses. In this context, we have already investigated essential proteins of the replication machinery of LASV, namely its polymerase, nucleoprotein and the Z-protein. So far, we have also been able to contribute to the structural analysis of CCHFV cap-snatching endonuclease, a part of the viral polymerase.

Poster: 93

Contributions of mass spectrometry to early drug discovery

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Peptide-based therapeutics are an important class of drugs. Due to their properties, they are usually administered by injection and therefore, they should be stable in aqueous formulations. To ensure that development candidates are stable enough to become a marketed drug, the peptides are submitted to forced degradation tests already during early drug discovery stages. While performing a screening, we observed a poorly reproducible degradation product in a family of peptides. We investigated its origin and concluded that one possible cause is the presence of traces of Fe(II) in the solution.

Poster: 94

Highly reproducible and accurate label free quantification using the PASEF method on a TIMS-QTOF mass spectrometer

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The quality of quantification is mainly dependent on three different parameters: performance of the instrument, optimized acquisition methods, and sophisticated data analysis software.
To evaluate the reproducibility of PASEF on the timsTOF Pro mass spectrometer (Bruker Daltonics), we analyzed HeLa digest using a 90min gradient. More than 5300 proteins families were identified in each run. Comparing LFQ intensities between replicates showed excellent reproducibility with a R2 = 0.98. While good reproducibility is a pre-requisite for good quantitative performance, accurate quantification of differentially expressed proteins remains challenging over a wide concentration range and requires a robust analytical platform. For evaluation of accuracy of quantitation we spiked E. coli and yeast digests in different

ratios into HeLa digest and measured each ratio in triplicate analyses. Peptides could be clearly separated by their expected ratios.

Poster: 95

Protein Interactions of the p53 Tumor Suppressor Probed by Chemical Cross-Linking and Mass Spectrometry

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The tumor suppressor p53, also referred to as the “guardian of the genome”, plays a key role in preventing the development of cancer. Despite the broad spectrum of known p53 interaction partners, the identification of p53-binding proteins has never been performed in the context of the full-length p53 on system-wide scale and detailed information on the binding sites is still missing. Our aim is to perform p53 interaction partner analysis with HEK293T cell lysates using a combined affinity enrichment cross-linking/MS approach utilizing the CID-MS/MS-cleavable cross-linker disuccinimidyl dibutyric urea (DSBU). Our studies will offer further insights into the protein interaction networks of p53 and its conformational versatility upon binding to different protein interaction partners.

Poster: 96

Investigation of the secondary structure isoforms of IgG2 type monoclonal antibody using LC-ESI-ToF-MS and orthogonal methods

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Biosimilars are defined as biological medicinal products comparable (but not identical) to their reference market products in quality, safety and bioefficacy. Monoclonal antibodies (mAbs) are subject to complex post-translational modifications which are sensitive to subtle changes in manufacturing processes, e.g. disulfide shuffling, N- and C-terminal protein chain heterogeneity (pyroglutamate formation, lysine clipping), and, therefore, require detailed and reliable analytical control testing.

In the present work, disulfide connectivity of IgG2 monoclonal antibody was accessed via native (non-reducing) LC-ESI-ToF-MS LysC/trypsin peptide mapping. IgG2 type antibody contains 18 disulfide bridges. Depending on the disulfide connectivity between the heavy and the light chains A, B, and A/B isoforms of IgG2 antibodies are known. The disulfide isoforms were investigated in IgG2 monoclonal antibody available on the market and compered to its biosimilar candidates. Disulfide variants were firstly separated via CEX-HPLC, digested under non-reducing conditions and analyzed using LC-ESI-ToF-MS. Relative amount of disulfide isoforms was calculated using EICs of the MS signals of the corresponding peptides. Conventional isoform A was present to 51%, isoform A/B was found to 47%, as well as low amount of B isoform (2%) was observed in the IgG2 market product. Comparable amounts of disulfide isoforms were found in the biosimilar candidates, confirming their biosimilarity regarding to the disulfide connectivity.

Taken together, our MS platform in combination with CEX-HPLC enables straightforward and highly effective assessment of mAb disulfide heterogeneities and assessment of safety and bioefficacy of biosimilar preparations and supports the development of biosimilar mAbs from early research to the final batch-to-batch consistency control.

Poster: 97

Short LC-gradients for high throughput and deep shotgun proteomics using PASEF on a TIMS equipped QTOF

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High sample throughput in proteomics is highly desirable to measure large sample cohorts in a reasonable time. The timsTOF Pro with trapped ion mobility spectrometry offers additional separation power and increased peak capacity. The powerful Parallel Accumulation Serial Fragmentation (PASEF) method for very high sequencing speed is perfectly suited for proteome analysis on short gradients.

We have optimized MS conditions, column lengths and LC overhead times of the nanoElute (Bruker Daltonics) HPLC to obtain runs of 28.8min (50 samples/day). 4200 proteins groups could be identified from 250ng of a proteolytic HeLa digest. HeLa and murine cerebellum digest were fractionated (high pH reversed-phase) into 24 concatenated samples. Subsequent measurement allowed the identification of more than 100.000 unique peptides and more than 9.000 protein groups in less than 12h of measurement time.

Poster: 98

Age-related changes in pea (*Pisum sativum*) root nodule: a proteomics approach

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Legumes represent an important source of food protein for human nutrition and animal feeding. Therefore, sustainable production of legume crops is an issue of global importance. For this, a high efficiency of legume-rhizobial symbiosis is a pre-requisite. Therefore, here we address age-related changes in legume and rhizobial proteomes of pea root nodules in the context of the molecular mechanisms, underlying development, and degradation of these symbiotic structures. Therefore, we considered the models of natural and accelerated aging, i.e. the setup, relying on pea FIX-7 mutants (gene *sym27*). The nodules were harvested after two – seven weeks post-inoculation, the total protein fraction was isolated by phenol extraction, and the resulted digests were analyzed by nanoLC-ESI-Q- and LIT-Orbitrap-MS in a data-dependent acquisition (DDA) mode. Database search was based on the SEQUEST algorithm, whereas quantification relied on the label-free strategy. The protein functions and localization were annotated by in house developed workflow, combined with MapMan, Mercator and LocTree3 tools. The analysis revealed totally 92 nodule proteins (46 of plant and 46 of rhizobial origin), regulated in an age-dependent manner. Thereby, plant proteins (38 species involved in signaling and protein synthesis) were mostly down-regulated, whereas the most of bacterial polypeptides (41 of 46, involved in energy metabolism and nitrogen fixation) were up-regulated. Interestingly, knockout in the gene *sym27* resulted in the suppression of signaling and protein biosynthesis in both partners of the symbiosis. The research was supported by the Russian Foundation for Basic Research (RFBR, project 18-016-00190).

Poster: 99

High Sensitivity Phosphoproteomics using PASEF on a TIMS-QTOF mass spectrometer

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Sensitivity, sequencing speed and peak capacity are prerequisites for deep identification and quantification into the phosphoproteome. Here, we evaluate trapped ion mobility spectrometry (TIMS) in combination with QTOF technology using parallel accumulation-serial fragmentation (PASEF) acquisition mode for deep phosphoproteomic analysis.

Increased peak capacity provided by the TIMS separation, PASEF sequencing speed (> 120 Hz) and sensitivity using low sample amounts enabled a deep phosphoproteome analysis where > 17,400 unique phosphopeptides were identified in a 90 min gradient. TIMS also enables mobility separation of co-eluting positional isomeric phosphopeptides, differing only by phosphorylation localization. Concatenated phosphopeptide enrichment strategies using primary hippocampal neurons demonstrated more than 32,800 unique phosphopeptides identified using PEAKS studio in a single injection.

Poster: 100

Comprehensive characterization of proteome changes accompanying ageing of common bean (Phaseolus vulgaris) root nodules

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Common bean (*P. vulgaris*) is widely cultivated in the world. Thereby, its productivity depends on the efficiency of legume-rhizobial symbiosis. Therefore, changes in protein patterns during ageing of root nodules might be crucial for crop productivity. Therefore, here, we characterize metabolic changes in ageing nodules, addressing their metabolism at the level of plant and bacterial proteomes. To address nodule development, we selected three time points, corresponding to young, mature and aged plants (28, 48 and 53 days). Proteomic analysis relied on LC-based bottom-up strategy. The MS/MS data were performed against a combined legume database comprising *Phaseolus vulgaris, Medicago truncatula* and

Lotus japonicus sequences and rhizobial database (*Rhizobium leguminosarum* bv. *Phaseoli*) using SEQUEST search engine and processed with Progenesis and Perseus software. Proteins in legume nodule were continuously down-regulated throughout the whole nodule ontogenesis. Functional annotation of cluster analysis revealed proteins in nucleotide and protein metabolism as the most affected, although many proteins could not be unambiguously assigned to some function. In agreement with the obtained result, prediction of intracellular localization showed, that most of the proteins were localized in nuclear and cytoplasm. Thereby, the most of the differentially regulated legume nodule proteins were represented by the molecules involved in regulation of translation. Thus, the clear tendency, observed in ageing nodules is decrease of protein biosynthesis and energy production. In contrast, rhizobial proteins mostly demonstrated up-regulation trend. Moreover, the most rhizobial part of the nodule proteins was related to DNA and protein metabolism functions and localized in cytoplasm and plasma membrane.

Poster: 101

A Comprehensive Strategy for Deep Glycomics

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Glycan biosynthesis is affected by disease more significantly than protein, which shows greater potential to develop as biomarker. Therefore, qualitative and quantitative analysis is indispensable for glycomics and mass spectrometry (MS) has been the most powerful analytical tool in this hot field. However, the native glycan has lower ionization efficiency and produces more complex fragments than peptide. To solve those problems, permethylation has been developed as the most efficient derivative approach with improved ionization efficiency and simple fragments in MS analysis. The classical solid-phase permethylation by microcolumns packed with sodium hydroxide powder is commonly used while the operation is complicated. Herein, we further simplify and optimize the solid-phase permethylation by different parameters, such as reaction time and ratio of water and dimethylsulfoxide. Then the optimized solid-phase permehylation workflow is applied on the glycan analysis using nanoLC-MS/MS system. A novel analytical workflow is also designed for glycan identification based on the classical SEQUEST algorithm. In this study, 89 N-glycan species (160 N-glycoforms) from etanercept and 245 N-glycan species (395 N-glycoforms) from acute promyelocytic leukemia cells are identified. This designed workflow is applicative on O-glycan analysis. Label-free quantification is also performed for glycan/glycome analysis with assistant of Skyline and Perseus and we prove that glycan biomarker will be more sensitive than protein. Finally, this study provides a comprehensive strategy for glycan identitification and further biomarker discovery.

Poster: 102

Accelerating DIA Studies to Extend Workflow Utility, Using Fast Microflow LC Gradients

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Background: Microflow LC has been used increasing in quantitative proteomics in combination with SWATH® Acquisition, to provide better robustness and higher throughput when measuring larger sample cohorts. Here, the impact of gradient length on protein quantitation results with DIA was explored.

Methods: Microflow LC was performed on the TripleTOF® 6600 System using the nanoLC™ 425 system plumbed in microflow mode. Trap-elute workflow was used and a range of gradient lengths from 5 – 45 mins was explored, and key acquisition parameters for SWATH® Acquisition were varied to optimize for the much faster run times. Data was processed with SWATH 2.0 microapp in PeakView® Software 2.2 and results were analyzed using the SWATH Replicates Template.

Conclusions: Using complex digested cell lysates, SWATH experiments were performed using gradient lengths ranging from 5-45 mins and protein quantitation results were assessed. Fast MS/MS acquisition rates were found to be critical because this enabled more smaller variable Q1 windows to improve S/N for quantitation. Even with the fastest gradients, methods with 60-100 windows with very fast accumulation times of 15 msec improved results. As expected, total # of protein quantified decreased when shortening the gradient from 45 to 5mins. However, with the 10min gradient, the peptide and protein quantified was ~60 and 70% respectively of results from the 45min gradient for the three matrices tested (on 2 instruments). The 5min gradient method still enabled the quantitation of >1600 proteins and >4000 peptides in a total run time of 18 mins.

Poster: 103

Chiral analysis of hypertrehalosaemic neuropeptides of cicadas

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For more than two decades it has been known that the neurosecretory glands of the cicadas synthesize two

isobaric peptides with hypertrehalosaemic activity. The amino acid sequence is identical (pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-Gly-Asn-NH_2), but some physical parameters like their retention time in RP-LC differ. Previously, it has been shown that the region Pro^6-Ser^7-Trp^8 contained a variable structural feature. The synthetic peptide with d-Pro^6 co-eluted with the more bioactive, more hydrophilic peptide. We have used acid hydrolysis and chiral derivatisation with a variant of Marfey`s reagent to verify racemization. However, the results unequivocally demonstrated the absence of d-Pro. This only leaves cis-trans isomerization at Pro^6 as a possible explanation for the presence of these neuropeptide hormone twins.

Poster: 104

Differential Proteomic Analysis for Validation of Truffle Origin

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Food fraud is becoming an increasingly critical topic. For expensive foods like truffle, incorrect labeling or contamination of premium products with inferior products are common. Truffles are scarcely researched and thus detection of food fraud challenging. Here, different protein extraction methods for proteomic analysis of truffles by bottom-up LC-MS/MS were tested. Then a LC-MS/MS based differential proteomics approach utilizing data independent acquisition (DIA) was developed for determining reference LC-MS/MS data of authentic truffles.

Homogenates from authentic European and Asian truffles were obtained by lyophilization and grinding. The homogenates were incubated with different detergents / chaotropes for protein extraction. Protein extracts were enzymatically degraded by trypsin. Peptides were analyzed by LC-MS/MS in data-dependent acquisition (DDA) mode. The most convenient extraction from ground truffle achieved with sodium deoxycholate (SDC) was then compared to homogenates obtained by picosecond infrared-laser (PIRL) ablation. This showed an at least twofold change in relative amount for 15 % of extracted proteins with no preference towards a certain method. Therefore the SDC extraction from ground truffle was chosen for the differential proteomic approach.

Next, generated peptides were fractionated by high pH reversed phase LC and the fractions analyzed by low pH reversed phase LC-MS/MS in DDA mode to generate a spectral library. For obtaining quantitative reference LC-MS/MS data, measurements in DIA mode for the individual samples were done. By this approach, truffles from Europe and Asia could be distinguished by origin.

This is the first study presenting LC-MS/MS-based data as a starting point for truffle origin validation.

Poster: 105

Fast Microflow Chromatography for Accelerating Protein Identification Experiments

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Background: Proteomics has typically been done using nanoflow LC for sensitivity but long analysis times. With higher flow rates gradients are formed faster, allowing much faster run times to be achieved. When sample run times are important, microflow LC offers a great alternative for higher-throughput proteomics. Here, the impact on protein identification results due to faster gradients was explored.

Methods: Microflow LC was performed on the TripleTOF® 6600 System using the nanoLC™ 425 system plumbed in microflow mode. Trap-elute workflow was used and a range of gradient lengths from 5 – 45 mins was explored, and key acquisition parameters were varied to optimize for the much faster run times. Data was processed with ProteinPilot™ Software 5.0 as well as a prototype version of ProteinPilot running in the Cloud environment.

Conclusions: Using digested cell lysates, gradient lengths ranging from 5-45 mins were compared and protein identification results were assessed. Fast MS/MS acquisition rates were found to be critical for highest peptide IDs; The use of 90 MS/MS per cycle with 15msec accumulation times was found to be optimal. As expected, total # of protein identifications decreased with shorter gradients, however the drop was not as large as expected. Only a small drop was observed when shortening the gradient from 45 to 20mins. With the 10min gradient, the peptide and protein ID rates dropped by about 60 and 75% respectively relative to the 45min gradient for the three matrices tested, but still provided >1500 protein and >8500 peptide IDs.

Poster: 106

High-throughput phenotypic characterization of colorectal cancer tumor tissue with SWATH-MS

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Introduction

Here, we demonstrate high-throughput measurement of protein expression profiles for colorectal cancer tumors (CRC) using SWATH acquisition mass spectrometry.

Methods

FFPE colon tissue samples (95 cancer, 10 healthy) from subjects with colorectal cancer were acquired from commercial biobanks. Peptides for each sample were injected on a Triart C18 column (YMC) coupled to a NanoLC 425 system (SCIEX) using a 43 min gradient at a flow rate of 5 µl/min. The eluted peptides were then analyzed with a TripleTOF® 6600 system (SCIEX) operated in SWATH mode. Data were analyzed in Spectronaut X (Biognosys) with a project specific library. All data were filtered with a 1% FDR on peptide and protein level.

Results

Across all samples, >4,500 protein groups were quantified. ~1,000 proteins were differentially expressed in the cancer cohort. Unsupervised clustering of the data separated healthy and tumor tissues and revealed three main proteomic subtypes within in the cancer cohort (A, B and C) which were largely distinguished by expression of cell adhesion proteins. Hepatocyte nuclear factor 4-alpha (HNF4A) was most significantly overexpressed in subtype B, which correlates with protein signatures from MSI high samples from previous studies (Zhang et al., 2014). Additional analysis of key protein networks related to CRC and MSI high status, as well as analysis of the mismatch repair proteins MSH2 and MSH6 expression, will be presented from this work.

Conclusions

Proteomic profiling of FFPE tissues using SWATH-MS enables the deep phenotypic characterization of tumor tissues.

Poster: 107

Inhibitor-sensitive autophosphorylation sites of *Staphylococcus aureus* S/T kinase detected by target ion mobility mass spectrometry

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Protein kinases have a key role in staphylococcal persistence and virulence. *S. aureus* encodes for one eukaryotic-type S/T kinase, PknB. Inhibiting cellular MAP kinases like MEK1 was shown to result in reduced influenza virus (IV) load. Treatment impact on secondary bacteria was not analyzed yet; thus, the anti-pathogenic action of the MEK-inhibitor ATR-002 on IV and *S. aureus* was assessed. Five phosphorylated threonine sites were detected. The level of phosphorylation of two sites could be lowered by the inhibitor. Reduced phosphorylation at T146 and T230 might lead to a disturbed interaction with other bacterial pathways involved in growth and virulence of *S. aureus.* ATR-002 is promising in acting against IV and *S. aureus* at the same time.

Poster: 108

Phosphoproteome analysis of the near-haploid cell line HAP1 to reveal phosphorylation events originating from PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK)

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Introduction: PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK) is a serine/threonine mitotic kinase that belongs to the mitogen-activating protein kinase kinase (MAPKK) family. It is involved in mitotic cell division through phosphorylation of hundreds of C2H2 zinc finger protein linker sequences resulting in their removal from the condensing chromatin. PBK/TOPK shows only low activity in differentiated cells but is highly active in highly proliferative normal fetal cells and malignant cells. **Aim:** The identification of novel cellular PBK/TOPK substrates and dependent phosphorylation events to identify new targets for restricting malignant cell proliferation and cancer progression. **Methods:** In order to identify PBK-regulated events in the phosphoproteome of wild-type and PBK knockout cells of the near-haploid HAP1 cell line, cells were arrested in mitotic phase by treatment with okadaic acid. Tryptic digests of respective

protein extracts were subjected to proteomic and phosphoproteomic analysis after TiO_2/Ti-IMAC phosphopeptide enrichment. A Synapt G2-S mass spectrometer was employed to analyze the phosphopeptides using both data-independent (DIA) and data-dependent (DDA) acquisition mode. **Results:** Wild-type and PBK knockout HAP1 cells comparison shows 23 differentially regulated phosphorylation sites belonging to 21 proteins. Among them, 16 proteins are DNA- and RNA-binding proteins (DRBPs). Several phosphorylation sites were identified solely in HAP1 wild-type and absent in PBK knockout HAP1 cells. **Conclusion:** These results suggest that PBK phosphorylates directly or indirectly multiple DRBPs in addition to C2H2 zinc finger proteins. Future experiments will use in-vitro kinase proteome phosphorylation and mass spectrometry to distinguish between PBK direct and indirect phosphorylation events.

Poster: 109

Profiling of walnut kernels: a proteomics approach

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Food authenticity is nowadays highly relevant since food fraud of expansive foods is a common problem. Differential proteomics approaches are powerful tools in this regard. Every kind of food needs a tailored sample preparation for proteomic analyses. Especially foods with a high content of lipids like nuts are challenging.

In this study two different sample preparation methods suitable for downstream LC-MS/MS analysis were compared regarding the quantity of the extracted protein amount in relation to the weighed portion of nutmeat and the number of unambiguously identified proteins and peptides respectively. For the 1st approach nutmeat was grinded and defatted with hexane. Proteins were extracted by boiling in a sodium deoxycholate (SDC) containing buffer and then proteolytically digested with trypsin. In the 2nd approach grinded nutmeat was boiled in SDC containing buffer for protein extraction. Proteins were precipitated with acetonitrile (ACN) and then digested with trypsin.

The protein recovery was approx. 38 % higher if the nutmeat was defatted first. Although the ACN precipitation procedure yielded less total protein, it performed superior in terms of the number of identified peptides (695 ACN precipitation/208 defatting) and proteins (226 ACN precipitation/46 defatting) across all replicates. Approximately 3.5 times more peptides and about six times more proteins were identified by the 2nd approach.

The results display using ACN for precipitation of extracted proteins shows advantages over firstly extracting lipids with hexane for walnut sample preparation in proteomics applications, thus leading towards a reliable quantification for generating reference protein profiles as fingerprints for authentic foods.

Poster: 110

Stoichiometric analysis of the mitochondrial glycine decarboxylase multienzyme complex using isotope-labeled concatenated peptides

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The ubiquitous glycine decarboxylase (GDC) consists of four subunits, the P-, T-, L- and H-protein, which form an enzymatic complex splitting glycine into CO_2, NH_3, $NADH_2$, and methylene-tetrahydrofolate, the latter plays an important role in C1 metabolism. Knowledge of subunit stoichiometry of the GDC complex is mandatory for its in-depth functional characterization. The successful isolation of the pure GDC complex has not been reported so far, therefore, we analyzed the stoichiometry of GDC subunits in mitochondria of plant leaves, where the GDC represents up to 30% of matrix proteins. For this purpose, a synthetic protein (QconCAT) comprising a concatenation of peptides from GDC subunits of *Arabidopsis thaliana* and *Pisum sativum* was designed for GDC quantification. The QconCAT encoding gene was expressed in *E. coli.* The stable-isotope-labeled form was obtained by growing cells with $^{15}NH_4Cl$ as sole nitrogen source. Mixtures of mitochondria extracts and purified QconCAT were digested with trypsin and analyzed by data-independent acquisition using nanoULPL (nanoAquity, Waters) on-line coupled to a Synapt G2 mass spectrometer (Waters). The Progenesis QI software was used for MS^1 intensity-based quantification. Stoichiometric ratios of about 1L/4P/4T/10-14H were calculated for Arabidopsis and pea. Comparison of QconCAT-based to label-free Hi3 quantification showed consistent ratios for the P-, T-, and L-proteins. However, substantial differences were found for the small H-proteins (approx. 14 kDa), because the limited number of peptides caused erroneous results if the label-free approach was used.

Poster: 111

Tracing the Proteolytic Cascade of the Contact System by Migration Profiles

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Many signaling pathways are activated when proenzymes are proteolytically cleaved releasing the active enzyme (e.g.auto-activation of Factor XII by contact with negatively charged surfaces, starting pathways of coagulation and inflammation). In this work, we use migration profiles for differential analysis of Factor XII contact activation by dextran sulfate (DXS) and poly-phosphate (polyP) and downstream effects. Plasma was incubated with DXS, PolyP or PBS as sonrol. Migration profiles were obtained for all identified proteins from each sample, allowing a comprehensive study of the activated proteolytic pathways. It's shown that the use of different activators has an impact on the mechanism of FXII activation providing an explanation for the fact that PolyP activates coagulation and activativates of the kallikrein-kininsystem, whereas DXS only activates the kallikrein-kininsystem.

Poster: 112

Rapid identiy assays for mAB development, production control and release

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Fast analysis is required for pharmaceutical development and production to accelerate decisions and reduce costs. We utilized rapid protein digestion methods and integrated MALDI-TOF analysis to compare measurements against reference profiles. Medicinal formulations of antibodies were digested by IdeS (Genovis) for clone selection and run in linear mode MALDI-MS on an autoflex max (Bruker) for Fc-glycan profiling. Antibody identity was confirmed based on the peptide profile similarity while rapid glycan profiling was based on Fc-linked glycans.BioPharma Compass allows the comparison of target profiles with references. Supported applications are screening in early development (clone selection), rapid release ID testing (fill & finish) with run times of 15 min, QC of incoming goods like Tween differentiation and LC-free 2-AB glycan profiling.

Poster: 113

Clinical Diagnostics of Neuronal Ceroid Lipofuscinoses (NCL-1, NCL-2 and NCL-10) using New Substrates for Tandem Mass Spectrometry and Fluorimetry

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Neuronal ceroid lipofuscinoses (NCLs) are a group of neurodegenerative diseases in childhood, signs and symptoms include a combination of psychomotor deterioration, epilepsy, dementia, vision loss and early death of patients. Detecting and correctly diagnosing this disease is of high importance, particularly since enzyme replacement therapy of some NCLs has become available in recent years. Here we report the development and validation of specific assays in clinical diagnostics triplex clinical assay for CLN1 (PPT1), CLN2 (TPP1) and CLN10 (Cathepsin D) by enzyme activity determinations on dried blood spots in NCL patients. The activity was monitored and measured by using specific and highly sensitive tandem mass spectrometry tools with multiple-reaction-monitoring (MS-MRM) in combination with fluorimetric determination. Specific substrates suitable for mass spectrometric as well as fluorimetric assays were synthesized by coupling coumarine- type building blocks with the corresponding peptidyl- substrate moieties recognized by the target enzyme; so that in each a 4(7)alkyl-umbilleforone derivative is formed as the product of the enzymatic reaction to be used for MS-MRM. Suitable peptidyl-substrates for enzymatic determinations were synthesized for different cathepsins (cathepsin- D; -F; -B) in order to provide a differentiation of proteolytic cleavages in enzyme activity determinations. Enzyme determinations obtained by fluorimetry and MS-MRM showed good correlation in single assays. Duplex and triplex assays for CLN1, CLN2 and CLN10 were established by MS-MRM.

Poster: 114

Investigation of post-biopsy degradation processes with laser ablation sampling and differential proteomics

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Removing tissue from a living organism, triggers immediate release of enzymes, leading to degradation of biomolecules, including the proteome. Thus, its composition may change significantly. For focusing on this assumption, in this study the impact of time after removing tissue from a living organism on the proteome was investigated.

Primary tumor formation of a human small cell lung carcinoma (H69AR cell line) was simulated using a xenograft model consisting of 18 mice. Sampling of the tissue was performed in vivo on the anesthetized mouse (T = 0), after tissue removal and subsequent resting for 5 minutes (T = 5) and after resting for 10 minutes (T = 10). Proteome analysis of all 54 samples was performed using Data-independent-acquisition (DIA) LC-MS/MS on an Orbitrap Fusion Tribrid mass spectrometer.

DIA MS revealed 2861 proteins quantified in all of the 54 samples. Relative quantities of the individual proteins were imported into Perseus Software for statistical analysis. Principal component analysis (PCA) showed a significant change in protein composition depending on the different sampling time points. A Student's T-Test (FDR < 0.05) comparing T = 0 with T = 5 identified 85 proteins significantly changed in their amounts, whereas comparing T = 0 with T = 10 identified 210 proteins significantly changed in their amounts.

This study showed that the proteome of tissue is changing quickly after having been removed from the living organism. This aspect should be taken into account to avoid false results in the future.

Poster: 115

Maximized throughput and analytical depth for shotgun proteomics using PASEF on a TIMS equipped QTOF and a novel LC system

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The timsTOF Pro with trapped ion mobility spectrometry offers additional separation power and increased peak capacity and the powerful Parallel Accumulation Serial Fragmentation (PASEF) method provides very high sequencing speed. The Evosep One is an optimized front-end that enables large-cohort experiments by minimizing overhead-time and thereby allows efficient use of short gradients. Here we combine the speed of the timsTOF Pro with high chromatographic turnover rates of the Evosep One to achieve, high depth at high throughput analysis.

More than 1200 protein families and 8000 peptides could be identified by using only a 5.6min gradient. At this sample rate we can analyze 200 injections of 50 ng HeLa and identify at least 1.600.000 peptides and 250.000 proteins per day.

Poster: 116

Multi-attribute monitoring (MAM) to identify differences in Trastuzumab from 2 manufacturers.

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Introduction:

Biosimilars are a growing industry, which requires a comparison of the safety and efficacy of the new biotherapeutic to the original. Certain quality attributes, such as high mannose content glycans, can have a detrimental effect on the safety and efficacy of the product. Using trastuzumab from two different manufacturers, a comparison of the biotherapeutics was performed by acquiring the data using SWATH® acquisition and data was processed using BioPharmaView™ MAM workflow to identify the levels of change in the sample.

Methods:

Trastuzumab from two manufacturers were run as intact and reduced, alkylated and digested forms on an X500B QTOF system using an ExionLC™ AD. Data for peptide mapping and MAM workflow was acquired using SWATH acquisition. All data was processed using BioPharmaView™ MAM and intact workflow.

Results:

The data was processed in BioPharmaView™ with the glycosylations setup. The subsequent glycosylation percentages were calculated and compared against each other for the two manufacturers versions of trastuzumab. Differences in the glycosylations were identified at the peptide level. The same samples were

acquired as intact protein and the levels of glycosylations and the variances were compared to the data acquired in peptide mapping. The result was a confirmation that some levels of glycans differed between the two samples showing how the workflow can be used to monitor for changes in biologic in production or can be used to compare biosimilar to the original biotherapeutic.

Poster: 117

Quantitative LC-MS/MS proteomics of FFPE Medulloblastoma tissue reveals new molecular signatures for different cancer subtypes

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Medulloblastomas are highly malignant pediatric brain tumors, genetically divided into 4 subgroups, WNT, SHH, Group 3 and Group 4. Routinely used clinical biomarkers and target specific therapies for each subtype are not available, making proteomic research indispensable. Large proteomic studies are limited due to the availability of fresh frozen tissue, while formalin fixed paraffin emended (FFPE) tissue sections are available in a higher quantity. In recent years, methods have been developed that allow mass spectrometric analysis of formalin fixed tissue, opening a new avenue for biomarker detection.

Here we use quantitative proteomics in Data-Independent-Acquisition (DIA) mode and Tandem-Mass-Tag 10-Plex (TMT) mode to analyze 42 tryptic FFPE Medulloblastoma samples, representing the 4 subtypes. All measurements were performed on an Orbitrap Fusion™ Tribrid™ Mass Spectrometer.

The DIA analysis of 2 SHH and 2 Group 3 tumors was performed to determine, whether analysis of FFPE tissue sections is feasible to identify molecular profiles in different Medulloblastoma subtypes. In total more than 1900 proteins were quantifiable. A Student's T-Test with a false discovery rate smaller than 0.05 identified 229 proteins that showed significant changes between the two subgroups, indicating that differential proteomic analysis can be applied, despite formalin fixation. The TMT approach was used to profile all 42 FFPE Medulloblastoma samples and led to the identification of distinct molecular profiles, representing aberrant dysregulated molecular pathways within the 4 subgroups. Furthermore, we were able to identify additional subdivisions within the predefined molecular subgroups.

Poster: 118

Sampling of Tissues with Laser Ablation for Proteomics: Comparison of Picosecond Infrared Laser (PIRL) and Microsecond Infrared Laser (MIRL)

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It was recently shown that sampling of tissues with a picosecond infrared laser (PIRL) for analysis with bottom-up proteomics is advantageous compared to mechanical homogenization (Kwiatkowski et al. *Angew. Chemie* 2015, *54* (1), 285–288.). Since the ablation of tissues with PIRL is soft, proteins remain intact and the adjacent tissue is without burning residue. In contrast, it was observed that irradiation of tissues with a microsecond infrared laser (MIRL) is heating the tissue thereby is causing burning residue in the adjacent tissue (Boettcher et al., *Eur. Arch. Oto-Rhino-Laryngology* 2015, *272* (4), 941–948).

In this study we followed the question if sampling of tissues with MIRL for analysis of their proteomes via bottom-up proteomics is possible and how the results are differing from sampling of tissues with PIRL. Comparison of the proteomes of the MIRL- and PIRL-tissue-homogenates showed that the number of identified proteins by bottom-up proteomics was larger in PIRL-homogenates of liver tissue whereas the number of identified proteins was higher in MIRL-homogenates of muscle tissue. In the PIRL-homogenate of renal tissue enzymatic activities were detectable whereas in the corresponding MIRL-homogenate enzymatic activities were absent. In conclusion, MIRL and PIRL are suited for sampling tissues for bottom-up proteomics. If it is important for bottom-up proteomic investigations to inactivate enzymatic activities already in the tissue before its ablation, MIRL-tissue-sampling is an option, because the proteins in the tissues are denatured and inactivated by the heating of the tissue during irradiation with MIRL whereas heating effect is absent during irradiation with PIRL.

Poster: 119

Visualizing chemical defense mechanisms of *Asclepias curassavica* against herbivores using autofocusing MALDI mass spectrometry imaging

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Autofocusing atmospheric-pressure matrix-assisted laser desorption/-ionization mass spectrometry imaging (AP-SMALDI5 AF; TransMIT GmbH, Giessen, Germany) was employed to unravel the spatial distribution of defensive metabolites of *Asclepias curassavica* against herbivores. Upon mechanical wounding (mimicking attacks by herbivorous insects), cardiac glycosides were exclusively detected in damaged leaf tissue, indicating that wounding might increase latex flowrate to the point of damage. While the concentration of cardiac glycosides showed no significant differences between 10 and 300 minutes after wounding, a decrease of the cardenolide content was observed after 24 hours. To confirm the MSI results obtained, the bulk cardenolide content of intact and injured leaf samples was analyzed using HPLC-ESI-HRMS showing that cardenolide concentrations increased 3- to 5 fold.

Poster: 120

Integrating MALDI imaging and ESI metabolomics for broadband identification and validation

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Metabolomics is a huge field with many different technical approaches being used to gain insight about the metabolic processes. Here we describe results from an integrated spatial metabolomics workflow that combines MALDI imaging and ESI-based metabolomics.

Frozen mouse brain was sectioned to 10 µm thickness, mounted onto ITO slides. For infusion 40 µm slices were cut and pieces of rat brain from different regions homogenized and extracted to a constant w/v tissue/solvent. Images and direct infusion experiments were performed using a MRMS solariX 2xR 7T system with a mass resolution of 650.000 at m/z 400.

The combination of direct infusion Electrospray measurements of brain extracts and MALDI imaging gives additional insight into the localization of metabolites in specific tissue regions.

Poster: 121

Comparison of MALDI-MS imaging and quantitative HPLC-FLD of *Pseudomonas* quinolone signal molecules

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Pseudomonas aeruginosa is a major cause of nosocomial bacterial infections. Adaptation of this microorganism to the local environment is orchestrated by Quorum Sensing (QS) molecules, including the prominent Pseudomonas quinolone signal (PQS). “Microbial MALDI-MSI” is an emerging technique that can visualize the lateral distribution of QS molecules across bacterial colonies. However, MALDI-MSI is generally a far cry from being quantitative. Here, we combined MALDI-2-MS imaging and quantitative HPLC-FLD analyses of extracts. We compared the PQS intensities from bacterial colonies grown at different temperatures and harvested at different time points. Although the MALDI and HPLC-derived PQS intensities generally showed a similar trend, a clear linear correlation was so far not found, pointing to the need for further refinements.

Poster: 122

Development and characterisation of novel MALDI-matrices based on polycyclic aromatic hydrocarbons

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Due to their physico-chemical properties (e.g., low polarity and high potential as electron acceptors), polycyclic aromatic hydrocarbons (PAH) constitute an interesting class of alternative MALDI-matrices. Here, we tested eight PAH for their potential for QTOF-MALDI mass spectrometry. Within our study we discovered a number of exciting features for 5/8 of these compounds: For example 9,10-di-2-naphthalenyl-anthracene (ADN) and 9,10-diphenylanthracene (DPA) produced remarkably high sensitivities for MALDI-MS of free fatty acids (FFAs) in the negative ion mode. In the positive ion mode, the nonpolar analyte β-carotene was detected with LODs close to the femtomole range by use of ADN or 9-cyanoanthracene

(9-CA). The MALDI-QTOF-MSI analysis of a cross-section through a carrot highlighted these advantages for ultra-sensitive MALDI-MS imaging.

Poster: 123

Analysis of tattoo pigments in human skin tissue with µXRF and LDI-MS

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Popularity of tattoos has grown worldwide. Around 9 % of the German population was tattooed in 2016. Despite the grown popularity of tattooing, the regulations on tattoo inks are insufficient. Tattoo inks consist of different ingredients, such as different pigments, suspending agents and preservatives. The coloring components are insoluble inorganic and organic pigments. The applied pigments are often developed for car lacquers or printing inks and are not intended for intradermal use. Therefore, a detailed risk assessment with regard to intradermal applications and long-term exposure in the human body is necessary. In some cases, side effects occur weeks or even years after tattooing. The trigger is often unknown and the patients usually have little knowledge which type of tattoo inks or pigments was used. Former studies identified the use of some prohibited pigments, which are listed in the German “Tätowiermittel-Verordnung”.

In this study, different tattoo pigments in human skin tissue after an allergic reaction were investigated with micro X-ray fluorescence (µXRF) and laser desorption ionization-mass spectrometry (LDI-MS). µXRF was used to detect the elemental distribution within the tissue. Especially the distribution of copper, chlorine and titanium in the pigment region was of great interest. Due to this non-destructive method, the same tissue sample was also analyzed by LDI-MS and LDI-tandem MS for structure elucidation. This enabled the identification of compounds on the molecular formula level with information about fragmentation. In correlation with the microscopic image and the allocation of the dye regions, the identification of pigments was achieved.

Poster: 124

Lipid analysis on tissue with improved lateral resolution using nanospray desorption electrospray ionization (nano-DESI)

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Nanospray desorption electrospray ionization is a liquid extraction method combined with electrospray ionization in front of the MS inlet capillary. The highly localized liquid extraction allows for mass spectrometry imaging (MSI) of lipids on a variety of sample surfaces.

The achievable lateral resolution is affected by various parameters, such as capillary diameters and geometry, solvent flow rate and sampling speed. Imaging of sections of porcine liver and mouse brain, as standardized systems, was used for optimization of these parameters. The solvent flow rate and mixture were adjusted for highest signal intensities. Sampling conditions were optimized for different stage speeds. Stage speed and flow rate combinations were optimized for detectability of signals and for minimized spreading along the scan direction. The resulting achievable lateral resolution was in the range of 35-50 µm. Besides the higher lateral resolution, the overall optimization resulted in a strong increase in signal stability. This allows for larger scan areas and extended measurement times. Additionally, the reduced liquid flow rates overcame measurement time restrictions based on the syringe pump setup.

Characteristic phospholipid signals were detected. The resulting ion images show expected analyte distributions, in accordance with microscopic images. The improvements make nano-DESI a highly specific and reproducible method for ambient MSI of larger sample areas with a reasonable resolution.

Poster: 125

MALDI-2-mass spectrometry and multimodal imaging of globotriaosylceramide (Gb3Cer) and further lipids in human colorectal cancer tissue

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The upregulation of globotriaosylceramide (Gb3Cer/CD77), the main receptor for bacterial Shigatoxins (Stx), in colorectal and further gastrointestinal cancers has spurred a high interest of using this glycosphingolipid as target for both diagnostic and therapeutic tumor interventions. Here, we used MALDI imaging combined with laser-induced postionization (MALDI-2-MSI) to simultaneously visualize the distribution of Gb3Cer lipoforms and of further lipids in tissue sections of six patients diagnosed with colorectal cancer. Comparison with H&E stained adjacent tissue sections revealed a tight association of several phospholipids with cancer cells arranged in glandular structures, a finding indicating a high potential

of the method for molecular histology and the analysis of the altered tumor lipidome. Comparison with Gb3Cer-targeted immunohistochemistry moreover revealed an excellent correlation between these two imaging modalities.

Poster: 126

Metabolic changes in the brain of sleep-deprived and rested *Drosophila melanogaster* flies, investigated by high-resolution atmospheric pressure MALDI-MSI

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Sleep is a fundamental and vital process in almost all organisms. We developed analytical methods for detecting metabolic changes linked to sleep-regulatory neurons of the fly brain.

To understand the biochemical mechanisms of homeostatic sleep control, well-rested and sleep deprived *Drosophila melanogaster* flies were investigated. Brains were collected and thin-sectioned. High-resolution matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) was performed using an AP-SMALDI5 AF ion source (TransMIT GmbH, Giessen, Germany) coupled to an orbital trapping mass spectrometer (Q Exactive HF-X, Thermo Fisher Scientific, Bremen, Germany). Metabolic changes were determined and topographically correlated with GFP-marked neurons in the dorsal fan-shaped body of the brain, known to play a central role in sleeping behavior.

Poster: 127

LipidCompass

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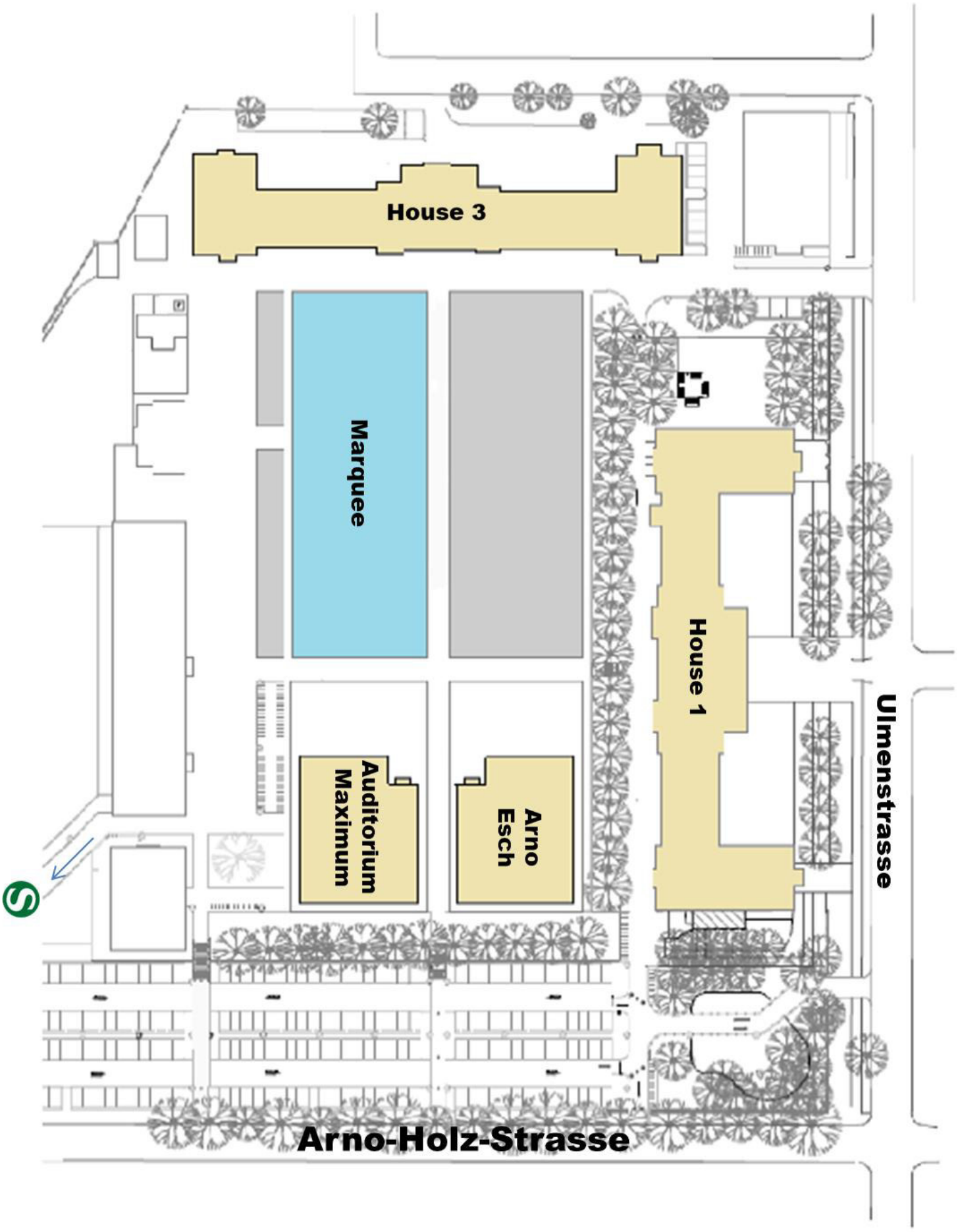
We present LipidCompass, a database that simplifies the exploration of the lipidomic structural space from different angles, following the structural hierarchy, as established by LipidMaps and the proposed extension to further levels based on high-resolution MS technologies, as proposed by Liebisch et al.. LipidCompass also supports the lipid hierarchy taxonomies used by the LipidHome database for theoretical lipids and by SwissLipids.

Lipids in the database have an associated level of confidence according to their identification status, e.g. being mentioned in the literature, having evidence from actual experimental data on the MS/MS level, or even having quantitative data available. Additional taxonomic information on e.g. species and tissue where lipids have been identified are linked in the database for cross-cutting analysis and queries, together with external links to PubChem, Chebi and other relevant resources.

The database provides convenient query and visualization tools to compare qualitative and quantitative lipidomics data within and across experiments. Submission of experimental data is possible using the mzTab-M data format and will be extended to support import of data via MetaboLights. LipidCompass will furthermore be an integration point for multiple lipid-related web services, such as LUX Score and LipidXplorer as part of the Lipidomics informatics for life-science (LIFS) project.

List of Participants

Overview Ulmencampus



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