



31ST MASSSPEC FORUM 2020 VIENNA
25TH – 26TH, FEBRUARY 2020



On behalf of the organizing committee, we are delighted to welcome you in Vienna for the 31st MassSpec Forum Vienna, a conference dedicated to all fields of mass spectrometry. We are confident that you will appreciate the scientific program and hospitality of the city.

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PROGRAM HIGHLIGHTS

REGISTRATION is open from 7:45, in the lobby of TUtheSky, 11th floor.

KEYNOTE SPEAKERS:**Prof. Kevin Pagel***Freie Universität Berlin*

Sugars in the Gas Phase – Novel Techniques to Unravel the Glycocode

TUESDAY, 8:40 – 9:25

TUESDAY, 14:45 – 15:30

Prof. Edwin De Pauw*Université de Liège*

Molecular identification in complex samples using very high mass resolution, Kendrick mass defects and ion mobility, concepts and applications

**Prof. Liam McDonnell***Fondazione Pisana per la Scienza Onlus*

Regio-specific proteomics of the Twitcher mouse model of Krabbe Disease

WEDNESDAY, 8:30 – 9:15

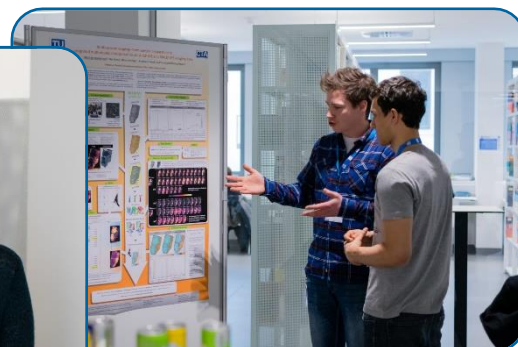
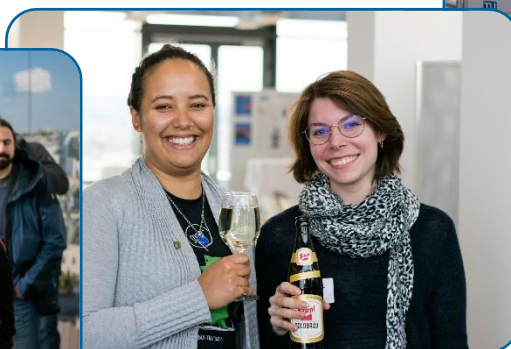
WEDNESDAY, 11:50 – 12:35

Prof. Anneli Krueve*Stockholm University*

Application of non-targeted screening for uncovering the overlooked contaminants in environmental samples

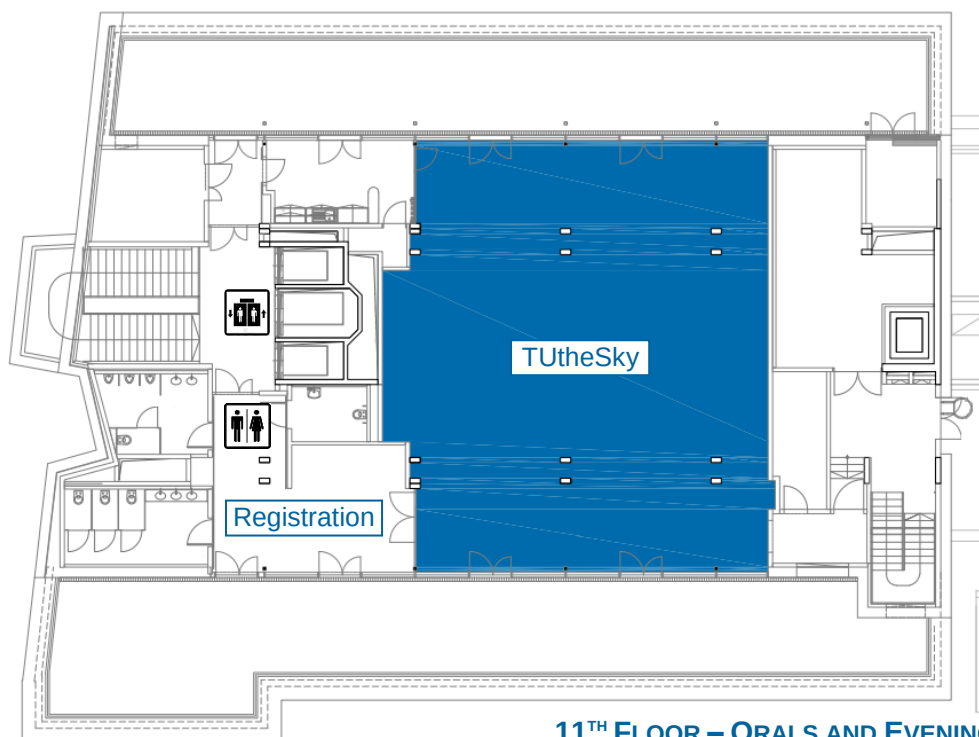


EVENING EVENT, register and join us for dinner buffets and drinks, network and enjoy the amazing panoramic view of Vienna by night. On Tuesday, from 18:45 at TUtheSky.

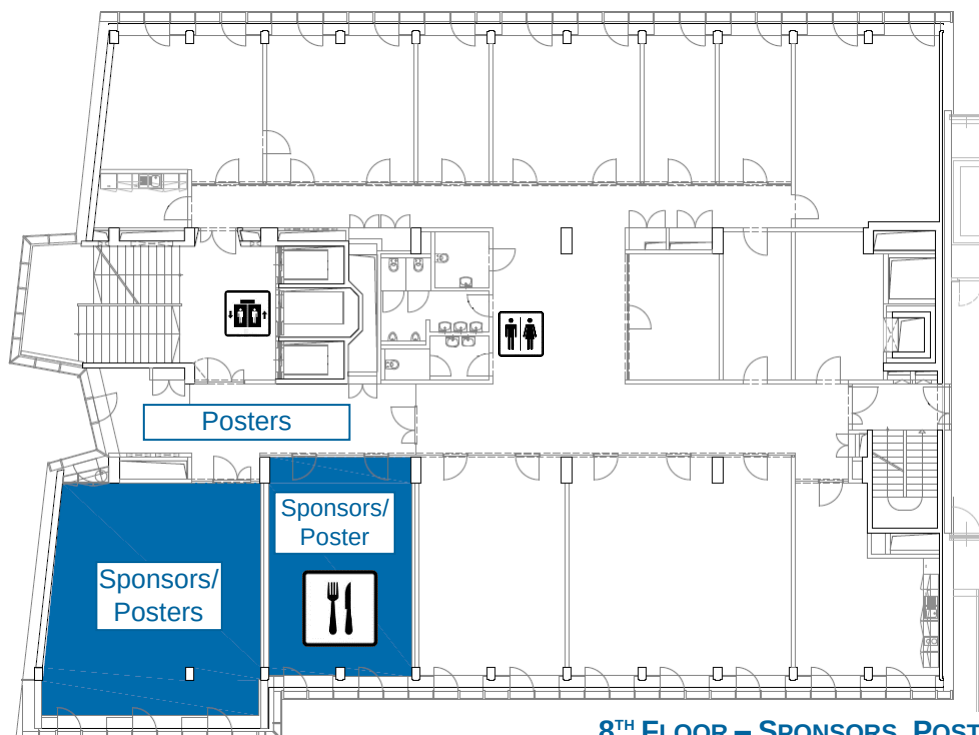




VENUE FLOOR PLANS



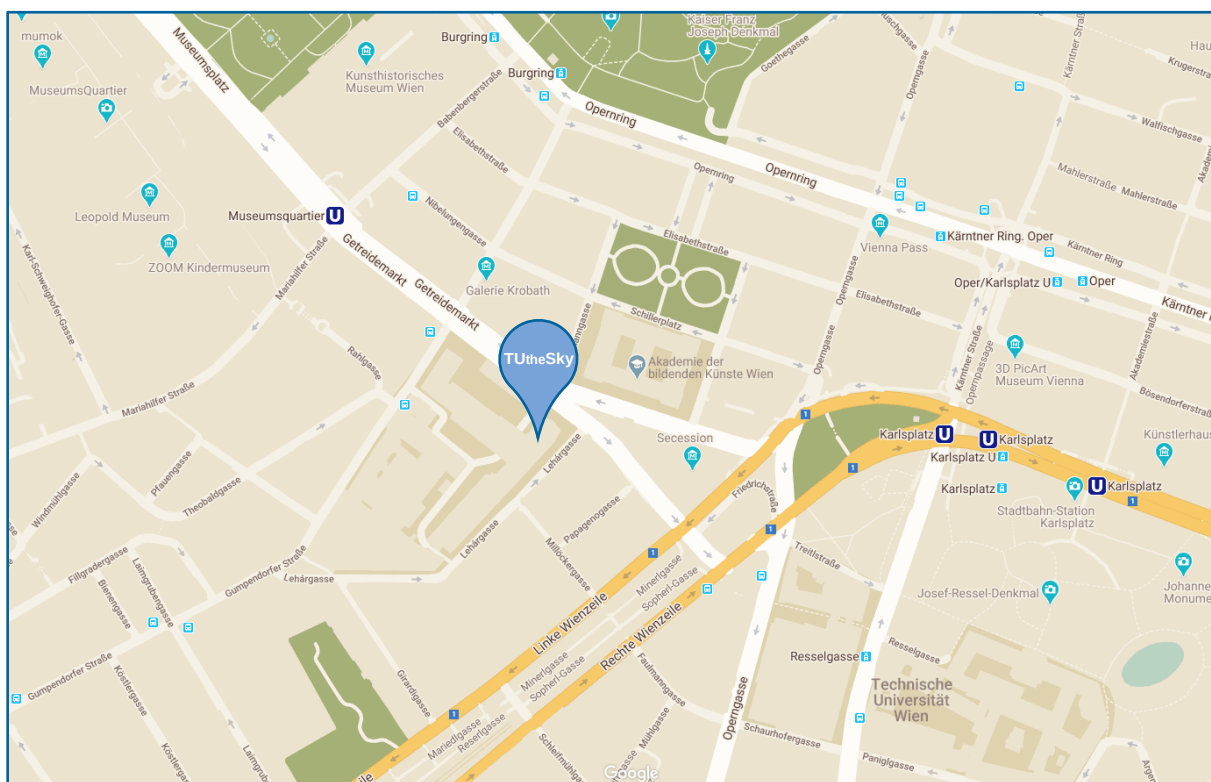
11TH FLOOR – ORALS AND EVENING EVENT



8TH FLOOR – SPONSORS, POSTERS AND COFFEE BREAKS



GENERAL INFORMATION



CONFERENCE REGULATIONS

Please review these policies which are intended to assure the comfort and privacy of all conference participants.

Name badge is required for all conference sessions, including the Poster/Sponsor Hall and at the evening event (registration required).

No smoking is permitted in the convention center.

All devices must be silenced during oral sessions.

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Please note that photographs will be taken throughout the event. These will be used by the organizers for publicity in our publications, or on our website. If you wish to be exempted from this activity, no-photography stickers are available at the registration booth.



PROGRAM OF TUESDAY, 25.02.2020

07:45 - 08:30	REGISTRATION
08:30 - 08:40	WELCOME
SESSION 1	
08:40 - 09:25	Kevin Pagel , <i>Freie Universität Berlin</i> Sugars in the Gas Phase – Novel Techniques to Unravel the Glycocode
09:25 - 09:45	Armin Guntner , <i>Johannes Kepler University Linz</i> Ion mobility collision cross sections as a new descriptor to predict blood-brain barrier permeation by drugs
09:45 - 10:05	Elizabeth Crawford , <i>Thermo Fisher</i> AcquireX...A Deep Dive into the Sea of Metabolic Features
10:05 - 10:25	Max Feuerstein , <i>University of Natural Resources and Life Sciences Vienna</i> Advanced data acquisition and data processing strategies for metabolomics applications using IM-QTOFMS
10:25 - 11:05	COFFEE BREAK
SESSION 2	
11:05 - 11:25	Mirela Sarbu , <i>National Institute for R&D in Electrochemistry and Condensed Matter</i> Ion mobility mass spectrometry for ganglioside biomarker discovery in human cerebrospinal fluid
11:25 - 11:45	Robert Ahrends , <i>University of Vienna</i> LipidCreator: A workbench to probe the lipidomic landscape
11:45 - 12:05	Arnd Ingendoh , <i>Bruker</i> Proteomic profiles of Breast Cancer Tumor Sub-populations defined by MALDI guided SpatialOMx
12:05 - 12:25	Sophie Honeder , <i>Medical University Graz</i> Tumor suppressive function of lipolysis in lung cancer cells
12:25 - 12:45	Giorgia Del Favero , <i>University of Vienna, Service Facility</i> Moving perspectives: bridging multi-imaging to multi-omics
12:45 - 14:45	LUNCH AND POSTER SESSION (even numbers: 12:45 - 13:45, odd numbers: 13:45 - 14:45)
SESSION 3	
14:45 - 15:30	Edwin De Pauw , <i>Université de Liège</i> Molecular identification in complex samples using very high mass resolution, Kendrick mass defects and ion mobility, concepts and applications
15:30 - 15:50	Yasin El Abiead , <i>University of Vienna</i> mzRAPP - a tool for reliability assessment of non-targeted LC-HRMS data pre-processing
15:50 - 16:10	Djuro Josic , <i>Brown University</i> Proteomic analysis of pyridoxal oxime derivatives (POD) treated <i>Listeria monocytogenes</i> reveals downregulation of the main virulence factor, Listeriolysin O
16:10 - 16:25	Marcus Macht , <i>MSVision</i> Modular tools for top down mass spectrometry of intact proteins
16:25 - 17:05	COFFEE BREAK
SESSION 4	
17:05 - 17:25	Sarah Theiner , <i>University of Vienna</i> LA-ICP-TOF-MS imaging as high-throughput tool for metal-based anticancer drug research
17:25 - 17:45	Ann-Christin Niehoff , <i>Shimadzu</i> Mass Spectrometry Imaging – From MS/MS to multimodal approaches
17:45 - 18:05	Anton Skriba , <i>Czech Academy of Sciences</i> Urinal Siderophores on the Rise of Infection Metallomics
18:05 - 18:25	Victor Weiss , <i>TU Wien</i> Determining native virus and virus-like particle molecular weights in the gas-phase – an update
18:45 - 21:00	EVENING EVENT



PROGRAM OF WEDNESDAY, 26.02.2020

SESSION 1	
08:30 - 09:15	Liam McDonnell , <i>Fondazione Pisana per la Scienza Onlus</i> Regio-specific proteomics of the Twitcher mouse model of Krabbe Disease
09:15 - 09:35	Corina Itze-Mayrhofer , <i>University of Veterinary Medicine Vienna</i> Targeted analysis of autophagy-based protein secretion - Insight into the ovarian cancer cell secretome
09:35 - 09:55	Christof Mitterer , <i>Pharma Fluidics NV</i> Development of capillary flow micro pillar array columns (μ PAC TM CapLC): Combining nano flow sensitivity with analytical robustness and throughput for proteomics LCMS
09:55 - 10:15	Benjamin Neuditschko , <i>University of Vienna</i> The challenge of classifying metastatic cell properties by molecular profiling of a melanoma brain metastasis model
10:15 - 10:55	COFFEE BREAK
SESSION 2	
10:55 - 11:10	Tamara Tomin , <i>TU Wien</i> Introduction of Austrian Proteomics and Metabolomics Association (APMA) by APMA Junior Board
11:10 - 11:30	Iva Cobankovic , <i>University of Vienna</i> Overcoming Modafinil Plasma Instability towards neuroPK of Modafinil and CE-123 by LC-HRMS
11:30 - 11:50	Moritz Wagner , <i>Agilent</i> Analytical methods to Address the Pharmaceutical Industry Nitrosamine Crisis
11:55 - 12:35	Anneli Krueve , <i>Stockholm University</i> Application of non-targeted screening for uncovering the overlooked contaminants in environmental samples
12:35 - 13:45	LUNCH AND POSTER SESSION (even numbers: 12:35 - 13:00, odd numbers: 13:00 - 13:30)
SESSION 3	
13:45 - 14:05	Michael Przybylski , <i>Steinbeis Transfer Centre</i> New Approaches for Enzyme Replacement Therapy of Lysosomal Diseases with high Immunogenicity by Mass Spectrometric Identification of Antibody Epitopes
14:05 - 14:25	Jorick Vanbeselaere , <i>University of Natural Resources and Life Sciences Vienna</i> Sulphated and sialylated N-glycans in the echinoderm <i>Holothuria atra</i> reflect its marine habitat and phylogeny
14:25 - 14:45	Goran Mitulovic , <i>Medical University of Vienna</i> Native Peptidomics- An HPLC-MS/MS Approach for Analysis of Tumors' Signature
14:45 - 15:05	Mira Flasch , <i>University of Vienna</i> Unravelling the fate of xenobiotics in human cell culture by stable isotope-assisted metabolomics
15:05 - 15:25	Rutuja Patil , <i>Czech Academy of Sciences</i> On the threshold between <i>Aspergillus fumigatus</i> colonization and proliferation in a host
15:25 - 16:00	CLOSING OF THE EVENT



ABSTRACTS OF ORAL PRESENTATIONS - TUESDAY



Sugars in the Gas Phase – Novel Techniques to Unravel the Glycocode

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Introduction Oligosaccharides or glycans are essential in nature and central participants in virtually every biological process. The extensive structural diversity enables glycans to encode rich information in biological functions; however, it also creates major challenges in almost all aspects of the glycosciences. The structural analysis of glycans is often challenging due to the coexistence of multiple isomers. Also the synthetic formation of glycosidic bonds during glycan assembly is mechanistically still not fully understood. This is largely a result of highly-reactive, but short-lived cationic intermediates, which are difficult to study using established techniques. However, the structure of these intermediates dictates the stereochemistry of the resulting glycosidic bond, the control of which is absolutely crucial for a successful synthesis.

Methods Here we show, how ion mobility-mass spectrometry (IM-MS) and gas-phase IR spectroscopy can be used to unravel the structure, fragmentation pathways and reaction mechanisms of glycans. IM-MS data was collected using a commercially available Waters Synapt G2S instrument. Cold-ion IR spectroscopy was performed using a homebuilt instrument in which molecular ions are encapsulated in superfluidic helium nanodroplets and subsequently irradiated using the Fritz Haber Institute's Free Electron Laser (FEL). Resonant absorption of multiple photons leads to ejection of ions from the droplets. Plotting the ejection yield as a function of the laser wavelength yields an IR spectrum.

Results Proof of principle experiments showed that glycan linkage- and stereoisomers, which are difficult to distinguish using established techniques, can be unambiguously identified using IM- MS.¹ Further analyses revealed that also glycopeptides, which merely differ in the regiochemistry of the attached glycan can be distinguished using fragmentation and subsequent IM-MS analysis. Furthermore, cold ion IR spectra of a series of synthetic standards were recorded. For each of these oligosaccharides, unique and highly diagnostic absorption patterns with well-resolved bands were obtained.² This unprecedented resolution reveals remarkable differences in the overall IR signatures and allows a simple, fingerprint-based discrimination between isomers. Encouraged by these results, we recently used cold-ion IR spectroscopy to study short-lived cationic intermediates that are relevant in sugar synthesis.³ From the obtained fingerprints, high-resolution structures of elusive cationic species were generated, which in turn provide a novel level of mechanistic understanding of sugar chemistry.

Innovative aspects

- IM-MS can distinguish glycan and glycopeptide isomers
- Cold-ion IR spectroscopy provides highly diagnostic fingerprints for glycans
- High-resolution structures of reactive cationic intermediates can be derived

References [1] Hofmann, J.; Hahm, H. S.; Seeberger, P. H.; Pagel, K.; Identification of Carbohydrate Anomers Using Ion Mobility-Mass Spectrometry, *Nature* **2015**, 526, 241-244. [2] Mucha, E.; González Flórez, A. I.; Marianski, M.; Thomas, D. A.; Hoffmann, W.; Struwe, W. B.; Hahm, H. S.; Gewinner, S.; Schöllkopf, W.; Seeberger, P. H.; von Helden, G.; Pagel K.; Glycan Fingerprinting using Cold-Ion Infrared Spectroscopy, *Angewandte Chemie International Edition* **2017**, 56, 11248–11251. [3] Mucha, E.; Marianski, M.; Xu, F.-F.; Thomas, D. A.; Meijer, G.; von Helden, G.; Seeberger, P. H.; Pagel, K.; Unraveling the Structure of Glycosyl Cations via Cold-Ion Infrared Spectroscopy, *Nature Communications* **2018**, 9, 4174.



Ion mobility collision cross sections as a new descriptor to predict blood-brain barrier permeation by drugs

Armin S. Guntner¹, Christian W. Klampfl¹, Wolfgang Buchberger¹

¹ Johannes Kepler University Linz, Institute of Analytical Chemistry, Linz, Austria.

Introduction Constant effort on defeating diseases and new types of cancer leads to a permanently growing knowledge in medicine and a persistent need for novel pharmaceuticals. Especially when treating the human brain, the complexity of this organ and its protecting layer, the blood-brain barrier (BBB), constitute major obstacles that need to be taken into account when brain-active drugs are developed. Considering the big financial expenditure associated with drug design, new methods to evaluate the ability of a drug to permeate the BBB are needed already at an early stage of development. So far existing methods for this purpose do not claim universal validity. Therefore we aimed to implement a new descriptor based on ion mobility mass spectrometry (IMS) experiments to illustrate branching and molecular volume of a molecule - parameters which are known to influence penetration properties.

Methods Within the scope of this study we determined the collision cross sections (CCS) of 46 pharmaceutically active substances (with known penetration properties regarding BBB) in a high throughput dilute-and-shoot approach. In detail, a 1200 Series HPLC instrument operated in a flow-injection mode without a separation column hyphenated to a 6560 Ion-Mobility Quadrupole Time-of-Flight MS (IMS-Q-TOF MS), both from Agilent Technologies (Santa Clara, California), was used. In addition to the instrumental ascertainment of the CCS values we used supporting software-based prediction of this parameter.

Results Using IMS, CCS values were determined for 46 pharmaceutically active substances with known permeation properties. Thereby a distinctive cut-off at around 200 Å was observed, as substances above this limit are lacking sufficient ability for BBB permeation. Besides, software tools were used to predict CCS values, enabling a preliminary statement without the use of costly IMS instruments. Summing up, our study revealed a new promising additional molecular descriptor accompanying lipophilicity, molecular weight and others to assess the cerebral pharmacokinetic properties of pharmaceutically active substances. Considering the threshold region in particular, an additional supporting parameter is beneficial to minimize the number of false positive/negative results and further to judge the suitability of a certain substance for treating diseases of the central nervous system or to avoid possible unwanted side effects there. Keeping in mind the substantial time and cost expenses related to the development of pharmaceuticals and the fact that CCS databases are recently growing, our approach is a new and reasonable step towards time efficiency and effectiveness in medicinal research.

Innovative aspects

- CCS values determined with IMS are a promising new descriptor to predict the BBB penetration ability of a drug.
- The CCS of a substance is the first measurable parameter to quantitatively analyse molecular branching and molecular volume that are known to influence penetration properties.
- Software-based prediction tools can easily support the instrumental determination of CCS.

References Guntner et al. Collision cross sections obtained with ion mobility mass spectrometry as new descriptor to predict blood-brain barrier permeation by drugs, Scientific Reports 9, 19182 (2019)



AcquireX...A Deep Dive into the Sea of Metabolic Features

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² Thermo Fisher Scientific GmbH, Dreieich, Germany.

Introduction Compound identification remains a challenge in untargeted metabolomics. In LC/MS based untargeted metabolomics experiments, detection of thousands of features in a single sample is routinely accomplished. However, this shouldn't be equated to "global" metabolome coverage, as only a small percentage of those metabolites are of biological origin. Data-dependent acquisition (DDA) often provides information for the most abundant ions. Recently developed AcquireX acquisition software can determine on-the-fly features corresponding to background contaminants and compound degeneracy, such as isotopes, adducts, and dimers, enabling more efficient MS/MS and MS_n sampling of unique metabolites. Unlike traditional DDA, where the fragmentation of background ions dominates the duty cycle, AcquireX selects precursors intelligently by excluding background ions and targeting unique metabolites of biological relevance for fragmentation.

Methods

Sample Preparation: Standard Reference Materials SRM1950 (human plasma), SRM3673 (non-smoker's urine), and SRM3225 (green tea extract) were purchased from NIST. Yeast and E. coli cell extracts were purchased from Cambridge Isotope Laboratories, Inc. Metabolites were extracted with methanol at a ratio of 3:1 (methanol:sample). Extracted metabolites were centrifuged and metabolite containing supernatants were evaporated. Dried samples were resuspended in water containing 0.1% formic acid.

Instrumentation: Two microliters of resuspended metabolites were injected on a Hypersil GOLDTM column (15cm x 2.1mm ID). Chromatographic separation was achieved on a VanquishTM UHPLC system coupled with an Orbitrap ID-X Tribrid mass spectrometer using AcquireX intelligent data acquisition software.

Data Analysis: Traditional DDA was compared to the intelligent acquisition approach, AcquireX using Compound Discoverer software.

Results We used samples of varying matrix and complexity, to demonstrate the utility of AcquireX acquisition across several sample types. By excluding background and degenerate signals, the total number of fragmentation targets was reduced without compromising metabolite coverage. By focusing acquisition on biologically relevant compounds, more time could be spent collecting multistage (MS_n) fragmentation data, without affecting experiment length. MS_n provided additional structural information and confidence for compound annotations and, in the case of flavonoids, isomeric compound annotation candidates could be differentiated without the need for additional experiments. Ultimately, AcquireX intelligent acquisition enabled annotation of non-biological and redundant features on-the-fly, resulting in comprehensive MS_n coverage regardless of sample type, complexity, and concentration.

Innovative aspects

- Utilization of the AcquireX DeepScan workflow on the Orbitrap ID X platform has shown to deliver improved MS/MS acquisition with real time generation of exclusion and inclusion lists allowing for the focus to be on acquisition of true sample components.
- Sequential injections show an increased depth of MS/MS fragmentation coverage with the AcquireX acquisitions. Leading to less redundant acquisitions and more on low abundant compound fragmentation.
- Implementing MS_n capabilities, we gain confidence in compound identification of isomeric species with searching the mzCloud spectral library.



Advanced data acquisition and data processing strategies for metabolomics applications using IM-QTOFMS

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² Agilent Technologies Inc., Santa Clara, CA, USA.

Introduction High resolution tandem or two-stage mass spectrometry (MS) coupled to liquid chromatography (LC) separation is the method of choice in non-targeted metabolomics experiments. Technical developments in recent years eased coupling ion mobility (IM) devices to MS. Besides offering an additional dimension of separation, low-field drift tube IM-MS allows rugged and accurate determination of collision cross sections using nitrogen as drift gas ($^{DT}CCS_{N_2}$) and its application as an additional identification marker. Additionally, the used instrument (Agilent 6560) offers the possibility to reestablish precursor-fragment relationships in data independent acquisition (DIA) methods for non-targeted metabolomics based on their arrival times (tA). Together with the accessibility of curated $^{DT}CCS_{N_2}$ databases, this allows increasing confidence in identity confirmation workflows for discovery oriented non-targeted metabolomics.

Methods The implementation of a prototype fast scanning quadrupole driver suitable for Quadrupole Resolved All Ions (QRAI) acquisition using an Agilent 6560 IM-QTOFMS and its application on a stable isotope label (SIL)-supported non-targeted metabolomics workflow are presented. This novel approach allows drift time-directed wideband quadrupole isolation by scanning through the entire m/z space in the time scale of a single ion mobility transient (i.e. <100 ms) with isolation window widths of up to 100 AMU. QRAI acquisition for IM-QTOFMS allows increasing confidence in fragment information for DIA strategies in non-targeted metabolomics. Using metabolic extracts of natural and uniformly ^{13}C ($U^{13}C$) labelled *Pichia Pastoris* relevant to fermentation biotechnology as a model, its applicability for advanced non-targeted metabolomics tasks is illustrated.

Results Recently developed QRAI acquisition increases the selectivity of fragment spectra in high energy frames for DIA using IM-QTOFMS without suffering from substantial losses in signal intensity, mass accuracy, or duty cycle. Additionally, $^{DT}CCS_{N_2}$ accuracy is maintained. In a toggled operation mode, drift time-directed isolation in the high energy frame is combined with full-scan low energy frames and enables increasing selectivity for fragment spectra of a subset of analytes (i.e. singly charged small molecules), while total information in the low energy frame is maintained. Combining the selectivity enhancements offered by chromatographic deconvolution of DIA-fragment spectra with IM-QTOFMS data acquisition strategies maximizes confidence in the quality of fragment spectra in DIA applications for discovery-oriented non-targeted metabolomics. MS-DIAL was used to perform peak picking, chromatographic deconvolution, alignment and identity confirmation by searching fragment spectra against community high resolution MS libraries, e.g. MassBank of North America (MoNA). Additionally, $^{DT}CCS_{N_2}$ were compared to reference values obtained from the CCS Compendium, a curated database of $^{DT}CCS_{N_2}$ values, and number of carbons were confirmed to ensure highest confidence in annotated identities of metabolites in ethanolic cellular extracts of *P. pastoris* from fermentation biotechnology.

Innovative aspects

- Quadrupole Resolved All Ions acquisition (QRAI) for IM-QTOFMS
- SIL-supported workflow for IM-QTOFMS
- Elaboration of new data processing workflows

Acknowledgments Vienna Business Agency and EQ BOKU VIBT GmbH are acknowledged for providing mass spectrometry instrumentation.



Ion mobility mass spectrometry for ganglioside biomarker discovery in human cerebrospinal fluid

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Introduction The proximity of cerebrospinal fluid (CSF) with the brain, its permanent renewal, and better availability for research than tissue biopsies, as well as ganglioside (GG) shedding from brain to CSF, impelled lately the development of protocols for the characterization of these glycoconjugates and discovery of central nervous system biomarkers expressed in CSF. In the present study, we have implemented for the first time ion mobility separation mass spectrometry (IMS MS) for the exploration of human CSF gangliosidome, in order to generate the first robust mass spectral database of CSF gangliosides [1].

Methods The normal lumbar CSFs (21 mL) investigated here were obtained from adult individuals exhibiting no signs of tumors, or acute inflammatory process of the CNS. The extracted GG sample of 5 pmol/μL in methanol was infused into a Synapt G2s and the signal was acquired in the negative ion mode at 1.6kV ESI voltage. To enhance the separation, IMS wave velocity was set at 650 m/s and IMS wave height at 40 V. CID was performed after mobility separation in the transfer cell, using energies between 30-40 eV.

Results IMS MS separation and screening revealed 113 distinct GG species in CSF; of these, more than 76% were found polysialylated, which is similar with the percentage of the polysialylated ganglioside species (over 78%) detected in human brain using same approach. As evidenced by IMS MS, the ganglioside classes found in CSF are GT1>GQ1>GD1>GA3>GD3>GD2=GT2 >GM3>GM2=GT3>GM4>GM1=GT4, given here in descending order, based on their abundances. In comparison with the brain tissue, we have discovered in CSF several components containing fatty acids with odd number of carbon atoms and/or short glycan chains. In addition, IMS MS evidenced the presence of four O-Fuc GGs and five O-Ac GGs. Further, the structural confirmation of GD3(d18:1/18:0) and GD2(d18:1/18:0), exhibiting shorter carbohydrate chain, a feature of CSF, was achieved in the transfer cell by tandem MS. Using variable collision energy within 30-40 eV, a significant number of fragment ions diagnostic for both the glycan core and ceramide moiety of the postulated GD3(d18:1/18:0) and GD2(d18:1/18:0) were generated. Remarkably, the occurrence of a single mobility feature for each fragmented species discloses that only a single structural conformation exists for each case. According to the present results, human CSF and brain show a similar ganglioside pattern, a finding that might be useful in clinical research focused on discovery of ganglioside species associated to neurodegenerative diseases and brain tumors.

Innovative aspects

- Human CSF gangliosidome was explored to generate a mass spectral database of GGs
- Over 100 distinct GG species were detected and identified in CSF
- Human CSF and brain show a similar ganglioside pattern

References [1] Sarbu M, Raab S, Henderson L, Fabris D, Vukelic Z, Clemmer DE, Zamfir AD. Cerebrospinal fluid: Profiling and fragmentation of gangliosides by ion mobility mass spectrometry. *Biochimie* 2020; 170:36-48.

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LipidCreator: A workbench to probe the lipidomic landscape

Bing Peng^{1,2}, Dominik Kopczynski¹, Brian S Pratt³, Christer S Ejsing^{4,5}, Bo Burla⁶, Martin Hermansson⁴, Peter Imre Benke⁷, Sock Hwee Tan^{8,9}, Mark Y. Chan^{8,9,10}, Federico Torta⁷, Dominik Schwudke^{11,12,13}, Sven Meckelmann¹⁴, Cristina Coman^{1,16}, Oliver J. Schmitz¹⁴, Brendan MacLean³, Mailin-Christin Manke¹⁵, Oliver Borst¹⁵, Markus Wenk^{6,7}, Nils Hoffmann¹, Robert Ahrends^{1,16, *}

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Introduction We have developed LipidCreator [1], the first software that fully supports targeted lipidomics assay development. LipidCreator offers a comprehensive framework to compute MS/MS fragment masses for over 60 lipid classes. LipidCreator provides all functionalities needed to define fragments, manage stable isotope labeling, optimize collision energy and generate in silico spectral libraries. The generated assays were computationally and analytically validated and they proved to be an efficient tool for investigating phospholipid-derived mediators to dissect lipid-signaling pathways of human platelets during activation.

Methods LipidCreator is written in the C# language and can be run on both MS Windows (stand-alone or as a Skyline plugin) and on Linux (using the Mono framework). It contains multiple level of structured data to create lipid transition lists. To underline the wide applicability of LipidCreator, and to prove the correctness of the generated transitions, we performed computational, analytical, and biological validation experiments for different use cases. We therefore generated validation-specific assays with LipidCreator, monitored the lipid targets with mass spectrometry, and obtained quantitative results.

Results LipidCreator facilitates the development of customized targeted lipidomics assays considering the entire analytical process by providing all necessary information for its optimization. LipidCreator is well integrated into Skyline for small molecules, which makes it the first vendor-independent software for fast assay development in targeted lipidomics. It can be easily extended and customized due to its platform independence and open source codebase. Method development with LipidCreator is well documented, user-friendly and will not only foster basic research but also has the potential to pave the way for clinical investigations.

Innovative aspects

- First targeted lipidomics pipeline.

References [1] DIO: 10.5281/zenodo.3629321



Proteomic profiles of Breast Cancer Tumor Sub-populations defined by MALDI guided SpatialOMx

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¹ Bruker Daltonik GmbH, Bremen Germany.

² M4i, Univ. Maastricht, Netherlands.

Since tissue and disease are correlated, the SpatialOMx concept provides the unique opportunity to combine region-specific information of MALDI Imaging with deep LC-MS/MS driven proteomic coverage for biomarker discovery and molecular characterization.

Here, we present SpatialOMx for the micro-proteomic characterization of tumor sub-populations in breast cancer. In this workflow, unsupervised segmentation of MALDI Imaging data is used to define ROIs. Image processing strategies provide boundary information of segments of tumor sub-populations for laser capture microdissection. Protein extraction and tryptic digestion of small micro-dissected material is followed by proteomic analysis using PASEF LC-MS/MS.

Fresh-frozen breast tumor sections were cut at 12 µm thickness and mounted on PEN (polyethylene naphthalate) membrane slides. After drying, the slides were coated with Norharmane matrix (7 mg/mL in CHCl₃:MeOH, 2:1, v:v) using a TM-sprayer (HTX Technologies) and measured on a timsTOF flex (Bruker). After defining ROIs from lipid MALDI Imaging analysis in the SCiLS lab software (Bruker), the data was further processed in Matlab. Areas containing approximately 2000 cells were dissected from each segmented tumor sub-population with a Leica LMD 7000 and directly transferred to reaction tubes for micro-extraction and tryptic digestion. Micro-extracted peptide samples were LC-separated and analyzed on the same instrument using PASEF.

SpatialOMx is a novel workflow for in-situ characterization of tissue subtypes. Lipid imaging from tumor sections was used to define ROIs by segmenting intra-tumor heterogeneity for laser microdissection (LMD) based micro-proteomics. This allows for an in-depth proteomic analysis of small microdissected tissue pieces. In average, about 3500 protein IDs were obtained from the three microextractions to provide a deep insight into the proteome from just approximately 160 µg sample material. Interestingly, the proteomic profiles of the tumor sub-population differed largely. The gene ontology terms “Biological regulation” and “Developmental processes” were under-represented in sub-populations 1 and 2, respectively, while “cellular component organization” was over-represented in tumor sub-population 3.

In summary, SpatialOMx provides guidance to specific regions of interest for microextraction and in-depth proteomics analysis to characterize region-specific molecular changes in very detail.

Innovative aspects

- Combination of MALDI imaging and nanoLC/MS/MS on a single platform
- In-situ characterization of tissue subtypes by selection via imaging
- High-sensitivity identification of proteins from a limited number of selected cells



Tumor suppressive function of lipolysis in lung cancer cells

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Introduction Adipose Triglyceride Lipase (ATGL) catalyzes the initial step of triacylglycerol breakdown into glycerol and free fatty acids in adipocytes but is expressed in most tissues. The gene encoding ATGL is lost in many human tumors, with high deletion frequency in lung cancer. Moreover, ATGL (-/-) mice develop lung neoplasia.

Methods To elucidate possible tumor suppressive cellular functions of ATGL we deleted and overexpressed ATGL in A549 lung carcinoma cells and analysed their phenotype by 3D cell culture, proteomics, lipidomics, imaging and biochemical assays.

Results This led as expected to triacylglycerol accumulation in lipid droplets but surprisingly also to higher levels of cellular phospholipid and bioactive lipid species (lyso- and ether- phospholipids), preferentially containing arachidonic acid. Proteomics revealed elevated expression of the pro-oncogene SRC kinase in ATGL depleted cells, which was also found on mRNA and phosphorylated (active) SRC (Y416) protein level. Cells depleted of ATGL migrated faster, which was dependent on SRC kinase activity and reversible by lentiviral reintroduction of ATGL. Pharmacological inhibition of ATGL in LLC1/LL2 Lewis lung carcinoma cells reproduced the observed phenotype of SRC activation and increased migratory potential suggesting a general link between loss of ATGL activity, accumulation of lipids, activation of SRC and increased migration [1]. To identify lipid metabolic enzymes which may be regulated upon loss of ATGL we performed activity-based and quantitative proteomics of ATGL-KO compared to ATGL overexpressing lung cancer cells and identified Lysophospholipid Acyltransferase LPCAT3 to be upregulated in ATGL-KO, which is known to incorporate arachidonic acid into phospholipids. Interestingly, arachidonic acid can activate SRC in a dose dependent manner. Thus, elevated free arachidonic acid levels may be responsible for activation of SRC. In line with this hypothesis, enzymes involved in very long chain fatty acid desaturation were found to be upregulated while fatty acid oxidation was downregulated in ATGL-KO spheroids which grew much faster than control spheroids. Moreover, glucose transporters and enzymes involved in glycolysis were upregulated and cells took up more glucose.

Innovative aspects

- Loss of lipolysis enhances oncogenic signalling via Src kinase promoting faster cell migration in lung cancer cells.
- Src kinase activation may be due to altered fatty acid signalling when lipolysis is lost.
Loss of lipolysis may lead to a metabolic switch affecting fatty acid and glucose metabolism supporting faster tumor growth.

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Moving perspectives: bridging multi-imaging to multi-omics

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Introduction Technological and scientific advances often occur at the interface between disciplines. In line, in order to cope with the complexity of the questions posed by modern science increasing effort is needed to approach problems from different angle and complementary methods.

Methods Imaging and microscopy-based techniques offer unique complementary capabilities with proteomics-metabolomics approaches. In particular, fluorescence based-live cell imaging allows to monitor and describe intracellular motility, organelle distribution and activation at resolution in the nm range.

Results Depiction of cell heterogeneity in controlled cell culture condition, especially in association to pathophysiological changes, is often a challenging task. Almost as challenging is the description of common molecular mechanisms describing disease progression. [1] In line, cellular changes can result in morphological changes and associated functional status. As example, mitochondria metabolic status is tightly connected to morphometric features with constant equilibrium maintained between fusion and fission. This can also be accompanied by movement within the cells in relation to functional status with broader distribution or perinuclear accumulation. [2,3] Similarly, autophagy activation or endoplasmic reticulum (ER) stress response can be followed by the morphological adaptation of the respective organelles, as well as with the respective proteome profile. [4] However, live cell imaging and image analysis experiments are often limited by the number of cells observable for every experimental condition and a certain bias in the selection of the optical fields has to be considered. On the other side, proteomics and metabolomics offer unbiased data-sets over a considerably broader sample size distribution, albeit being limited to description of “static” conditions and relatively limited spatial resolution. In conclusion, cross validation and combination among the disciplines allows novel opportunities to ease data interpretation and deepen comprehension of complex biological processes.

Innovative aspects

- Cross validation between morphological changes and proteome-metabolome profiling
- Complementary data interpretation supported by multimodal image analysis

References [1] Neuditschko, B. et al. The challenge of classifying metastatic cell properties by molecular profiling exemplified with cutaneous melanoma cells and their cerebral metastasis from patient derived mouse xenografts. *Molecular & cellular proteomics: MCP*, doi:10.1074/mcp.RA119.001886 (2019). [2] Del Favero, G., Hohenbichler, J., Mayer, R. M., Rychlik, M. & Marko, D. Mycotoxin Altertoxin II Induces Lipid Peroxidation Connecting Mitochondrial Stress Response to NF-kappaB Inhibition in THP-1 Macrophages. *Chemical research in toxicology*, doi:10.1021/acs.chemrestox.9b00378 (2020). [3] Gerner, M. C. et al. Proteome Analysis Reveals Distinct Mitochondrial Functions Linked to Interferon Response Patterns in Activated CD4+ and CD8+ T Cells. *Frontiers in Pharmacology* 10, doi:10.3389/fphar.2019.00727 (2019). [4] Del Favero, G. et al. Deoxynivalenol induces structural alterations in epidermoid carcinoma cells A431 and impairs the response to biomechanical stimulation. *Scientific reports* 8, 11351, doi:10.1038/s41598-018-29728-5 (2018).

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Molecular identification in complex samples using very high mass resolution, Kendrick mass defects and ion mobility, concepts and applications

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Mass spectrometry is a unique method in molecular Sciences. It can handle all types of samples, providing separation, structural analysis, identification and quantification. It is usually coupled with upfront separation methods. Intense efforts were made to alleviate sample preparation and separation requirements to speed up analysis and keep trace of the maximum of information for potential reexamination of data. Thanks to technical developments MS can be now seen as a technique for standalone rapid analysis of complex samples either in screening or targeted analysis.

The access to high-resolution and accuracy mass spectrometer is increasing. The introduction of ion mobility with improved separation is also widely spreading. Together, they allow rejuvenating historical methods as the Kendrick mass scale and rough parallel separation methods such as Thin Layer Chromatography.

Mass Spectrometry Imaging has been extensively developed for the molecular imaging of histological sections. Non-medical applications are also growing. Rapid imagers have a direct consequence: the handling of the large amount of data produced is the bottleneck. Preliminary examples of integration of high resolution Kendrick filtering and mobility data in MSI analysis in both medical (histological sections) and non-medical applications such as the analysis of host-pathogens interactions and TLC plate for food safety will be presented making both target and untargeted analysis available.

References Anal. Chem. 2019, 91, 20, 13112-13118

**mzRAPP – a tool for reliability assessment of non-targeted LC-HRMS data pre-processing**

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Introduction Today liquid chromatography-high resolution mass spectrometry (LC-HRMS) states the most comprehensive technology for non-targeted (NT) analysis of biomolecules. This implies a huge amount of data which has to be dealt with during downstream analysis. There is a huge variety of open-source and commercial software tools enabling NT data pre-processing (NPP) and further downstream data analysis in NT metabolomics. However, those tools are widely considered as black boxes, and their outputs are therefore often viewed with skepticism. Here we present mzRAPP an R-based tool for reliability assessment of NPP via automated generation and application of benchmark datasets (BM).

Methods mzRAPP was developed in R and ease of use was ensured via shiny interface. Metabolomics datasets from different LC-HRMS platforms (each with retention time information on at least 50 compounds (target compounds)) were submitted to mzRAPP. Thereby all visible isotopologues of target compounds were automatically extracted from mzML files for generation of BMs. This fully automatized procedure involved multiple steps including region of interest extraction and parameter-free peak shape-insensitive peak picking. The quality of picked peaks was evaluated considering the predicted isotopic patterns of respective target compounds. mzRAPPs infrastructure was then used to utilize BMs for reliability assessment of NPP done by different tool such as XCMS, mzMine2, MS-DIAL and Compound Discoverer.

Results mzRAPP aims at enabling routine NPP-performance checks via automatically from mzML-files generated BMs. This performance is addressed with regard to number and nature of recovered peaks as well as reliability of reported abundance values, sample alignment and gap filling process. For this purpose more than 10 LC-HRMS datasets acquired using different types of LC (reversed phase and hydrophilic interaction) as well as HRMS including time of flight and orbitrap instruments were used to generate BMs via mzRAPP. Resulting BMs included peaks with abundances ranging over more than five orders of magnitude enabling performance evaluations for peaks with different intensities. After generating BMs for all collected datasets (2000 – 15000 BM peaks each) different AGs as implemented in XCMS, MS-DIAL, mzMine2 and Compound Discoverer were used to generate NPP outputs from the same datasets. Applying mzRAPP it was obvious that optimal NPP-parameters, cannot be generalized, but are highly specific to the acquired data. Although this can be intuitive for some NPP-parameters like setting a minimum and maximum peak width it might be less so for choosing the correct smoothing level or retention time/mz weighting during sample alignment. Since mzRAPP is returning performance measures for peak detection, alignment and final output individually problematic NPP-steps and thereby parameters can be identified.

Innovative aspects

- An approach to automatically generate and validate benchmark datasets from LC-HRMS datasets
- Introduction of a strategy to assess the reliability of non-targeted data pre-processing in LC-HRMS
- Presentation of mzRAPP, a tool enabling those performance checks on a routine basis

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Proteomic analysis of pyridoxal oxime derivates (POD) treated *Listeria monocytogenes* reveals downregulation of the main virulence factor, Listeriolysin O

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A proteomic analysis of food borne pathogen *Listeria monocytogenes* after treatment with three disinfectants based on ammonium salts of pyridinium oxime was performed. Changes of the proteome of this Gram-positive bacterium give a view in perturbation of cellular processes under stress conditions caused by the influence of investigated antibacterial agents. It was demonstrated that the main bacterial toxin Listeriolysin O (LLO) is down regulated after treatment with each of three investigated inhibitors [1, 2]. These inhibitors caused an additional disturbance in the synthesis of plasma membrane proteins and cell wall proteoglycans. These investigations confirm already postulated mechanism of action of POD-based inhibitors that results in disturbance of key cell surface proteins and proteoglycans in Gram-positive bacteria. Additionally, the use of some proteins such as LLO, as potential biomarker candidates of food poisoning with this bacterium is discussed, and recent methods for their fast detection are presented.

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Modular tools for top down mass spectrometry of intact proteins

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Introduction Over the past couple of years top-down mass spectrometry has become a vital tool for the study of protein quaternary structures and modifications, in particular in biopharmaceutical industry [1,2]. Commonly these systems are complex and consequently expensive FT based high resolution MS systems due to the required mass resolution. The presentation will describe how this can be set up in a modular, scalable way at moderate costs.

Methods We present a setup which comprises out of several modules from us and/or collaboration partners which assemble into systems suitable for top down mass spectrometry. The modules consist out of (modified) mass spec systems, data processing tools and dedicated fragmentation devices. Additionally, a (not described) sample preparation system based on cIEF is available.

Results The combination of various tools allows to create a dedicated top-down analysis capable system in a dedicated way. In particular, this is also possible for older systems already in the lab. These can either be Orbitrap-based FT-systems – or with better capability for large noncovalent protein complexes but some restrictions in resolution – QTOF-based systems.

We will show which components assemble into the final systems and show examples of results obtained from both types of systems. All components of the system are individually available and will be supported by MS Vision.

The upgrade possibility of older systems opens up a possibility to dedicate older systems for a specific application, which might otherwise go out of operation will still being valuable instruments.

Innovative aspects

- A system enabling the design of a top-down mass spec system in a modular way

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Acknowledgements Joe Beckman, eMSion, Corvallis, OR, USA Friedel Drepper, Bettina Warscheid, Universität Freiburg Yuri Tsybin, SpectroSwiss, Lausanne, Switzerland



LA-ICP-TOF-MS imaging as high-throughput tool for metal-based anticancer drug research

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Introduction Recent advancements in the development of low-dispersion ablation cells have significantly improved throughput and sensitivity for high-resolution laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) imaging [1]. The scope of LA-ICP-MS applications was hereby expanded to single cell analysis and (sub-)cellular imaging. Quantitative assessment of sample heterogeneity is hampered by accurate and precise quantification due to sensitivity drift, elemental fractionation, matrix effects and the lack of available matrix- matched reference materials. Therefore, matrix-matched standards are typically prepared based on homogenized tissue/gelatin sections or droplet-based approaches.

Methods A low dispersion high-throughput LA-ICP-MS ablation system, consisting of the COBALT ablation chamber (Teledyne Photon Machines, MT, US) and the ARIS laminar flow aerosol transport system was used. This setup provides single pulse responses of less than 5 ms and pixel acquisition rates of > 200 pixels s⁻¹. The combination with an ICP-TOF-MS instrument (icpTOF 2R, TOFWERK AG, Thun Switzerland) permits visualization of the distribution of endogenous trace elements with biological key functions and exogenously introduced metals (e.g. metallodrugs or metal-conjugated antibodies) on single cell level.

Results Calibration standards were developed based on gelatin micro-droplets, which were generated by a micro-droplet spotter with high accuracy and with high-throughput. Linear calibration curves were obtained for a variety of elements with biological key functions using LA-ICP-TOF- MS detection. The calibration approach was applied for multi-element quantification in 3D multicellular tumor spheroids after treatment with clinically used Pt(II)-based drugs. Quantitative platinum accumulation was assessed in parallel to the distribution of endogenously present elements (Mg, P, Cu, Zn etc.) in the different compartments of the tumor spheroids with a pixel size of around 2.5 µm. In a second example, single cell analysis by LA-ICP-TOF-MS was performed for multi-element imaging of blood cells of a patient undergoing cisplatin chemotherapy [2]. Blood cells were differentiated based on their elemental composition without the need for cell fixation or labelling approaches. Platinum accumulation was assessed in different blood cells and the plasma fraction.

Innovative aspects

- Automated, fast and reproducible standard production for bioimaging by LA-ICP-MS
- High-throughput multi-element analysis in biological samples by LA-ICP-TOF-MS
- Label-free single cell imaging by LA-ICP-TOF-MS

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Mass Spectrometry Imaging – From MS/MS to multimodal approaches

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Introduction Mass spectrometry imaging (MSI) has developed as a powerful tool for spatially resolved elemental or molecular analysis. The application of MSI in biological, pharmaceutical and medical sciences, is developing to an important strategy due to the high sensitivity and spatial resolution as its major advantages. Here, we present two studies; first one focussing on the analysis of human tissue samples showing inflammatory bowel diseases, such as Crohn's disease (CD) using MALDI-MS(/MS), second one on the multimodal analysis of tumor spheroids after incubation with a photosensitizer using MALDI-MS and LA-ICP-MS.

Methods From respective samples cryo-sections were prepared using gelatin or cellulose based embedding media to avoid ion suppression for MALDI-MSI. Samples for MALDI-MSI were mounted on ITO slides. Measurements were performed using either a AP-MALDI-IT-TOF or a MALDI-TOF/TOF instrument. Elemental imaging experiments were performed with a 213 nm Nd:YAG laser, which was coupled to a single quadrupole based ICP-MS system by means of a transfer line. For quantification, standards based on gelatin were prepared containing different concentrations of the respective element. For molecular imaging experiments, CHCA, or DAN were applied via sublimation and recrystallization to detect lipids and drugs in positive or negative ion mode.

Results CD: Different lipids were detected in negative ion mode. The lamina propria, crypts and muscularis mucosae tissues are clearly distinguishable via different lipids. Statistical analysis was performed on multiple samples comparing infected and control samples. A preliminary analysis suggests that the largest changes in lipid expression related to Crohn's are produced in the lamina propria. Among the species that experience the largest changes, PI(38:4), m/z 885.550, seems to be a relevant candidate. Structural analysis was performed via high-energy CID to identify chains and double bonds, to identify the lipid. Tumor spheroids: Spheroidal cell cultures were used as a model system for malignant tissues and were incubated with a photosensitizer. To visualize the drug by means of ICP-MS, a Pd- tagged photosensitizer was used. The enrichment of the drug occurs within the first cell layers of the spheroid. Different phospholipids were used as marker for zones in the spheroid and verified by MS/MS analysis.

Innovative aspects

Multimodal imaging, structural identification, statistical analysis.



Urinal Siderophores on the Rise of Infection Metallomics

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Introduction The detection and quantitation of siderophores in urine represents an innovative early indication of pathogenic microorganisms in a host [1]. In this presentation we document why the general application of siderophores in invasive infectious diagnostics will soon represent the same clinical breakthrough like ribosomal protein typing 12 years ago. In addition to handling coinfections or polymicrobial frenemies, with mass spectrometry we can monitor the antimicrobial drug metabolism and microbial resistance in a single analysis.

Methods A database of 700 microbial siderophores was used for screening patient's status with invasive infections caused by genera *Aspergillus*, *Mycobacterium*, *Pseudomonas*, and various *Candida* and *Zygomycetes*. Human urine samples were examined by liquid chromatography and Fourier-transform ion cyclotron resonance (FTICR) mass spectrometer. The biomarkers were quantified against matrix-matched standards with ferrioxamine E as internal standard, and our in-house tool called CycloBranch (<https://ms.biomed.cas.cz/cyclobranch>) was used for compound dereplication.

Results For the diagnosis of aspergillosis we used an array of ferri-triacetylfulvarinine C (Fe-TAFC)/ferriferrocrocine (Fe-FC)/gliotoxin (GTX) in a cohort of 36 patients (13 positive and 23 negative controls with other infectious diseases). LOD for TAFC in human urine was 0.3 ng/mL. TAFC exceeding 10 ng/mL could be lethal, max experienced concentration was 1.6 µg/mL. In the working dataset of critically ill, mostly non-neutropenic humans, the TAFC/FC/GTX sensitivity in urine was 100% compared to standard GM in serum (55%). The kinetic profiles upon voriconazole & posaconazole treatment showed a quick drop in siderophore production in vivo. In the same cohort, ferri-pyoverdines and pyochelin were detected as urinal biomarkers in a patient with *Pseudomonas aeruginosa*, which is a Gram-negative bacterium. The concentration of PVD E, D, and C varied between 8- 92 ng/mL. Less stronger siderophore, pyochelin, was detected at 18 ng/mL. In addition to clinical data, we will show the performance of microbiologydedicated tool CycloBranch in our presentation.

Innovative aspects

- Urinal samples are analytically attractive due to less chemical background compared to serum and clinically interesting due to non-invasive application.
- Optimized sample preparation could soon move siderophore analysis from FTICR equipment to standard MALDI-Biotyper-type labs.
- Meet Infection Metallomics as an emerging standard representing the next generation diagnostics standards

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Determining native virus and virus-like particle molecular weights in the gas-phase – an update

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Introduction Nano Electrospray Gas-phase Electrophoretic Mobility Molecular Analysis (nES GEMMA) [1] separates single-charged nanoparticles in the gas-phase according to their electrophoretic mobility diameter (EMD) after a nES process followed by surface drying of analytes and charge equilibration. Relating EMD values of standards to their respective molecular weight (MW) enables MW determination of analytes based on their EMD as shown for the first time by Bacher et al. [2] for proteins. However, also for viruses [3] and virus-like particles (VLPs) [4] very promising results are obtained, if corresponding EMD / MW correlations are applied.

Methods Gas-phase electrophoresis was carried out on a nES GEMMA instrument (TSI Inc, Shoreview, MN, USA) consisting of a nES aerosol generator (model 3480) equipped with a 210Po α -particle source, a nano Differential Mobility Analyzer (nDMA, model 3080) and a n-butanol-based ultrafine condensation particle counter (CPC, model 3025A). Analytes were electrosprayed from ammonium acetate under conditions yielding a stable Taylor cone for the nES.

Results Correlation of a (bio-)nanoparticle's EMD to its molecular weight (MW) is possible within an analyte class enabling MW determination based on gas-phase electrophoresis data. Turning our attention on icosahedral, non-enveloped viruses and virus-like particles (VLPs), the latter being proteinaceous shells encapsulating an aqueous interior applied for shielded cargo transport or for vaccination, we set up corresponding EMD / MW correlations. Significant differences between correlations for proteins, viruses and VLPs were observed. EMD based calculation of a particle MW represents an interesting alternative to native ESI MS. Both methods relate bionanoparticle MW values but differ significantly in (i) sample purity and analyte homogeneity requirements, (ii) ease of instrument handling, (iii) costs of instrumentation and (iv) accuracy of obtained MW values. Therefore, both methods – gasphase electrophoresis as well as native ESI MS – can be regarded as being complementary.

Innovative aspects

- MW determination of viruses and VLPs based on gas-phase electrophoresis data
- Comparison of nES GEMMA derived MW values to calculated values based on virus and VLP building blocks as well as native ESI MS data

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ABSTRACTS OF ORAL PRESENTATIONS - WEDNESDAY



Regio-specific proteomics of the Twitcher mouse model of Krabbe Disease

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Introduction Krabbe disease is a rare, childhood lysosomal storage disorder caused by a deficiency of galactosylceramide beta-galactosidase (GALC). The major effect of GALC deficiency is the accumulation of psychosine in the nervous system and widespread degeneration of oligodendrocytes and Schwann cells, causing rapid demyelination. The molecular mechanisms of Krabbe disease are not yet fully elucidated and a definite cure is still missing. Here we report the first in-depth characterization of the Twitcher mouse, a spontaneous mouse model of Krabbe disease, to investigate changes in the Central and Peripheral Nervous System.

Methods A quantitative microproteomics workflow for the analysis of small tissue samples was developed for regio-specific tissue analysis based on single-pot, solid-phase enhanced sample preparation. The conditions for in-solution and on-column tandem mass tags (TMT) labeling, for accurate relative quantification, were first optimized. An automated high-pH fractionation protocol for low sample amounts was then developed for TMT multiplex samples: fractionation of 6x1 µg of protein doubled the number of unique peptides and increased proteome coverage 1.43 fold compared to a single long gradient experiment. These capabilities were then exploited for the region specific analysis of the Twitcher mice.

Results Laser capture microdissection was performed to isolate small, 1mm², regions of the corpus callosum and motor cortex, and the sciatic nerves isolated from Twitcher mice (N=5) and litter mate WT controls (N=5). A modified SP3 based protocol was used to analyze the volume- limited samples. More than 400 protein groups exhibited differences in expression and included proteins involved in pathways that can be linked to Krabbe disease, such as inflammatory and defense response, lysosomal protein accumulation, demyelination, reduced nervous system development and cell adhesion.

Innovative aspects

- Molecular analysis of Krabbe disease
- Histology defined microdissection for region specific proteomics



Targeted analysis of autophagy-based protein secretion - Insight into the ovarian cancer cell secretome

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Introduction Secreted proteins have multifaceted functions in vital biological processes, while ovarian cancer cells utilize them to govern survival mechanisms including drug resistance [1]. Thus, these proteins present valuable therapeutic targets. In addition to the conventional endoplasmic reticulum to Golgi-dependent secretory pathway, cells release molecules by different unconventional mechanisms, which include parts of the autophagy machinery (autophagy related, ATG) [2]. In the present study we specifically analysed the ATG-dependent secretome of ovarian cancer cells to obtain a more detailed insight into the cellular microenvironment and related molecular mechanisms.

Methods A label-free based quantitative proteomic approach was conducted to analyse the conditioned media of epithelial ovarian cancer (EOC) cells. The contribution of autophagy-related (ATG) molecules to the secretory profile of EOC cells was investigated by siRNA mediated knockdown versus non-targeting controls. Different culture conditions were evaluated and optimized for the subtractive secretome analyses. Serum-reduced conditioned media were collected for 24 hours. Samples were analysed by nanoLC-MS/MS (Q ExactiveTM Plus system, Thermo Fisher). Raw Data were processed by MaxQuant and Perseus [3].

Results As a model system, two human epithelial ovarian cancer (EOC) cell lines with varying chemoresistance levels were analysed and further compared to a non-cancerous epithelial cell line (human fallopian tube epithelial cells). Cluster analysis revealed distinct ATG-dependent secretory protein profiles. The secretome of ATG7 deficient cells was characterized by the decreased abundance of proteins that were classified amongst others as cell adhesion molecules, proteases, calcium binding proteins, and enzyme modulators. Functional protein network analysis highlighted the involvement of ATG-dependent secreted proteins in “extracellular matrix organization” and “regulated exocytosis”. Moreover, comparative data analysis demonstrated a cell-line specific composition of the sub-secretomes and thus, allow further the distinction between different cancer and non-cancerous epithelial cells.

Innovative aspects

- Quantitative analysis of ATG-specific sub-secretomes of epithelial ovarian cancer cells
- MS/MS based identification of ATG-specific secreted proteins potentially opens up new ways for therapeutic intervention

References [1] Wang, Y., et al., Autocrine production of interleukin-6 confers cisplatin and paclitaxel resistance in ovarian cancer cells. *Cancer Lett*, 2010. 295(1): p. 110-23. [2] Rabouille, C., Pathways of Unconventional Protein Secretion. *Trends Cell Biol*, 2017. 27(3): p. 230-240. [3] Tyanova, S., et al., The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat Methods*, 2016. 13(9): p. 731-40.

Acknowledgments The MS/MS system was acquired within the HRSM project BM4BR (2016) funded by the Austrian Federal Ministry of Education, Science and Research (BMBWF).



Development of capillary flow micro pillar array columns (μ PACTM CapLC): Combining nano flow sensitivity with analytical robustness and throughput for proteomics LCMS

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Introduction Mass-spectrometry based proteomics has become an essential tool in biological, biomedical and biopharmaceutical research. Due to the increased sensitivity that can be achieved compared to analytical flow LC-MS, the majority of LC-MS based proteomics research is performed within the nanoflow LC regime, with flow rates typically below 1 μ l/min and total run times exceeding 60 min. However, capillary and microflow LC-MS solutions are recently gaining interest: the demand for large quantitative proteomics studies requiring increased throughput and robustness is growing and the sensitivity of MS instrumentation has increased significantly. By working at higher flow rates, the impact of gradient delay and sample loading volumes on the total analysis time can be reduced and low flow rate or column dimension related technical errors (such as electrospray instability, column clogging or the presence of void volumes in the analytical flow path) are minimized.

Methods A micro pillar array column-based solution for capillary flow LC-MS is presented. In contrast to conventional LC columns that contain randomly packed beads as their stationary phase, microchip-based pillar array chromatography columns have a separation bed of perfectly ordered and freestanding pillars obtained by lithographic etching of a silicon wafer. The regular mobile phase flow pattern through these micro-chip pillar array columns adds very little dispersion to the overall separation, resulting in better peak resolution, sharper elution and increased sensitivity. The freestanding nature of the pillars also leads to much lower back pressure buildup, and makes it possible to operate longer columns.

Results This presentation will explain the principles of the micro-chip based pillar array columns. Their contribution to LC-MS workflow robustness will be illustrated with high resolution DIA data of 500 ng human proteome analyzed using either packed bed or micro-chip based pillar array columns operated at different flow rates and gradients, as an opening to an alternative future in proteomics: highly reproducible label-free quantification of 1000's of peptides over large sample batches using capillary LC-DIA. In addition to proteomics experiments, the μ PACTM CapLC column is perfectly suited for many LCMS-based separations where high reproducibility and robustness is desired.

Innovative aspects

- Micro pillar array columns (μ PACTM) offer an innovative chromatographic separation backbone that overcomes the limitations of conventional packed-bed alternatives
- In addition to the well-accepted μ PACTM nano-LC range, PharmaFluidics has now transferred the technology to higher flow rate regimes.
- The performance of the new μ PACTM CapLC column is illustrated for high-throughput proteomics applications.



The challenge of classifying metastatic cell properties by molecular profiling of a melanoma brain metastasis model

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Introduction Molecular profiling of cells aims at the prediction of relevant cell properties eventually supporting the optimal choice of therapy based on individualized data related to a given patient. The prediction of metastatic properties from molecular analyses however, still poses a major challenge. Here we aimed at the classification of metastasis-related cell properties by proteome profiling making use of cutaneous and brain-metastasizing variants from single melanomas sharing the same genetic ancestry. Previous experiments demonstrated that cultured cells derived from these xenografted variants maintain a stable phenotype associated with a differential metastatic behavior: The brain metastasizing variants produce more spontaneous micro-metastases than the corresponding cutaneous variants.

Methods Four human melanoma cell lines (YDFR, DP, M12, M16) were transformed in mouse models to create cutaneous tumors (C) and brain metastasis (CB). These eight cell lines were then used for molecular profiling experiments, namely proteomics, eicosanomics, glutathione measurements as well as morphological evaluations and immunofluorescence. For proteomics a FASP protocol was used and a QExactive Orbitrap coupled to a Dionex 3000 nano-HPLC was employed. Eicosanoids of the supernatant were analysed after solid phase extraction using a QExactive Orbitrap HF. Intracellular glutathione levels were determined with a 6490 Triple Quadrupole MS after derivatisation with N-Ethylmaleimide.

Results Among the differentially expressed proteins we identified cell adhesion molecules, immune regulators, epithelial to mesenchymal transition markers, stem cell markers, redox regulators and cytokines. Similar results were observed with regard to eicosanoids, considered relevant for metastasis, such as PGE2 and 12-HETE. Multiparametric morphological analysis of cells also revealed no characteristic alterations associated with the cutaneous and brain metastasis variants. However, no correct classification regarding metastatic potential was yet possible with the present data. We thus concluded that molecular profiling is able to classify cells according to known functional categories, but is not yet able to predict relevant cell properties emerging from networks consisting of many interconnected molecules. The presently observed broad diversity of molecular patterns, irrespective of restricting to one tumor type and two main classes of metastasis, highlights the important need to develop meta-analysis strategies to predict cell properties from molecular profiling data. Such base knowledge will greatly support future individualized precision medicine approaches.

Innovative aspects

- Identification of differentially regulated metastasis-associated molecules
- Evidence for individually distinct patterns of metastasis-associated molecules
- Highlighting the evident need for establishing meta-analyses strategies



Overcoming Modafinil Plasma Instability towards neuroPK of Modafinil and CE-123 by LC-HRMS

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Introduction Cognitive enhancement is one of the major concerns in neuropharmacology as cognitive impairment is a symbol of aging and neurodegenerative disorders. The need for neurotherapeutics for the treatment of cognitive impairment is highly demanded. However, the central nervous system (CNS) remains a complex organ with many factors to be considered. Combinatory Mapping Approach (CMA) was proposed to evaluate neuropharmacokinetic (neuroPK) parameters of unbound-drug CNS exposure making it useful for potential neurotherapeutics discovery [1]. We synthesized the novel modafinil analogue CE-123 for which in-vivo experiments suggest beneficial effects on cognitive performance [2]. To accurately understand neuroPK parameters of modafinil and CE-123, using CMA, a valid LC-HRMS method for their quantification in rat plasma and brain needs to be developed.

Methods Stability of modafinil and CE-123 in rat plasma was examined by spiking blank plasma with analytes and incubating for 6 h at 37 °C. Inhibition studies for modafinil were conducted at same conditions however adding 10% dimethylformamide (DMF) to plasma [3]. SPE-recovery in plasma was assessed by comparing quality control samples at low and high levels.

Results Assessment of modafinil and CE-123 stability in rat plasma revealed that during 6 h incubation at 37°C there is formation of modafinil acid, while CE-123 was found to be stable most likely due to its thiazole ring leading to metabolic stability. Furthermore, detection of modafinil acid in *in-vitro* samples may underestimate the real *in vivo* concentration. To assess modafinil neuroPK parameters, drug metabolism needs to be inhibited. Addition of 10% DMF to plasma completely inhibits the enzymatic degradation of modafinil and no metabolites were detected.

The applied SPE sample preparation protocol for removing buffer and lipids demonstrated recoveries of all analytes in plasma samples, ranging from 77% to 91%. For quantification of modafinil and CE-123 in plasma samples a dynamic range between 20 to 2000 ng/mL was evaluated.

Innovative aspects

- Modafinil plasma instability can be inhibited by adding 10% DMF to rat plasma most probably inactivating the enzyme amid esterase.
- For neuroPK studies a LC-HRMS method was developed for the quantification of Modafinil and its analogue CE-123 in rat plasma and brain samples.

References [1] Loryan, I. et al. (2014) Mechanistic Understanding of Brain Drug Disposition to Optimize the Selection of Potential Neurotherapeutics in Drug Discovery. *Pharm Res* 31(8):2203-19. doi: 10.1007/s11095-014-1319-1 [2] Nikiforuk, A. et al. (2017) A Novel Dopamine Transporter Inhibitor CE-123 Improves Cognitive Flexibility and Maintains Impulsivity in Healthy Male Rats. *Front. Behav. Neurosci.* doi: 10.3389/fnbeh.2017.00222 [3] Gorman, S. H. (2002) Determination of modafinil, modafinil acid and modafinil sulfone in human plasma utilizing liquid-liquid extraction and high-performance liquid chromatography. *J. Chromatogr. B* 767, 269–276.



Analytical methods to Address the Pharmaceutical Industry Nitrosamine Crisis

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Introduction After the June 2018 US FDA announcement confirming the presence of potential genotoxic nitrosamine impurities in Angiotensin II Receptor Blocker (ARB) medicines there have been several drug recalls to reduce public health risks. Since then the problem has been exacerbated and the risk extended to histamine-2 (H-2) blocker ranitidine medicines and other drug classes

Methods Most recently, in October 2019, the FDA published the latest paper validating Agilent Triple quadrupole LC/MS on trace level nitrosamine analysis for NDMA and NDEA in different formulations. As a further safeguard, we have since developed a multi-target nitrosamine solution utilizing the Agilent Ultivo LC Triple Quadrupole LC/MS to quantify not only for the six nitrosamines listed by the FDA, but also for the simultaneous detection of six additional nitrosamine impurities. Furthermore, we have extended this high sensitivity capability to High Resolution Q/TOF LC/MS systems for the quantitative determination of 11 nitrosamine compounds with the added capability to deliver deeper insights during retrospective investigations if desired.

Results Agilent Technologies has developed several analytical methods to detect these impurities in DP and API's at levels lower than current FDA recommendations using routine GC/MS or LC/MS technologies.



Application of non-targeted screening for uncovering the overlooked contaminants in environmental samples

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The applicability of suspect and non-targeted screening with LC/ESI/MS in metabolomics, pharmaceutical analysis, environmental analysis, and monitoring illegal substances is growing fast. The non-targeted analysis allows detecting hundreds or thousands of features simultaneously; while classical targeted methods allow detecting only previously selected compounds. The most significant obstacles for non-targeted LC/HRMS screening has been the scarcely defined scope of the method and, more importantly, the inability to provide quantitative information. To overcome this issue, we have developed a quantification approach based on in silico predicted electrospray ionization efficiencies. The ionization efficiency values are predicted based on 2D descriptors with the random forest algorithm. The model was developed based on ~6000 ionization values and accounts for both analyte and eluent properties. The model can be transferred between different labs with the aid of a small set (6 compounds) of calibration compounds. The method has been tested for quantification of pesticides and their transformation products analysis in surface water and food samples, as well as for general non-targeted screening of surface and groundwater. In all of the applications, the concentration prediction accuracy of ~3 times has been observed. This means that the reported concentration of 3 ppm is likely to be in the range of 1 to 9 ppm (3 ppm/3 times to 3 ppm * 3 times). This accuracy is suitable for prioritization purposes, e.g. combining the concentration prediction with the toxicity predictions and identifying the most relevant contaminants in groundwater or in food. We have also used this method to quantitatively compare the results of suspect screening of different laboratories. Based on the suspect screening of pesticides in cereals we have seen that the concentrations estimated in two independent labs are in good agreement. The R² of 0.85 was observed and on the average, the concentration difference was 3.2 times. Based on the concentrations estimated by one of the labs we were able to identify the root cause of inconsistency in the tentatively identified compounds between the two labs: one of the labs used a method with significantly lower LoD values.



New Approaches for Enzyme Replacement Therapy of Lysosomal Diseases with high Immunogenicity by Mass Spectrometric Identification of Antibody Epitopes

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Introduction Enzyme replacement therapies (ERT) have been successfully developed for different Lysosomal Storage Diseases (LSDs) such as Gaucher (GD), Fabry (FD), and Pompe (PD) Disease. While effective for several LSDs, substantial problems are caused by development of antibodies. Patients who develop antibodies upon ERT can have allergic reactions, from mild symptoms to life threatening events. IgG antibodies may neutralize the infused lysosomal enzyme and prevent successful treatment. Here we report new therapeutic approaches by identification and affinity characterization of antibody epitopes upon ERT, using synthetic epitope peptides that block antibodies directed against the infused enzyme. It is expected that the application of epitopes to block neutralizing antibodies will substantially improve efficiency and safety of ERT in LSD patients.

Methods For epitope identification, blood from FD and PD patients who had developed antibodies was analyzed using proteolytic excision- MS and surface plasmon resonance (SPR) biosensor combined with electrospray (ESI) mass spectrometry. SPR-MS provided the concomitant structural identification and affinity quantification of epitopes. The epitope from FD patients was identified with a human anti- α -galactosidase antibody immobilized on an activated sepharose microcolumn by incubation for 2 hrs at 37°C. The tryptic peptide mixture resulting from digestion (12 h, 37°C, protein:trypsin 1:30) was loaded onto the SPR-MS via the SPR injector. After washing out the nonbinding peptides, epitopes were eluted with 0.1 % TFA into the MS interface equipped with a C4-desalting microcolumn; ESIMS was performed with a Waters Quattro Ultima instrument.

Results The SPRMS combination was successfully applied to the epitope elucidation and affinity characterization of antibodies against α -galactosidase in 3 FD patients, and provided identical peptide sequences, α Gal (309-332). The epitope (309-332) was synthesized by solid phase peptide synthesis (SPPS), and purified by reversed phase HPLC. SPR Analysis provided high affinity to the antibody (KD, 39 nM) [1]. For cell culture studies skin fibroblasts were used to evaluate the epitope peptides on the uptake of lysosomal enzymes in the presence of the patient's antibodies. The results showed that antibodies were blocked by tight binding to the epitope peptide, thus opening a new concept to reconstituting therapeutic efficiency of ERT.

Innovative aspects

- Epitope identification is opening a new therapeutic concept to hyposensitizing patients from immunogenicity and reconstituting therapeutic efficiency of ERT.
- Individual epitopes of patient antibodies basis for personalized medicinal approaches
- Epitopes and affinities are concomitantly identified by SPR-MS.

References [1] Kukacka Z, Iurascu M, Lupu L, Rusche H, Murphy M, Altamore L, Borri F, Maeser S, Papini AM, Przybylski M, Molecular Structure of the Human α -Galactosidase A Antibody Epitope Revealed by Affinity-Mass Spectrometry: A Basis for Reversing Immunoreactivity in Enzyme Replacement Therapy of Fabry's Disease, ChemMedChem. 2018, 13: 909-915.



Sulphated and sialylated *N*-glycans in the echinoderm *Holothuria atra* reflect its marine habitat and phylogeny

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Introduction Among the earliest deuterostomes, the echinoderms are an evolutionary important group of ancient marine animals. Within this phylum, the holothuroids (sea cucumbers) are known to produce a wide range of glycoconjugate biopolymers with apparent benefits to health; therefore, they are of economic and culinary interest throughout the world. Other than their highly modified glycosaminoglycans (e.g. fucosylated chondroitin sulfate and fucoidan), nothing is known about their protein linked glycosylation.

Methods Here we used multistep *N*-glycan fractionation to efficiently separate anionic and neutral Nglycans before analysing the *N*-glycans of the black sea cucumber (*Holothuria atra*) by MS in combination with enzymatic and chemical treatments.

Results These analyses showed the presence of various fucosylated, phosphorylated, sialylated, and multiply sulphated moieties as modifications of oligomannosidic, hybrid, and complex-type Nglycans. The high degree of sulphation and fucosylation parallels the modifications observed previously on holothuroid glycosaminoglycans. Compatible with its phylogenetic position, *H. atra* not only expresses vertebrate motifs such as sulfo- and sialyl-Lewis A epitopes but displays a high degree of anionic substitution of its glycans, as observed in other marine invertebrates. Thus, as for other echinoderms, the phylum- and order-specific aspects of this species' *N*-glycosylation reveal both invertebrate- and vertebrate-like features.

Innovative aspects

- The *N*-glycome discovery of *Holothuria*.
- New phosphorylated and sulphated structures.
- In depth MS based sulfo-glycomics analyses.

References Vanbeselaere, J., et al. (2020), 'Sulphated and sialylated *N*-glycans in the echinoderm *Holothuria atra* reflect its marine habitat and phylogeny', J Biol Chem. doi: 10.1074/jbc.RA119.011701.

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Native Peptidomics- An HPLC-MS/MS Approach for Analysis of Tumors' Signature

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Introduction Tumor cells secrete both peptides and proteins that could be used as biomarker for specific recognition of cancer. The analysis of native peptides from tumor sample immediately after resection is fast-approximately one-hour total running time - and can help characterizing the type of the tumor. Currently, we are populating the native peptidomics database in order to facilitate future targeted analysis of brain tumor samples.

Methods Tumor tissue was extracted and immediately thoroughly rinsed in the OR using ice cold PBS in order to remove as much blood as possible. Tissue was snap frozen using liquid nitrogen and stored at -80°C until further processing. Upon lysis, the sample was filtered using MW cutoff filters of 10kDa and the filtrate was treated with DTT before nano LC-MS/MS was performed in data-dependent mode using an Q-Exactive Plus instrument upon separation on the 50cm µPillar-Arrayed-Column. Data analysis was performed using MaxQuant and Perseus.

Results Sample preparation based on molecular weight cut-off filtration of lysed tumor samples significantly shortened the time prior to sample injection and analysis and the use of short µPillar-Arrayed-Columns (50 cm length) enabled the use of fast and shorter separation gradients. Depending on the tumor, between 200 and 5000 peptides were identified in analyzed samples. Based on these results, it was possible to detect, identify, and differentiate glioma by isolated peptides. Furthermore, it was possible to distinguish between glioma and other brain or pituitary tumors. Further work is needed to classify and quantitate the glioma peptides and further optimization of sample preparation is needed in order to speed up the process.

Innovative aspects

- Fast sample preparation
- Fast LC-separation and MS detection
- Adjunct method for tumor identification

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Unravelling the fate of xenobiotics in human cell culture by stable isotope-assisted metabolomics

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The human body is exposed to a wide range of xenobiotics. Known biotransformation pathways in humans include sulfation, glucuronidation, acetylation and glutathione conjugation. A possibility to reduce data complexity in LC-HRMS measurements is the bioinformatics tool MetExtract II. In combination with stable isotopic labeling the algorithm makes it possible to search for biotransformation products of xenobiotics. The mycotoxin deoxynivalenol (DON) serves as a model toxin to demonstrate the applicability of the approach to study xenobiotic metabolites in human cell culture.

Human colon adenocarcinoma cells (HT-29) and liver hepatocellular carcinoma cells (HepG2) were incubated for 3 h with an equimolar concentration of ¹²C- and ¹³C-DON. The extracellular medium and the cell lysates were extracted and analysed. Measurements were performed on a Vanquish UHPLC coupled to a Q Exactive HF quadrupole-Orbitrap mass spectrometer operated in the positive and negative ionization modes. Full scan MS and ddMS2 were acquired. Data processing was carried out with MetExtract II [1].

The workflow applying stable isotopic labeling assisted metabolomics enabled the detection of known as well as afore unknown biotransformation products of the investigated xenobiotic in mammalian cell models. Eight phase II- biotransformation products of the mycotoxin DON were revealed. The previously undescribed metabolites include conjugates of the toxin with amino acids and the antibiotic penicillium G. Manual inspection of MS2-spectra supported the structure of the novel biotransformation products. The approach facilitates the elucidation of human xenobiotic metabolites and thus can improve the comprehension of from in vitro experiments drawn conclusions.

Innovative aspects

- An adapted workflow for the comprehensive identification of known and unknown xenobiotic biotransformation products in mammalian cell culture was established.
- DON is detoxified mainly by conversion into sulfur-containing metabolites and by formation of novel amino acid conjugates.
- DON-sulfonate formation in human cells was reported for the first time.

References [1] Bueschl, C., et al., MetExtract II: A Software Suite for Stable Isotope-Assisted Untargeted Metabolomics. *Analytical Chemistry*, 2017. 89(17): p. 9518-9526. [2] Flasch, M., Bueschl, C., et al., Stable Isotope-Assisted Metabolomics for Deciphering Xenobiotic Metabolism in Mammalian Cell Culture. *ChemRxiv*, 2019, Preprint. <https://doi.org/10.26434/chemrxiv.10293224.v1>

Acknowledgments We would like to thank Maria Doppler, Heidi Schwartz-Zimmermann, and the team of the Mass Spectrometry Center at the University of Vienna. Furthermore, we would like to thank the Austrian Science Fund (SFB Fusarium #F3715 and #F3718) as well as the Provincial Government of Lower Austria (projects NoBITUM, OMICS 4.0) for financial support.

On the threshold between *Aspergillus fumigatus* colonization and proliferation in a host

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Introduction The application of microbial siderophores in *Aspergillus* diagnostics does not require previous microscopy, culture or PCR. Modulation of host functions is one of the non-canonical roles of siderophores in an invaded host, which can establish a new borderline between asymptomatic colonization and proliferation on a host mucosa.

Methods In a working dataset of fourteen patients with putative or probable invasive aspergillosis (IPA) we used an array of urinal triacetylfusarinine C (uTAFC), ferricrocine (uFC), gliotoxin (uGTX) and several other mycotoxins to quantitatively probe the patients' status. *Aspergillus* hydrolytic (triacetylfusarinine B) products were also monitored both in urine and serum. Conventional serology (sGM; s(1,3)- β -Dglucan, sBG) was performed both on serum or bronchoalveolar lavage fluid samples. Urinal samples were creatinine-standardized and quantified against matrix-matched standards by liquid chromatography (LC) and Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS). Isotope data filtering was used for allocation of iron-containing species in the LC-FTICR-MS dataset.

Results In a host, the colonist is characterized by copy numbers of its cellular components including siderophores, toxins, or soluble GM. Their qualitatively and quantitatively different profiles are characteristic for both isotropic and polarized *A. fumigatus* morphotypes. We defined the beginning of fungal proliferation in a host by secretion of *Aspergillus* extracellular virulence factors. In agreement with recent literature and own observations, all monitored TAFC, FC, or GLX metabolites are extracellular (FC to at least some extent). In our set of critically ill patients, which consisted of mostly non-neutropenic subjects suffering from IPA, the uTAFC sensitivity was 92.9% compared to sGM (35.7%) and nonspecific sBG (85.7%). The limit of quantitation of uTAFC was 0.3 ng/mL, and the maximum observed concentration was 1.6 μ g/mL. The specificity of uTAFC was 100% compared to sGM (73%). In the presentation, the infection kinetics in a patient who developed an invasive disease from its "colonization" state will be reported.

Conclusions The threshold concentrations of intra and extracellular siderophores in a host can provide quantitative borderlines between isotropic and polarized morphotypes. The presence of mycotoxins in bodily samples may indicate a full mycelial development.

Innovative aspects

- Used mass spectrometry based identification and quantification of intra and extracellular fungal siderophores in patients
- Studied asymptomatic colonization and symptomatic infection in host

References [1] Skriba Anton., et al.: *Frontiers in Microbiology* 9, 2356 (2018). [2] Shankar Jata, et al.: *Frontiers in cellular and infection microbiology* 8 (2018):180.

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ABSTRACTS OF POSTER PRESENTATIONS



POSTER SESSION, TUESDAY: even numbers: 12:45 - 13:45, odd numbers: 13:45 - 14:45.
WEDNESDAY: even numbers: 12:35 - 13:00, odd numbers: 13:00 - 13:30.

POSTER CODE	AUTHOR/TITLE
01	Alina Zamfir , <i>National Institute for R&D in Electrochemistry and Condensed Matter</i> Microfluidics MS-based assay for studying the noncovalent interactions of α -Synuclein with glioma gangliosides
02	Ulrike Resch , <i>Medical University of Vienna</i> Comparative proteomic characterization of outer membrane vesicles (OMVs) isolated from multidrug resistant <i>Klebsiella pneumoniae</i> as basis for development of type-specific vaccines
03	Monika Cziferszky , <i>University of Innsbruck</i> Investigating Molecular Interactions of Organometallic Complexes with Model Peptides by Mass Spectrometry
04	Ica Raluca , <i>National Institute for R&D in Electrochemistry and Condensed Matter</i> Identification and characterization of ganglioside structures associated to brain aging by Orbitrap MS
05	Alina-Iulina Petrut , <i>National Institute for R&D in Electrochemistry and Condensed Matter</i> High resolution mass spectrometry profiling and fragmentation of glycolipids expressed in human hemangioma
06	Clemens Grünwald-Gruber , <i>University of Natural Resources and Life Sciences Vienna</i> Analysis of bacterial arabinosyl transferase reaction products of Kdo2-lipid A by LC-ESI-MS
07	Āgnes Horváthová , <i>Slovak Academy of Sciences</i> MALDI-TOF analysis for identification and classification of yeast associated with meadow plants in Central and Western Slovakia
08	Patrik Mehlsack , <i>University of Graz</i> <i>Andiperla morenensis</i> : insect lipidome adaptation to life on a glacier
09	Bassam Lajin , <i>University of Graz</i> Non-metal speciation analysis by HPLC-ICPMS/MS
10	Filip Pančík , <i>Slovak Academy of Sciences</i> MALDI TOF/TOF analysis of oligosaccharides from seaweed samples
11	Marek Šebela , <i>Palacky University in Olomouc</i> MALDI-TOF mass spectrometry of inhibitor-treated cyanobacteria
12	Veronika Lukáčová , <i>MEDIREX Group Academy n.o.</i> Proteomic and glycomic characterization of microvesicles by mass spectrometry
13	Dmitry Kechasov , <i>Norwegian Institute of Bioeconomy Research</i> Effect of organic fertiliser on metabolic profile of hydroponically cultivated tomato
14	Kurt Varmuza , <i>TU Wien</i> ICP-MS of meteorite samples: chemometric evaluation of diversity and discrimination
15	Stephanie Steinberger , <i>TU Wien</i> MALDI MS supports method development for gas-phase electrophoresis of extracellular vesicles
16	Oliver Greitbauer , <i>Biomim Holding GmbH</i> Quantification of bacitracin in broiler feed and digesta – MRM3 vs. QTOF
17	Anna Turyanskaya , <i>TU Wien</i> Correlation of μ XRF and LA-ICP-MS in analysis of human bone-cartilage sample
18	Barbora Stratilová , <i>Slovak Academy of Sciences</i> MALDI spectra database for Culture Collection of Yeasts (CCY)
19	Fanni Bugyi , <i>Research Center for Natural Sciences Budapest</i> The effects of PTMs on proteomic searches
20	Gábor Tóth , <i>Research Center for Natural Sciences Budapest</i> HPLC-MS investigation of glycosaminoglycan disaccharides
21	Cristina Coman , <i>University of Vienna</i> Modified hippocampal lipid signaling pathways triggered by lifestyle conditions: a multi-omics perspective
22	Elisavet Kunz-Vekiru , <i>Biomim Research Center</i> The <i>B. subtilis</i> CotA laccase metabolizes Aflatoxin B1 into Aflatoxin Q1 and epi-Aflatoxin Q1



POSTER SESSION, TUESDAY: even numbers: 12:45 - 13:45, odd numbers: 13:45 - 14:45.

WEDNESDAY: even numbers: 12:35 - 13:00, odd numbers: 13:00 - 13:30.

POSTER CODE	AUTHOR/TITLE
23	Georg Weingart , <i>Biomim Holding GmbH</i> Quantitative determination of phytochemical compounds in chicken breast muscle, skin and fat by GC-MS/MS
24	Pavel Rehulka , <i>Faculty of Military Health Sciences, University of Defence</i> Fractionation of complex peptide samples using manual HILIC chromatography
25	Ivana Karmelic , <i>University of Zagreb</i> LC-MS/MS Characterization of Sphingoid Bases from Neutral and Acidic (Glyco)Sphingolipids in Glioblastoma Multiforme
26	Martina Marchetti-Deschmann , <i>TU Wien</i> Bacterial Responses to Different Cultivation Conditions – A Correlated Multimodal Imaging Approach



Microfluidics MS-based assay for studying the noncovalent interactions of α -Synuclein with glioma gangliosides

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Introduction Gangliosides (GGs), sialylated glycosphingolipids are valuable biochemical markers in early diagnosis of central nervous system pathologies. At this level, the interest in the study of GG interactions with peptides and proteins was stimulated by findings indicating that the formed complexes might play roles in the molecular mechanisms of neurodegenerative diseases, the action of bacterial toxins, progression of malignant brain tumors and discovery of novel species based on their high binding affinity. We have developed here a robust method based on chip-nanoESI MS for studying the noncovalent interactions of GGs extracted from human glioblastoma with α -Synuclein (α Syn).

Methods α Syn was dissolved to a final concentration of 1 pmol/ μ L in 10 mM ammonium acetate buffered with acetic acid, pH 6.0. The native GG mixture extracted from human glioblastoma was also dissolved in buffer to the concentration of 10 pmol/ μ L, calculated for an average MW of 2000 g/mol. 100 μ L of α Syn in buffer was mixed with 100 μ L of GG solution and incubated at 37°C under stirring. Aliquots were collected after 10, 30 and 60 min directly into the 96-well plate of a NanoMate robot and submitted to MS analysis. MS was conducted on a QTOF instrument in-laboratory coupled to a fully automated chip-based nanoESI system (Nanomate robot) operated in the positive ion mode.

Results MS and MS/MS analyses have shown that α Syn interacts with GG species belonging to G1 class, exhibiting the longest glycan chain. Furthermore, the highest affinity of α Syn was observed for the polysialylated structures. We have discovered α Syn complexes formed with species containing several Neu5Ac units: GD1, GT1, GQ1 and GO1. Some of these species, were not detected in glioblastoma by direct chip-nanoESI screening of the GG extract, mainly because of their low expression. From the technical-methodological point of view, the data have demonstrated the high performance of the microfluidics-QTOF MS approach in terms of sensitivity, reproducibility, mass accuracy, compatibility of the designed buffer, which allowed the detection of 89 different species of gangliosides associated with human glioblastoma as well as of the noncovalent G1 class- α Syn complexes. From the biological point of view, the results have shown that, in glioma, there are minor, however, biologically relevant species, not previously identified and which could be for the first time detected via their interactions with α Syn. Although α Syn is intensively studied in relation to neurodegenerative diseases, recent studies have evidenced α Syn overexpression in U251 glioblastoma cells [1] and the ability of GM1 to protect against the toxicity of this protein. Our results suggest for the first time that α Syn has affinity for the entire G1 class, not only GM1; therefore the entire G1 class is to be tested against α Syn toxicity possibly with effects of reducing glioma aggressiveness.

Innovative aspects

- Chip-nanoESI MS was developed for the determination of α Syn-GGs interactions;
- α Syn interacts with glioblastoma GG species belonging to G1 class;
- Novel, biologically relevant GGs could be detected via their complexes formed with α Syn.

References [1] J. Duan, Z. Ying, Y. Su, F. Lin, Y. Deng, α -Synuclein binds to cytoplasmic vesicles in U251 glioblastoma cells, *Neurosci. Lett.* 642, 148-152, 2017.

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Comparative proteomic characterization of outer membrane vesicles (OMVs) isolated from multidrug resistant *Klebsiella pneumoniae* as basis for development of type-specific vaccines

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Introduction *Klebsiella pneumoniae* is a Gram-negative bacterium causing severe lung injury associated with high morbidity and mortality in sensitive individuals including young, elderly and immunocompromised people. In addition, hospital-acquired infections with antibiotic/multidrug-resistant (MDR) *K. pneumoniae* are increasing worldwide and hence, new antimicrobial treatment options are urgently needed. Gram-negative bacteria continuously discharge lipid bilayered nanoparticles, termed outer membrane vesicles (OMVs), during growth. These OMVs facilitate intra-and interbacterial communication and cargo resistance determinants. OMVs are being exploited for vaccine-development, thereby offering new therapeutic opportunities to treat pan-resistant bacteria in a post-antibiotic era. This study aims to characterize MDR *K. pneumoniae*'s OMVs protein repertoire to identify immunogenic proteins and epitopes which could be exploited for type-specific vaccination.

Methods MDR- *K. pneumoniae*'s (strain ATCC 700603) was grown under antibiotics- and cytostatic (Daunorubicin) environmental stress and shotgun-proteomics analyses of OMVs, outer membranes (OM) as well as whole cell lysates (WCL) was performed using an UltiMate nano RSLC HPLC system coupled to a Q-Exactive plus mass spectrometer. Database search and label-free quantitation was performed using MaxQuant. Bioinformatic analyses were done using Perseus, the CNB-CSIC online tool Venny. The PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification system (www.pantherdb.org) was utilized to categorize proteins in "Protein Classes", using *Klebsiella pneumoniae* subsp *pneumoniae* strain ATCC 700721 (KLEP7) as reference dataset. ELISA and 1D-WB with patient's plasma proved differential IgG and IgM antibody-responses towards *K. pneumoniae*'s subcellular fractions.

Results We consistently identified 594 (569), 680 (429) and 1117 (1126) proteins in OMV, OM and WCL-fractions in absence of presence (numbers in parenthesis) cytostatic stress. Notably, we observed an enrichment of efflux pumps among other virulence factors in OMVs. ELISA and 1D-WB with patient's plasma proved differential IgG and IgM antibody-responses towards *K. pneumoniae*'s subcellular fractions.

Innovative aspects

- Comparative proteomic profiling of MDR-*K. pneumoniae*'s subcellular fractions and influence of environmental stress on protein composition.
- Immunogenic profiling of subcellular fractions using patient's plasma.
- Development of a platform for vaccine development.



Investigating Molecular Interactions of Organometallic Complexes with Model Peptides by Mass Spectrometry

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Introduction Platinum-based chemotherapy is widely used in clinical anticancer treatment despite its many severe side-effects and problems associated to the development of resistances. Current research in antiproliferative metallodrugs includes approaches like the combination of metal complexes with non-steroidal anti-inflammatory drugs, as well as the shift to other metals. A Ruthenium compound is currently in clinical trials. [1]

Mass Spectrometry is involved in different levels of the metallodrug development process. Both top down and bottom up methods are being used to investigate the molecular interactions of metal complexes with peptides, proteins and oligonucleotides. These studies provide information about the nature and stoichiometry of metal-biomolecule adducts, binding site preferences as well as the faith of the original ligands.

Methods Model peptides Angiotensin 1, Substance P, and Ubiquitin were incubated with Platinum and Ruthenium compounds in different ratios and monitored overtime. Top down MS studies employing higher energy collisional dissociation (HCD) delivered information about their molecular interactions. A bottom up experiment was performed on ruthenated Ubiquitin to establish whether Ruthenium-peptide bonds are stable during tryptic digestion and Liquid Chromatography

Results In a first study we investigated the fundamental differences between Zeise's salt and Cisplatin in their molecular interactions with peptides and demonstrated that *trans* labilizing effects play a crucial role in the overall peptide metalation by Platinum (II) complexes. [2]

The combination of Zeises's salt with acetyl salicylic acid via an alkenol linker led to a compound that was able to platinate and acetylate Ubiquitin at the same time. Unexpected peptide oligomerization was observed with Substance P. Interactions with Angiotensin 1 showed adduct formation with retention of the organic moiety. [3] Ruthenium (II) compounds formed adducts with Angiotensin I and Ubiquitin with partial loss of their original ligands. Binding site information obtained through top down and bottom up methods were compared and indicates sufficient stability of the Ruthenium-peptide bond.

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Identification and characterization of ganglioside structures associated to brain aging by Orbitrap MS

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Introduction Gangliosides (GGs) are found predominantly in the nervous system and involved in a series of biological and pathological processes at this level. Their expression, distribution and structure change with brain development, maturation and aging as well as in neurodegenerative diseases [1]. In this study we have developed a high resolution (HR) MS approach based on nanoESI Orbitrap MS and MSⁿ for GG mapping in normal human elderly cerebellum, in order to identify and structurally characterize the species associated to aged human brain. Such species could be further used in comparative healthy vs. diseased aged brain assays targeting the identification of GG biomarkers in neurodegenerative diseases.

Methods The GG mixtures were extracted and purified from normal human cerebellar tissues (65 years old, male subjects). For MS analysis the samples were dissolved in methanol up to final concentration of 10 pmol/μL and subjected one after the other to nanoESI Orbitrap MS and MSⁿ in the negative ion mode under identical and thoroughly optimized experimental conditions. For MS/MS the precursor ions were selected within an isolation width of 1 m/z unit. The product ion spectra were combined over scans acquired at variable collision energy within 30-65 eV range.

Results By HR MS screening, 63 distinct ganglioside species differing in the composition of their glycan core and/or lipid aglycone were identified. The high resolution and mass accuracy allowed here the identification and, therefore, the correlation with human aged cerebellum of GG components modified by CH₃COO⁻. No less than 54 species bearing more than one Neu5Ac were also detected. Next to the differences in the structure of the glycan chains, HR MS revealed dissimilarities in ceramide constitutions between the ganglioside species. Among these, the most interesting are related to the less common process of sphingoid base trihydroxylation and the unusual length of the fatty acid chains. Hence, no less than 19 species with trihydroxylated sphingoid base were found in the investigated GG mixtures. Further, (-)nanoESI HR HCD MSⁿ provided the detailed structural characterization of the following precursor ions: i) the [M-2H +]²⁻, which, according to the MSⁿ results corresponds to GD3(d18:1/18:0), a disialylated species characterized by a shorter glycan chain and ii) the [M-4H + +Na +]³⁻ identified at m/z 812.7035 by MS screening. Following the MSⁿ this ion was attributed to the d isomer of the tetrasialylated tetraose GQ1 (d18:1/18:0).

Innovative aspects

- We developed (-)nanoESI Orbitrap MSⁿ for GG analysis in aged human cerebellum;
- By (-)nanoESI Orbitrap MS we have identified 63 different species and evidenced the characteristic GG pattern in elderly brains;
- By HR MSⁿ we could discriminate GQ1d(d18:1/18:0) isomer in aged cerebellar tissue.

References [1] Ica R, Petrut A, Munteanu CVA, Sarbu M, Vukelić Ž, Petrica L., Zamfir AD. Orbitrap mass spectrometry for monitoring the ganglioside pattern in human cerebellum development and aging, J. Mass Spec. 2020 in press.

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High resolution mass spectrometry profiling and fragmentation of glycolipids expressed in human hemangioma

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Introduction Hemangioma is most commonly diagnosed by routine magnetic resonance imaging (MRI); specific detection can be accomplished by gradient-echo sequence MRI that is able to unmask small lesions that may otherwise remain undetected. A modern alternative is the early detection of hemangioma at an incipient stage, based on routine screening and biomarker discovery before manifestation of clinical symptoms [1]. In the present study, we optimized nanoESI Orbitrap MS and MSⁿ in the negative ion mode for the discovery and characterization in human brain hemangioma of the biomarkers in the class of sialylated glycosphingolipids, known as gangliosides.

Methods The native ganglioside mixture was extracted and purified from a hemangioma specimen. The tumor, located in the right hemisphere of the frontal cortex in an adult male patient, was removed by surgery. For MS analysis the sample was dissolved in methanol up to final concentration of 5 pmol/μL and subjected to high resolution (HR) nanoESI Orbitrap MS and MSⁿ in the negative ion mode under thoroughly optimized experimental conditions for screening and sequencing. For HCD MSⁿ the precursor ions were selected within an isolation width of 1 *m/z* unit. The product ion spectra were combined over scans acquired at variable collision energy within 35-65 eV range.

Results By HR MS screening of native hemangioma ganglioside mixture in the negative ion mode, no less than 150 ions, corresponding to 55 ganglioside species differing in the composition of their glycan core or ceramide moieties were detected. The mixture was found dominated by gangliosides exhibiting short oligosaccharide chains and a reduced number of Neu5Ac unit. Hence, the low sialylation degree of species appears as a feature characteristic to hemangioma. 46 gangliosides were found to belong to the monosialo GM and disialo GD class respectively, while only 11 structures were assigned to trisialylated GT class (GT1 and GT3). Another interesting finding is related to the rather unusual composition of the ceramides, which are mainly characterized by short fatty acid chains. The MSⁿ experiments carried out using as precursor the doubly deprotonated ion corresponding, according to mass calculation to GT1 (d18:1/20:0) provided valuable diagnostic ions which not only supported GT1 (d18:1/20:0) structure, but also helped in the discrimination of GT1c isomer associated to hemangioma. Similarly, by HCD MSⁿ GD1b (d18:1/20:0) could be identified in the hemangioma ganglioside extract.

Innovative aspects

- HR MS and MSⁿ was optimized for gangliosides extracted from brain hemangioma;
- HR MS revealed that hemangioma is characterized by gangliosides of short glycan chains and reduced number of Neu5Ac residues;
- MSⁿ provided data useful in structure elucidation and isomer identification in hemangioma ganglioside mixture.

References [1] McFaline-Figueroa JR, Lee EQ, Brain Tumors, *Am. J. Med.* 131, 874-882, 2018

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Analysis of bacterial arabinosyl transferase reaction products of Kdo2-lipid A by LC-ESI-MS

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Introduction The lipopolysaccharide (LPS) of Gram-negative bacteria serves as target for potent cationic antimicrobial peptides (CAMPs) and lipopeptides such as polymyxin B (PmB) or colistin, which are last-resort-antibiotics against multidrug-resistant bacteria. It is well known that many bacteria develop resistance mechanisms that counteract the action of CAMPs and polymyxins by covalent modification of the Kdo/Ko and lipid A with positively charged groups – among others, 4-amino-4-deoxy-L-arabinose (Ara4N). Inhibition of the biosynthetic pathway involved in the formation of the activated sugar and blocking the Ara4Ntransfer reaction, which is catalysed by the arabinosyl transferase (ArnT), would thus restore antimicrobial efficacy of PmB and CAMPs.

Methods L-Ara4N transferase from *B.cenocepacia* was recombinantly expressed in *E.coli* and crude membrane extracts were used for enzyme assays with different synthesized α and β -Ara4N- lipid phosphodiester derivatives as donor and Kdo2-lipid A (KLA) as acceptor. Purification of reaction products was carried out with TLC and subsequent extraction. LC-ESI-MS measurements were done using a RP C5 HPLC column directly coupled to a QTOF system (Bruker). KLA derivatives were identified by accurate mass measurements and MS/MS identification.

Results A sensitive and robust MS based method was developed to monitor enzymatic conversion of KLA to KLA+(2)AraN out of a mixture containing crude membrane and detergents. Different donors were tested, but only when the synthetic alpha-neryl substrate was used, ArnT converted KLA at high rates. These findings will be useful for studying not only the modification of the phosphate groups in the lipid A domain, but also for Ara4N glycoside formation in the core region of several Enterobacteriaceae.

References [1] Charlotte Olagnon, Julia Monjaras Feria, Clemens Grünwald-Gruber, Markus Blaukopf, Miguel Valvano and Paul Kosma. Synthetic phosphodiester-linked 4-amino-4-deoxy-L-arabinose derivatives demonstrate ArnT is an inverting aminoarabinosyl transferase. *Chembiochem*. 2019 Jun 24. doi: 10.1002/cbic.201900349



MALDI-TOF analysis for identification and classification of yeast associated with meadow plants in Central and Western Slovakia

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Introduction MALDI-TOF analysis is a method for identification and classification of microorganisms (yeast, fungi, bacteria) by mass spectrometry, where unique molecular fingerprint (of intracellular proteins) of each microorganism is measured. The fingerprints of each sample and reference are typical for every individual species. The strains with a sequenced D1 / D2 domain of the large subunit (26S) of ribosomal DNA are used as the reference spectra. This method is quick, with high sensitivity and good results, and it is relatively cheap compared to sequencing. Previous studies, which focused on the occurrence of yeasts in flower nectar, showed a narrow spectrum of yeast species, mostly of an ascomycetous character such as *Metschnikowia reukauffii*, *Candida* and *Debaryomyces* species [1] as well as basidiomycetous species belonging to the genera *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces* [2,3].

Methods 183 yeast strains were examined, which were collected in Martin (Central Slovakia, 164 strains) and Rusovce - Bratislava (Western Slovakia, 19 strains) from flowers and leaves of 22 plant species. 20 strains were used as standards. Every yeast strain was cultured on YPD for 4 days. The samples were washed with ethanol, then the intracellular proteins were released with formic acid, and as a matrix the mixture of sinapic and ferulic acid (SAFA) was used. MALDI-TOF MS measurements were made on an UltrafleXtreme (Bruker) in a linear positive mode. The instrument was calibrated on Cytochrome C (Sigma). Spectral data were processed by MALDI Biotyper 3.0 software (Bruker) [4].

Results MALDI-TOF measurements yielded quality spectra of 166 strains (90,7% success rate). In total, 5 yeast species were identified: *Cystofilobasidium macerans*, *Sporidiobolus metaroseus*, *Rhodotorula glutinis*, *Metschnikowia pulcherrima*, *Holtermanniella takashimae*. 39 yeast strains (26.7%), were suggested for sequencing, because their spectra did not match any reference spectra. All the obtained spectra will serve as references in our future work.

Innovative aspects

- MALDI biotyping helps with identifying and selecting strains into groups to make sequencing easier.
- This study proved wider diversity of yeast species associated with plants as was expected.
- The most of identified strains belong to *Sporidiobolus metaroseus*.

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***Andiperla morenensis*: insect lipidome adaptation to life on a glacier**

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Introduction *Andiperla morenensis* is a recently discovered stonefly species endemic to glaciers in Patagonia, Argentina [1]. This cryophilic insect spends its entire life cycle on the ice or in streams of glacier water. We used lipidomics to address how *A. morenensis* is adapted to the cold and compared its storage and membrane lipid profile to the lipidome of the domestic vinegar fly *Drosophila melanogaster*. In this study we want to analyse differences regarding the lipidome of an insect living at moderate temperatures and an insect that is adapted to the cold environment.

Methods *A. morenensis* abdomen and *D. melanogaster* total body samples were mechanically disrupted by adding glass beads and shaking the tubes in a mixer mill. Lipid extraction was conducted according to the MTBE method described by Matyash et al. [2]. The lipids were analysed using a C18-column in UPLC-QTOF-MS as described [3] and data analysis was performed using the Lipid Data Analyzer software [4].

Results Our results show a similar distribution of fatty acid chain lengths within the lipidome of *A. morenensis* and *D. melanogaster* with a much higher level of unsaturation within the *A. morenensis* lipidome in both phosphatidylcholine (PC) and triacylglycerol (TAG). The TAG species in *D. melanogaster* contain 0-5 double bonds with X:1 species being most abundant, while the TAG species in *A. morenensis* cover a huge range of 0-14 double bonds, peaking at 2-4 double bonds per TAG molecule. Corresponding observations were made for PC. These data suggest that the lipidome of *A. morenensis* is adapted to the cold environment through a high level of poly-unsaturation, potentially allowing the insect to maintain membrane fluidity despite very low surrounding temperatures. To prove the assumed presence of poly-unsaturated fatty acids (PUFA) such as 18:4, we had a closer look for the occurrence of acylium ions within the mass spectrum. Our analyses reveal and confirm the presence of 18:4 fatty acids within the lipidome of *A. morenensis*. Moreover, we also observed a significant amount of TAG species with odd-numbered cumulative FA carbon atoms for both species; however, the origin of these TAG species is currently unknown.

Innovative aspects

- *A. morenensis* has a much higher level of poly-unsaturation within its lipidome while having a similar distribution of fatty acid chain lengths compared to *D. melanogaster*.
- Both insects have a significant amount of odd-numbered fatty acid chains with *A. morenensis* having almost twice as much as *D. melanogaster*.
- The *A. morenensis* lipidome contains the highly polyunsaturated fatty acids 18:4 and 20:5, which are very unusual for higher organisms.

References [1] Pessacq. P. et al. A new *Andiperla* Aubert (Plecoptera, Gripopterygidae) species from the Perito Moreno Glacier, Argentina. *Zootaxa* 4664(2): zootaxa.4664.2.7 (2019) [2] Matyash, V. et al. Lipid extraction by methyl- tert -butyl ether for high-throughput lipidomics . *Journal of Lipid Research*. 49, 1137–1146 (2008). [3] Knittelfelder, O. L. et al. A versatile ultra-high performance LC-MS method for lipid profiling. *Journal of Chromatography. B. Analytical Technologies in the Biomedical and Life Sciences*. 951–952, 119– 28 (2014). [4] Hartler, J. et al. Lipid Data Analyzer: unattended identification and quantitation of lipids in LC-MS data. *Bioinformatics* 27, 572–7 (2011).



Non-metal speciation analysis by HPLC-ICPMS/MS

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Inductively coupled plasma mass spectrometry (ICPMS) is gaining increasing recognition as an alternative or complementary element-based mass spectrometric detector for chromatography. The employment of the triple quadrupole technology has been an impactful development in ICPMS, enabling trace level detection of problematic elements such as the non-metals. There have been few reports about the utilization of ICPMS/MS as a chromatographic detector since its introduction in 2012. In the present work, we show the great potential and versatility of ICPMS/MS as a chromatographic detector for the direct determination of chlorine- sulfur-, and phosphorous-containing compounds of interest in different matrices of varying complexity, including food, biological, and environmental samples. We show the clear advantage of ICPMS/MS over single quadrupole ICPMS for the studied non-metals under speciation analysis settings, including the improvement in the detection limits by 10-100 fold (down to 0.1 – 0.5 µg L⁻¹), high selectivity, and the capacity to identify new compounds at trace levels with exploratory non-targeted approaches. We also shed light on approaches to maximize the benefit of ICPMS/MS as a chromatographic detector and highlight important considerations in terms of potential matrix related effects (e.g. carbonate in hard water). Overall, ICPMS/MS offers several advantages over molecular mass spectrometric chromatographic detectors and can be exploited for quantitative as well as qualitative analysis.



MALDI TOF/TOF analysis of oligosaccharides from seaweed samples

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Introduction Seaweeds are the useful species of organisms used mostly in agricultural, medicine and food industry [1]. Their extracts have potential application as heparin analogues due to their sulphate groups [2, 3]. Therefore, it is important to characterize their structures in detail. This work is focused on MALDI TOF analysis of two red seaweed samples (from *Gigartina skottsbergii* and *Furcellaria lumbricalis* species) in both reflectron positive and negative ionisation mode. Primary goal of this work is to obtain signals characteristic for the carrageenan units in our samples.

Methods Samples were first prepared by hydrolysis of 10 mg of sample with 2 M trifluoroacetic acid (2 ml, at 80 °C for 10 or 30 minutes). To eliminate contaminants, samples were subjected to reverse phase SPE (Supelclean™ LC-18 SPE Tubes). After the clean-up, seaweed hydrolysates were concentrated, reconstituted in 10 µl of water and analysed by UltrafleXtreme MALDI TOF/TOF mass spectrometer with the addition of DHB or ATT as a matrix.

Results In the analysis of hydrolysate of the *Furcellaria lumbricalis* species, observed signals were attributable to β-carrageenan oligosaccharide chain (based on hexose and anhydrohexose dimer); and for κ-carrageenan oligosaccharide chain (based on the sulphated dimer). The disulfated hexose dimer is corresponding to μ- and ζ-carrageenan. In the mass spectra of analysed samples from *Gigartina skottsbergii*, signals characteristic for γ-carrageenan (based on monosulfated hexose dimer) and methylated 3, 6-anhydrohexose containing oligomers, were observed. This is in agreement with previous results observed on other species [4]. The presence of all carrageenan structures were confirmed in both reflectron positive and negative ionisation mode as well. Single or double sulphated structures were confirmed by MS/MS (LIFT) fragmentation analysis.

Innovative aspects

- Analysis of oligosaccharides from seaweed hydrolysates by MALDI TOF/TOF mass spectrometry
- Annotation of signals and characteristic fragments acquired from negative and positive ionisation mode
- Application of analytical methods used for characterisation of seaweed oligosaccharides and their modifications (such as rare sulphated forms) may contribute to the development of protocols for glycan analysis also in various other types of samples

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MALDI-TOF mass spectrometry of inhibitor-treated cyanobacteria

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Introduction Cyanobacteria represent a phylum of photosynthesizing bacteria. The whole system of their classification has been revised with the introduction of phylogenetic analyses based on molecular sequencing data. Modern approaches to characterize and differentiate microorganisms involve also matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). There is a general agreement that the identification of microbes based on MALDI-TOF mass spectrometric peptide/protein profiles of intact cells is highly reliable. But data inconsistencies for the same type of cells may appear due to differences in growth conditions, which can profoundly affect the microbial physiology and protein expression profile [1]. The influence of the metabolic status of cyanobacteria on the reliability of MS-based identification was studied in this work.

Methods We recently developed a procedure based on intact cell MALDI-TOF MS, which allows acquiring characteristic peptide/protein profile spectra (fingerprints) of cyanobacteria. The optimized matrix system contained ferulic and sinapinic acids. A reference library of the fingerprint spectra was constructed with representatives of the genera *Chroococcidiopsis* and *Synechococcus* and examined by bioinformatics to characterize similarities and differences, both applicable for taxonomic purposes [2].

Results Currently we are interested in the influence of various inhibitory compounds on the fingerprint spectra as the reliability and reproducibility of intact cell MALDI-TOF mass spectrometry have been disputed with a reference to the dynamic character of the metabolic status of microbial cells [1]. Antimetabolites and antibiotics represent some examples of the analyzed compounds. We show that the treatment generates differences in the representation of peptide/protein peaks but does not preclude a correct identification of the sample in the spectral library. The disturbing effect is more pronounced with antibiotics (e.g. streptomycin and chloramphenicol) than typical antimetabolites such as malonate.

Innovative aspects

- Cyanobacteria were cultivated in the presence of antibiotics and antimetabolites to measure alterations in MALDI-TOF mass spectrometric profiles of intact cells.
- Antibiotics were found more disturbing than antimetabolites.
- These alterations hampered but not precluded identification at the level of species.

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Proteomic and glycomic characterization of microvesicles by mass spectrometry

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Introduction Microvesicles are large membranous spherical vesicles with a diameter in the size range of 100 - 1000 nm. They originated directly from the plasma membrane by its budding followed by their detachment from the cell surface. Microvesicle cargo consists of proteins, lipids, and RNAs whereby its composition is dependent on the stimuli and cell type [1]. Therefore microvesicles isolation and subsequent analyzation of their biological content may be a precious source of information in case of severity, treatment, and prognosis of various diseases.

Methods 400 µl of plasma pool from healthy individuals was centrifuged at 16 000 x g for 40 min at room temperature. The pellet was washed three times to isolate the microvesicles without plasmatic content. For proteomic analysis, microparticle pellet was denatured, reduced, alkylated and digested with trypsin. Released peptides were subjected to reverse phase SPE and ethyl acetate extraction. Proteome was analyzed by nanospray LC-MS/MS Orbitrap Elite™ Hybrid Ion Trap-Orbitrap Mass Spectrometer coupled with UltiMate™ 3000 RSLCnano System and data were processed by MaxQuant software. N-glycans were released from tryptic peptides by PNG-ase F, isolated, permethylated and analyzed by UltrafleXtreme MALDI TOF/TOF mass spectrometer. N-glycan data were processed by GlycoWork Bench software.

Results Proteomic analysis of microvesicles isolated from pooled human plasma resulted in the discovery of 185 unique proteins, from which 158 was confirmed by the Extracellular Vesicle Database [2]. Many of them are considered as the potential biomarkers of various diseases (serotransferrin, alpha-1-antitrypsin, apolipoprotein CIII, etc.) and are bearing N- and O- glycosylation sites. Analysis of microvesicle specific N-glycoprofile, when compared to the serum, determined significant increase in fucosylated structures, with fucose units attached both to the core and antennae.

Innovative aspects

- proteomic and glycomic analysis of microvesicles can represent the innovative source of glycoprotein based biomarkers of inflammation, metabolic diseases, cancer, and other diseases
- preliminary data suggest the specific enrichment of fucosylated N-glycans in microvesicles
- further steps will be focused on the determination of microvesicle specific proteins and their posttranslational modifications

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Effect of organic fertiliser on metabolic profile of hydroponically cultivated tomato

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Introduction Organic waste from farms and greenhouses accumulates and pollutes aquatic ecosystems and atmosphere [1]. Recycling of organic waste fractions in production of vegetables might reduce environmental impact of food production industry. Former studies showed that organic fertilizers can be used for tomato cultivation in soil [2, 3]. Metabolic status of tomato roots receiving organic fertilizer has not been explored comprehensively, primarily because of difficulties during root extraction from soil. Hydroponic cultivation allows assessment of root metabolic and ionic status through analysis of root tissue and xylem sap for better understanding of observed phenotypic alterations.

Methods Tomato plants (*Lycopersicon esculentum* cv. Ailsa Craig) were cultivated in closed soilless cultivation system using organic and mineral fertilisers. Polar metabolites from xylem sap were extracted with water:chloroform mixture and derivatised with MSTFA [4]. Sample acquisition was performed on Agilent 6890 GC equipped with Rxi-5Sil MS column and coupled with 5975 MS detector operating at scan mode (5.5 scans/s) with a m/z range from 50 to 550 [5]. Signal deconvolution and peak annotation was performed with MSDIAL software [6] using alkane RI, integrated GC-EI-MS compound database and in-house library. Statistical analysis and pathway enrichment analysis was performed in MetaboAnalyst [7].

Results Plants grown on organic fertiliser had larger fruit size, yield, root proportion compared to mineral fertiliser. Higher proportion of amino acids and polyamines in xylem sap of organic treatment. Enhanced glutathione and alkaloid biosynthesis in plants received organic fertilisation. Plants grown with organic fertilisation shows elevated stress response which triggers more generative development and increased productivity comparative to treatment with mineral fertilisation.

Innovative aspects

- First report of applying GC-MS assisted metabolomics of xylem sap of tomatoes grown on organic hydroponic cultivation system.

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ICP-MS of meteorite samples: chemometric evaluation of diversity and discrimination

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Introduction The elemental composition of the surface of meteorite particles has been characterized by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Chemometric evaluation of the data comprises a comparison of the variation of the spectra for different meteorite types, and the discrimination of the meteorite types.

Methods Meteorite particles of size 200 - 1000 μm have been pressed on gold plates. Series of LA-ICP-MS spectra have been measured along lines of 200 - 800 μm with a spot size of 50 μm . Standard methods of multivariate statistics have been applied using software in the programming environment R.

Results Principal component analysis (PCA) was performed with a robust method (avoiding undue influence of outlying spectral data) using data transformations of the ion counts that consider the compositional nature of the data (relative abundances are relevant). The diversity of spectra measured on particular meteorites was characterized by the distribution of the Euclidean distances. Classification performance of meteorite classes was estimated by the k-nearest neighbor method (KNN).

Innovative aspects

- Preparation of meteorite samples and analysis by ICP-MS was successful.
- Multivariate data analysis provides powerful tools for an evaluation of the obtained data.

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MALDI MS supports method development for gas-phase electrophoresis of extracellular vesicles

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Introduction Cells release extracellular vesicles (EVs) in response to inflammation, injury, or stress. EVs are involved in a multiplicity of physiological processes, such as the regulation of the vascular function, or in pathological settings, such as cancer, inflammation and thrombosis [1]. Despite great efforts, purification, enrichment, and characterization of EVs poses a challenge as samples are heterogeneous. In addition, EVs and their similar-behaving matrix components are in a size / molecular weight range not easily targetable by most analytical methods. Hence, it was our intention to test the applicability of a nano Electrospray Gas-phase Electrophoretic Mobility Molecular Analyzer (nES GEMMA) [2] for their characterization, similar to liposomes [3].

Methods nES GEMMA was on a TSI Inc instrument (Shoreview, MN, USA) in the size range from 3.0 to 91.4 nm electrophoretic mobility diameter (EMD). MALDI MS was on an UltrafleXtreme (Bruker Daltonics, Bremen, Germany) in RTOF and TOF-PSD mode after SDS PAGE, MS compatible silver staining, and in-gel trypsin digestion followed by a MASCOT search in the NCBIprot database for protein identification.

Results A standard protocol for EV isolation and purification is based on differential ultracentrifugation. Subsequently, presence of vesicle material can be confirmed via nanoparticle tracking analysis and flow cytometry. However, taking corresponding samples to a nES GEMMA instrumentation revealed the occurrence of vast quantities of matrix-related proteins within samples, either resulting from co-purified material or material released after vesicle rupture. A MALDI MS based proteomics approach identified the major contaminations of EV preparations as haemoglobin, β -actin or β -actin-like protein and α -2-macroglobulin. Based on these results, a further purification step - size-exclusion chromatography (SEC) of EV material - was implemented. Obtained fractions were again checked for presence of EVs. Although gas-phase electrophoresis indicated removal of protein components and hinted presence of material with larger EMD, obtained particle count values were low. To date in our hands - even though standard protocols were employed - no satisfactory EV isolation and purification protocol was developed yielding EVs in sufficient quantity and purity for further in-depth characterization. Comparing orthogonal analysis methods, limitations and pitfalls of individual instrumental setups became obvious.

Innovative aspects

- Problems in EV isolation from human plasma and EV characterization employing several orthogonal analysis methods are described
- Proteins co-purified employing standard EV isolation protocols are identified via MALDI MS
- SEC is demonstrated to remove proteinaceous contaminations of EV containing samples

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Quantification of bacitracin in broiler feed and digesta – MRM3 vs. QTOF

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Introduction Bacitracin is a complex of several polypeptides produced by certain strains of *Bacillus subtilis* and *Bacillus licheniformis*. Bacitracin has antibiotic properties against Gram-positive and Gram-negative bacteria [1], and is used for the treatment of infections in farm animals, and as antibiotic growth promoter [2]. In the course of a feeding trial, broilers were fed diets supplemented with either a low growth promoter concentration of 20 ppm bacitracin for five weeks after hatching, or a treatment concentration of 200 ppm bacitracin during their third week of life. Digesta samples were taken on days three, 14, 21 and 35 to evaluate the impact on the intestinal microbiome, resistome and metabolome.

Methods The determination of bacitracin in feed and digesta samples described here, ascertained the presence of the antibiotic during the trial and was a necessary step to understand the observed changes. Two different LC-MS analyses for the quantitative determination of bacitracin in broiler feed and digesta were developed. The first method utilizes fragmentation of the bacitracin peptide using a QTrap instrument. The second method based on HR-MS detects the complete peptide. The presented work compares limit of quantification, selectivity and overall performance of those two methods.

Results Quantification of the target analyte was possible in the used broiler feed, as well as in the cecum, colon and ileum digesta samples gathered during the feeding trial. Both methods showed comparable performance, using the same chromatography method. The measured concentrations showed that most of the bacitracin remained in the digestive tract and was not absorbed by the animals.

Innovative aspects

- Quantification of bacitracin in chicken digesta and feed using LC-MS/MS

References [1] Smith, J.L., Weinber, E.D.; Mechanisms of antibacterial action of bacitracin; *Journal of General Microbiology*, (1962); 28: 559-569. [2] Engberg, R.M., Hedemann, M.S., Leser, T.D., Jensen, B.B.; Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers; *Poultry Science*, (2000); 79(9): 1311-1319.



Correlation of μ XRF and LA-ICP-MS in analysis of human bone-cartilage sample

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Introduction Within this project we aimed for the thorough elemental characterization of complex biological sample – biopsy of human femoral head, which includes both hard and soft tissue, i.e. bone and cartilage. Bone consists mostly of mineralized matter and its elemental composition is in focus of investigation (both in health and disease) of many research groups. Cartilage, however, is primarily composed of organic molecules – such as glycosaminoglycans, proteoglycans, collagen; and its elemental content is not well studied.

Methods Two elemental imaging methods employed - microbeam X-ray fluorescence (μ XRF) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). μ XRF and LA-ICP-MS are widely used in chemical analysis in various applications.

Results In this proof-of-principle study μ XRF was followed by LA-ICP-MS on the very same sample. The measurements resulted in the elemental maps of bone, as well as cartilaginous area of the biopsy (including the tidemark – interface between calcified and hyaline cartilage). In addition to the information on distribution of elements, we also obtained quantitative information by using matrix-matched standards.

Innovative aspects

- Combination of two elemental imaging techniques – LA-ICP-MS and μ XRF – on the very same sample and area (performed on samples of various thicknesses);
- Characterization of human bone and cartilage tissues in terms of elemental distribution;
- Quantification approach for both LA-ICP-MS, as well as μ XRF measurements.



MALDI spectra database for Culture Collection of Yeasts (CCY)

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Introduction MALDI Biotyping is a widely used routine method for microorganism identification utilised in many clinical laboratories all over the world. This method is based on (m/z) ratio fingerprint comparison of intracellular proteins which are unique for individual species [1]. The creation of MALDI spectra database for identification and validation of yeasts in CCY showed that the recommended protocol of protein preparation [1] should be amended for this purpose.

Methods Applicability of five growth media was tested, as well as two different methods of sample preparation, and two types of matrices, including those recommended by Bruker Daltonics [1]. The length of cultivation was also optimized. MALDI-TOF measurements were performed on UltrafleXtreme (Bruker). Obtained data were processed using MALDI Biotyper 3.0 (Bruker).

Results The best results were obtained using YPD, followed by malt extract agar. Some strains proved to show results only using one of these two media. The optimal cultivation was about 4 days, its prolongation led to increase of extracellular contaminants, especially in the case of basidiomycetous yeasts. Method using YPD medium, ethanol washing and combination of sinapic and ferulic acids had the highest success rate with 82.2% quality spectra obtained from measurements of 366 strains. Method with same medium and matrix, but water washing instead of ethanol yielded 69.4% quality spectra from which 25 were not measurable using ethanol washing sample preparation. In comparison, the use of α -cyanohydroxycinnamic acid as a matrix showed only 65% success. Database containing mass spectra obtained from samples cultivated on YPD medium and prepared using ethanol washing and combination of sinapic and ferulic acid matrixes currently consists of 301 high quality spectra including 122 strains identified by D1/D2 LSU (26S) rRNA sequencing (reference spectra) belonging to 69 species. 31 strains are not identified at present.

Innovative aspects

- Optimization of cultivation conditions for yeasts was provided as well as of conditions of sample preparation and measurement
- Database containing 301 spectra was created for CCY and it is continuously supplemented
- This database is used for validation of yeasts deposited in CCY and for identification of new isolates from various sources

References [1] MALDI Biotyper 3.0, User Manual, Revision 2 (2011) Bruker Daltonics, p. 15

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The effects of PTMs on proteomic searches

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Introduction Database search tools are commonly used to identify peptides and proteins in proteomics. Several input parameters are available to refine the identification, such as modifications. Theoretically, a longer modification list results a more sensitive peptide identification. The number of considered post-translation modifications (PTMs), however, also increases the search space, which may grow exponentially with the number of modifications. Therefore in classical search engines (like Mascot) only very few (maximum 3) modifications are recommended. Newer search engines like the “lookup hybrid approach” used by Byonic are better in this respect and modifications don’t increase the search space so dramatically, but it is not straightforward how many modifications should be considered to maximize the number of reliable peptide hits.

Methods In this research we have systematically investigated the effect of PTMs on the number of proteins identified. Several thousands of searches were performed to discover general trends and determine when it is worth to consider a PTM in protein search. We applied 2 different search engines (Byonic and Mascot), seven databases and 30 modifications in these searches altogether. Each search was performed with the same parameters/conditions except the considered PTMs.

Results Our results show that Byonic search engine can handle a lot of modifications and one of its real advantages is to keep search space under control. This makes it possible to use tens, even hundreds of modifications without dramatically increasing the search space in contrast to the traditional database search engines, like Mascot. In some cases 9 PTMs were needed to maximize the number of reliable peptide hits. We could demonstrate that the number of considered PTMs has linear relationship with the number of reliably identified peptides. Thus, increasing the number of PTMs considered will positively affect the sensitivity of peptide identification. Our results also show that as a rule of thumb it is worth considering a PTM if its occurrence is more than 2% in case of Byonic searches, otherwise they cause less sensitive peptide identification.

Innovative aspects

- There is a linear relationship between the number of considered PTMs and the number of identified proteins/peptides.
- PTMs which have >2% occurrence are worth considering in Byonic searches.
- PTM identifications using Mascot require the use of percolator.

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HPLC-MS investigation of glycosaminoglycan disaccharides

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Introduction Glycosaminoglycans, such as heparan sulfate (HS) and chondroitin sulfate (CS) play roles in different regulatory mechanisms and can be descriptive of pathological disorders. In the past years, their investigation came into the focus, but the applied instrumental analytical methods are usually particularly long and lack sensitivity. We decided to analyze HS and CS disaccharides using HILIC-WAX mixed-mode chromatography coupled to negative ionization mass spectrometry.

Methods HS and CS disaccharides were separated on a 250 µm i.d. column packed with Thermo Fisher GlycanPac AXH-1 resin. We mapped the effects of solvent composition (25%-50%-75% acetonitrile content) and salt concentration (5mM – 65 mM ammonium formate, pH=4.4) via isocratic measurements applying 3x10 factorial design. Then we developed fast ammonium formate gradients for optimal resolution. The MS was operated in negative ionization mode. CS and HS disaccharides were quantified in single ion monitoring mode, while optimized MRM transitions were used to determine the ratio of the monosulfated CS disaccharide isomers.

Results Solvent composition had dramatic effects on MS sensitivity, while salt concentration influenced mainly the chromatographic resolution. The combined use of 75% acetonitrile with ammonium formate had overall benefits regarding intensity, detection limits, and peak shape for all salt concentrations investigated. Results obtained with the isocratic measurements suggested the potential use of a salt gradient method in order to maximize separation efficiency. A 3-step gradient from 14 mM to 65 mM ammonium formate concentration proved to be ideal for the separation and quantitation of HS disaccharides. The LOQ for the individual disaccharides was between 2.5–5 fmol/µL and linearity could be observed up to 2 pmol. The salt gradient separation principle was also applicable for the CS disaccharide separation, but a slightly different gradient was necessary for optimal measurements. Using a short, 2-step salt gradient, we were able to separate and quantify CS disaccharides down to 30-50 fmol/µL. Linearity was observed up to 5 pmol/µL. The applicability of the disaccharide analysis methods was demonstrated by measuring heparan sulfate originating from bovine kidney and porcine intestinal mucosa, and chondroitin sulfate from human liver samples.

Innovative aspects

- Chromatographic salt gradients on a HILIC-WAX resin coupled to MS
- Detailed investigation of the retention mechanism of the HS disaccharides
- Fast and high-sensitivity methods developed for HS and CS disaccharide quantitation

References [1] Tóth et al. Salt and solvent effects in the microscale chromatographic separation of heparan sulfate disaccharides, *Journal of Chromatography A*, 2020 Jan 11;1610:460548

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Modified hippocampal lipid signaling pathways triggered by lifestyle conditions: a multi-omics perspective

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Introduction Environmental enrichment (EE) elicits various positive effects on the brain at the molecular, anatomical and behavioral levels including the induction of neural plasticity and enhanced learning and memory. Nevertheless, little is known about the molecular processes involving lipids and proteins of the hippocampal region of the EE animals. The synaptic junction, as central part of the pre- and post-synaptic junction, is the subcellular location where cell-to-cell communication and signal transduction is mediated. Simultaneous multi-omics extraction and analysis are highly informative technics to probe the interplay of proteins and lipids in neuronal signal transduction. Therefore, we employed the SIMPLEX [1] sample preparation workflow as a sensitive explorative approach to screen for lipids and proteins.

Methods Briefly, 225 µL of cold MeOH was added to the purified synaptic clefts and the samples were sonicated for 5 min at 4 °C. Next, 750 µL of cold MTBE were added and the mixture was incubated for 1 h at 4 °C under agitation. Phase separation was induced by adding 188 µL water with 0.1% ammonium acetate. The individual fractions containing lipids (top phase) and proteins (pellet) were then subjected to individual mass spectrometry based workflows. Structural lipids were determined by shotgun lipidomics (HR-MS/MS) while endocannabinoids and proteins were analyzed by nanoLC/ESI HR-MS/MS.

Results In this study, the molecular inventory of the synaptic junction is accessed and a novel mechanism of reduced endocannabinoid signaling by environmental stimuli was identified. Endocannabinoid signaling was linked to increased surface expression of AMPA receptors, which most likely regulates alterations in synaptic plasticity of mice exposed to an enriched environment.

Innovative aspects

- Molecular inventory of the synaptic junction.
- Linking quantitative MS-based multi-omics approaches to functional studies.

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The *B. subtilis* CotA laccase metabolizes Aflatoxin B₁ into Aflatoxin Q₁ and epi-Aflatoxin Q₁

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Introduction Laccase CotA is an endospore coat protein from *Bacillus subtilis* and belongs to multi copper oxidase family. A codon-optimized version of CotA fused to a Strep-tag was recombinantly expressed in *E. coli*. In accordance with literature the purified enzyme showed an >100 increase in activity towards the model substrate ABTS when expressed under microaerobic conditions, due to the improved inclusion of copper. CotA metabolizes Aflatoxin B₁ (AfB₁) into the 3-hydroxylated Aflatoxin Q₁ (AfQ₁, Masri et al., 1974) and its epimer epi-AfQ₁ (328 g/mol). Both substances are not stable in phosphate buffer and degrade into at least two to each other epimeric compounds with an assumed molecular weight of 346 g/mol. The genotoxicity of AfQ₁ is highly reduced compared to AfB₁.

Methods Time course experiments were conducted with CotA incubated with either 5 mg/L of AfB₁ or AfQ₁ as well as controls in sodium phosphate buffer (100 mM pH 7). The collected samples drawn after 0 h, 4 h, 24 h, 48 h and 72 h were diluted to 30% acetonitrile prior analysis. Measurements were performed with a QTrap 5500 (Applied Biosystems) equipped with a electrospray ionization (ESI) source and coupled to a UHPLC system. Chromatographic separation based on a water/methanol with 0.1% formic acid (v/v) gradient was performed on a C18-column. Q₁ scan data were recorded for all samples in the positive and negative ionisation mode and evaluated using Analyst 1.6.2. The genotoxicity of AfQ₁ was assessed and compared to AfB₁ using the SOS Chromotest (EBPI, Canada).

Results The data revealed the formation of several putative reaction products. Formation of additional reaction products cannot be excluded. The predominant formed products could unambiguously be assigned to epi-AfQ₁ and AfQ₁, respectively, by comparison to the respective standards. AfQ₁ standards were shown to be stable in acetonitrile/water (20/80) solution, however, recovery of AfQ₁ and epi-AfQ₁ in the sodium phosphate-buffer was about 74%, 64% and 54% after 24 h, 48 h and 72 h of incubation, respectively, and resulted in the formation of two new products (probably epimers) with suggested molecular weight of 346 g/mol. In general the recovery of the initial AfB₁ concentration in form of the detected metabolites was low, but this could be at least in part explained by the instability and rapid degradation of AfQ₁ itself.

The observed sensitivity threshold of the Chromotest was 125 µg/L for AfB₁. For AfQ₁ even at the highest used concentration of 10 mg/L no increased genotoxicity was observed. These findings are in agreement with literature using the Ames test (Hsieh et al., 1974).

Innovative aspects

- Laccase CotA metabolizes AFB₁ mainly into AFQ₁ and epi-AFQ₁.
- The SOS response on AFQ₁ showed no increased genotoxicity, thus enzymatic degradation by CotA can be considered a detoxification reaction.

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Quantitative determination of phytogetic compounds in chicken breast muscle, skin and fat by GC-MS/MS

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Introduction Phytogetics are plant derived functional substances. They are added to animal feed with the aim to improve palatability and acceptance of feed, support digestion and increase overall performance of farm animals. Phytogetics support the stabilization of the animals intestine leading to reduced stress. Therefore, the risk of sickness decreases which results in reduced or even completely eliminate use of antibiotics. In addition, phytogetics act as natural growth promotors so antibiotic growth promotors are not required any more. The use of antibiotics as growth promotors has been banned in the EU since 2016. At BIOMIN we are producing feed additives containing phytogetics. It is of interest to know the behaviour of such substances, for example, to see if accumulation of these phytogetic substances occurs in animal tissue. Therefore, we developed a method to extract phytogetic substances from chicken tissues: breast muscle, skin and fat. Due to the complexity of these matrices, the sample preparation is very labour-intensive. In addition, the vast number of substances require very selective analytical techniques which have to be very sensitive to be able to detect low concentrations (ppb) of the analytes.

Methods QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique is used very often for the extraction of analytes from complex matrices like animal tissue. We developed a QuEChERS based extraction method for five phytogetic compounds (menthol, methyl salicylate, carvone, thymol and carvacrol) followed by quantitation by GC-MS/MS operated in MRM mode. To increase the selectivity of the method, the substances were derivatized with N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide prior to GC-MS/MS analysis. For the skin and fat samples, a step to remove lipids using Agilent EMR Lipid Sorbent was added.

Results We could achieve low limits of quantitation (LOQ, 2.4 – 4.4 µg/kg) in chicken breast muscle. The precision of the method was always below 20% (within and between run). The recovery was between 76 and 99%.

The LOQ were between 2.7 and 5.9 µg/kg in chicken skin and fat matrix. The methods precision was in the range of 10 – 25% (within and between run) and the recovery between 54 and 106%. Considering the complexity of skin and fat samples the method performs very well.

Innovative aspects

- Use QuEChERS for extraction of phytogetics
- Determination of phytogetics in skin and fat tissue
- Increased sensitivity and specificity by derivatization and use of GC-MS/MS



Fractionation of complex peptide samples using manual HILIC chromatography

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Introduction For protein identification in complex peptide samples, a chromatography fractionation precedes the LC-MS/MS identification. Various types of chromatography are often applied for this purpose, e.g. ion exchange, reversed [1] or normal phase, HILIC etc. This work is focused on the adaptation of manual capillary chromatography with microgradient elution to the HILIC separation of peptide samples.

Methods A home-made capillary of i.d. 250 µm was used in combination with the binary elution gradient consisting of acetonitrile and water with 20 mM ammonium formate. The elution gradient manually formed in a microsyringe starting with higher concentration of acetonitrile was used to elute peptide into 17 fractions. The fractions were dried, resuspended and analysed using common nanoLC-MS workflow (Q Exactive, Thermo).

Results The sample of THP-1 cells lysed and digested with trypsin was analysed both unfractionated and after manual HILIC fractionation. The obtained results showed that the number of identified proteins (as well as peptides) increased about twice. This led to a higher coverage of the analysed sample in the LC-MS experiment. Although the reversed-phase fractionation in the high pH mobile phases under similar conditions provides better results as compared to the HILIC fractionation, a partially different set of proteins and peptides was identified in this case.

Innovative aspects

- a simple way for HILIC peptide fractionation with gradient elution
- easy and inexpensive implementation in the laboratory
- increased number of identified proteins and peptides

References [1] Gilar Martin et al. Journal of Separation Science. 2005, 28(14), 1694–703. DOI: 10.1002/jssc.200500116.

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LC-MS/MS Characterization of Sphingoid Bases from Neutral and Acidic (Glyco)Sphingolipids in Glioblastoma Multiforme

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Introduction Sphingolipids (SLs) are structural components of cell membranes, especially abundant in lipid rafts as complex glycosphingolipids (GSL), while their bioactive metabolites have important roles in fundamental cellular processes such as cell signalling, proliferation and cell death. Sphingoid bases (SBs) are long-chain aliphatic amino alcohols and represent the structural backbone of sphingolipid molecules. Sphingolipid content and metabolism are impaired during pathophysiological conditions such as oncogenic transformations and neurodegeneration. Glioblastoma multiforme (GBM) is the most aggressive primary brain tumour and the most common malignant brain tumour among adults. Aberrations in sphingolipid metabolism have been implicated in promoting the aggressiveness of glioblastoma multiforme.

Methods Sphingoid bases were quantitatively and qualitatively analysed in neutral sphingolipids (glycosphingolipids, sphingomyelin, ceramides) and acidic glycosphingolipids (GSL; gangliosides and sulfated GSL) isolated from three GBM samples and three samples of healthy human brain tissue. Total neutral SL and acidic GSL were extracted and purified from homogenized tissue samples by modified Folch partition and submitted to acid hydrolysis to obtain sphingoid bases, which were further analysed by LC-MS/MS using Dionex Ultimate 3000 RSLC coupled to Bruker amaZon ETD ion trap system. Quantification was performed through the calibration curve obtained by external standard.

Results The total sphingoid bases concentration detected in GBM is approx. 100 times lower than in healthy human brain. Sphingosine (d18:1) was predominant sphingoid base (>90%) in all analysed samples. The total SBs concentration in acidic GSL were significantly lower than in neutral SLs. Five different SBs were detected in neutral SLs of GBM and healthy human brain tissue and structurally characterized as d16:1, d18:2, d18:1, d18:0 and d20:1. In acidic GSL of GBM and healthy human brain only d16:1 was not detected. The SBs d18:1 and d20:1 were detected as two isomeric forms only in acidic GSLs but not in neutral SLs. The highest amount of d20:1 (5-15%) SBs was detected in acidic SLs of GBM, while only 0.01-0.07% of d20:1 was detected in neutral SLs.

Innovative aspects

- Over 100 times lower concentrations of SBs were detected in GBM compared to healthy human brain tissue.
- Differences in composition and amount of sphingoid bases were detected in neutral vs acidic sphingolipids, pointing out the diversity in biosynthetic pathways of simple and complex SLs.
- The important aspect of SL structural and functional diversity is the variability of sphingoid bases, besides the well-known variability of fatty acid residues in ceramide structures.

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Bacterial Responses to Different Cultivation Conditions – A Correlated Multimodal Imaging Approach

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Introduction *Lysobacter* spp. are common bacterial inhabitants of the rhizosphere. However, the impact of the rhizosphere conditions on their physiology is still relatively understudied. To provide clues on the behaviour of *Lysobacter* spp. in this ecological niche, we investigated the physiology of *L. capsici* AZ78 on LBA and on a growth medium containing components of the plant rhizosphere. A halo surrounding the AZ78 colony on RMA was a first visible effect. Metabolic profiling by mass spectrometry imaging revealed increased diversity of analytes produced on RMA as compared with LBA.

Methods AZ78 was grown on LBA in Petri dishes, cells were picked and suspended in sterile saline solution and precipitated by centrifugation. After washing cells were spotted on Rhizosphere- Mimicking Agar (RMA). AZ78 cells were also spot-inoculated on LBA. AZ78 growth was monitored over time. qRT-PCR was carried out to follow gene expression levels, cell motility was assessed by capturing stop-motion animation using a phase-contrast microscope, biofilm formation was studied by TEM and spatially resolved metabolite information was gathered by MALDI-MSI after preparing a miniaturized assay directly on a glass slide.

Results Having a picture of how AZ78 might behave in the rhizosphere provides useful insights on its ability to colonise this environment and to produce bioactive secondary metabolites. The appearance of AZ78 macrocolonies on RMA was different compared to that originated on LBA and the main visible differences consisted in the colour and formation of a surrounding halo. The quantity of AZ78 viable cells present in the macrocolony on RMA was strongly reduced compared to LBA. It was presumable that AZ78 cells face nutrient limitation during their growth on RMA. Bacteria respond to nutrient limitation through the activity of master regulators and a higher expression level of *clp* in AZ78 grown on RMA was observed. Colony morphology was characterised by the presence of many membrane vesicles whereas microcolonies on RMA did not show vesicles but a more extended monolayer outer-ring with cells disposed in small groups. Metabolite profiles investigated by MALDI MSI showed a higher quantity number of biologically relevant signals on RMA. Almost no spatial differentiation was observed for the LBA monolayer but for RMS a center-core and outer-ring were clearly distinguishable by different metabolite profiles. 35 metabolite signals were detected and annotated.

Innovative aspects

- MS Imaging of secondary metabolites directly from micro-assays
- Spatial distribution of metabolites correlates with biofilm formation
- RMA exclusive metabolites identified

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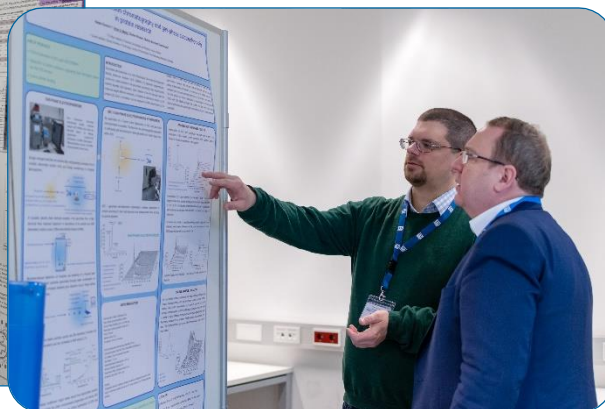
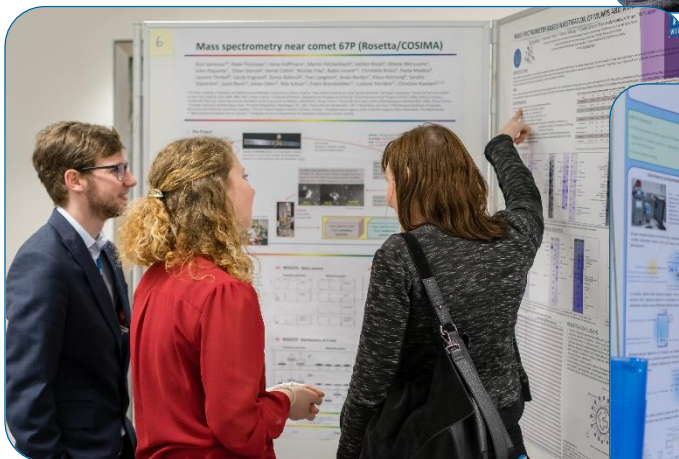
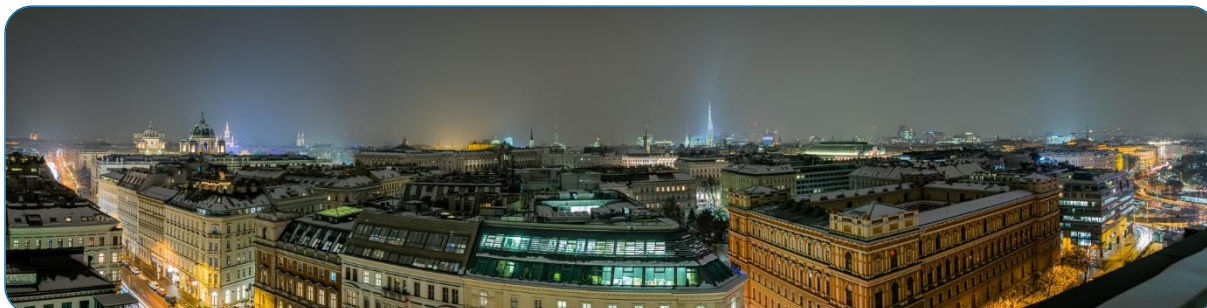


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