LIPIDOMICS FORUM

10. - 12. November 2019

VENUE
Research Center Borstel
Parkallee 1-40
23845 Borstel
Germany

ORGANIZERS
Robert Ahrends | ISAS
Nicolas Gisch | RCB
Nils Hoffmann | ISAS
Dominik Kopczynski | ISAS
Dominik Schwudke | RCB

Coordination: Jutta Passarger

AbstractBook

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Program at Glance

Sunday | **November 10**

10:00 – 16:30	**LIFS Workshop**	
16:00	REGISTRATION	
18:00	WELCOME	Dominik Schwudke, Stefan Ehlers
18:10	**Introduction to the International Lipidomics Society (ILS)**	Kim Ekroos, Robert Ahrends
18:30	**OPENING KEYNOTE** | Satyajit Mayor, National Centre for Biological Sciences - TIFR (Bangalore, India)	
	The skin of a living cell: an active actin-membrane composite.	
19:30	DINNER	
20:00	COME TOGETHER / SALON MUSIC AND TANGO	

PROGRAM AT GLANCE

Monday | **November 11**

9:00 – 10:00	**KEYNOTE 1** | Thomas Eichmann, University of Graz (Graz, Austria) **Explorative Lipidomics by Ion Mobility Mass Spectrometry.**	
10:00 – 12:00	**Talks from Abstract – SESSION 1** | Chair: Nicolas Gisch	
12:00 – 13:00	**KEYNOTE 2** | Lev Becker, University of Chicago (Chicago, US) **Adipose tissue-derived lipids promote a mechanistically distinct pro-inflammatory macrophage phenotype in obese patients and mice.**	
13:00 – 14:00	LUNCH	
14:00 – 15:00	**KEYNOTE 3** | Shane R. Ellis, Maastricht University (Maastricht, Netherlands) **A deeper view of localized lipid biochemistry using advanced mass spectrometry imaging approaches.**	
15:00 – 17:00	**Talks from Abstract – SESSION 2** | Chair: Robert Ahrends	
17:00 – 18:00	**KEYNOTE 4** | Hülya Bayir, University of Pittsburgh (Pittsburgh, US) **Redox Lipidomics: Clinical applications for mechanistic understanding of disease pathogenesis, biomarker discovery and treatment.**	
18:00 – 20:00	**POSTER SESSION**	
20:00	DINNER COME TOGETHER / JAZZ	

Tuesday | **November 12**

9:00 – 10:00	**KEYNOTE 5** | Karsten Hiller, Technische Universität Braunschweig (Braunschweig, Germany) **Metabolic crosstalk between mammalian cells and *Clostridium difficile* bacteria.**	
10:15 – 11:15	**Talks from Abstract – SESSION 3** | Chair: Nils Hoffmann	
11:15 – 11:30	**LIPIDOMICS FORUM AWARDS for best Talk and Poster**	Ronny Herzog, Dominik Schwudke
11:30 – 12:00	**Foundation of the International Lipidomics Society (ILS)** - Aims and mission statement - Introduction of the society structure - Overview of the different working groups - Presentation of the current board and their responsibilities	Kim Ekroos, Robert Ahrends
12:00 – 13:00	LUNCH	
13:00 – 14:30	**PANEL DISCUSSION** | **What is next?** Future steps of the ILS and the involvement of you!	

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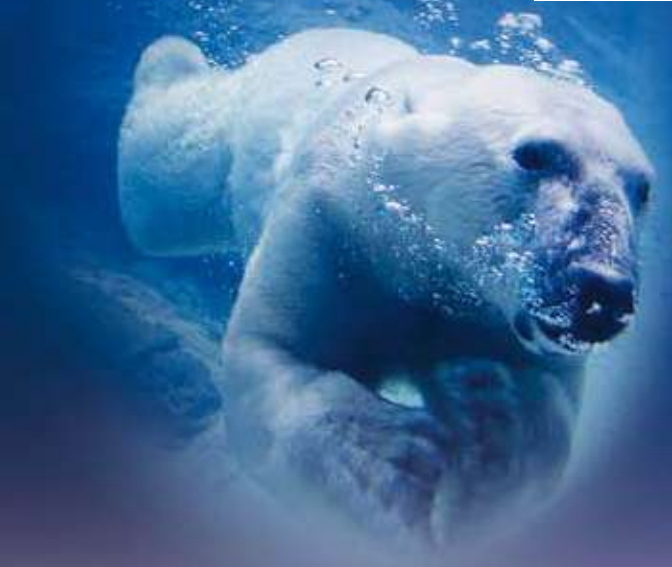

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Sunday | **November 10**

10:00 – 16:30	**LIFS Workshop**	Nils Hoffmann, Fadi Al Machot, Jacobo Miranda Ackermann
10:00 – 10:45	INTRODUCTION	
10:45 – 12:30	**PARALLEL SESSION** Workshop 1 and 2	
12:30 – 13:30	LUNCH	
13:30 – 16:30	**PARALLEL SESSION** Workshop 1 and 2	
16:00	REGISTRATION	
18:00	WELCOME	Dominik Schwudke, Stefan Ehlers
18:10	**Introduction to the International Lipidomics Society (ILS)**	Kim Ekroos, Robert Ahrends
18:30	**OPENING KEYNOTE** Satyajit Mayor, National Centre for Biological Sciences - TIFR (Bangalore, India) **The skin of a living cell: an active actin-membrane composite.**	
19:30	DINNER	
20:00	COME TOGETHER Salon Music and Tango	Duo Juhl / Reiling

OPENING KEYNOTE
The skin of a living cell: an active actin-membrane composite.

Satyajit Mayor

National Centre for Biological Sciences, Tata Institute for Fundamental Research, Bellary Road, Bangalore

The plasma membrane of a living animal cell is a lipid bilayer, composed of a remarkably complex mixture of lipid species and proteins, draped over a dynamic cortical actin mesh. The composition of the lipid layer of a given cell type is reproducibly maintained, influencing the functioning of the plasma membrane. The way the cell maintains such a precise lipid composition is not fully understood; a variety of extrinsic and intrinsic factors including dietary inputs and synthetic capacities, respectively, could contribute. The cell also regulates the local composition of its membrane at the meso scale by actively controlling the formation of lipidic nano-domains. These nano domains consist of clustered lipid anchored proteins at the outer leaflet and phosphatidylserine at the inner leaflet, engaged in trans-bilayer interactions via their lipid tails. These nanodomains are templated at the cytoplasmic leaflet by juxta membrane acto-myosin machinery. In my talk, I will describe how a specific membrane receptor, the fibronectin-binding integrin receptor activates a signaling cascade which triggers actin polymerization and myosin activation. In parallel, integrin-driven mechano-sensitive activation of the talin-vinculin axis couples the dynamic acto-myosin machinery to the construction of these nano-domains. This provides a cell the capacity to integrate both chemical and physical cues via the construction of mechanoresponsive membrane domains, which participate in fine tuning receptor output.

Monday | **November 11**

9:00 – 10:00 **KEYNOTE**
Thomas Eichmann, University of Graz (Graz, Austria)
K 01 **Explorative Lipidomics by Ion Mobility Mass Spectrometry.**

10:00 – 10:45 **SESSION 1 A** | Chair: Nicolas Gisch

Aiko Barsch
T 01 **Investigating increased lifespan in *C. elegans DAF-2* mutants by 4D Lipidomics**

Michael Witting
T 02 **A Lipid-Atlas of the Model Organism Caenorhabditis elegans**

10:45 – 11:00 COFFEE BREAK

11:00 – 12:00 **SESSION 1 B** | Chair: Nicolas Gisch

Kathrin Engel
T 03 **Lipocalin-2 and perilipin-5 have a significant impact on the hepatic lipid composition**

Olga Vvedenskaya
T 04 **Mutation-specific changes in fatty liver lipidome profile:**
An analysis of human non-alcoholic fatty liver by shotgun lipidomics

Lee Gethings
T 05 **Quantifying the lipidome for respiratory disease: A rapid and comprehensive HILIC-based targeted approach**

12:00 – 13:00 **KEYNOTE**
Lev Becker, University of Chicago (Chicago, US)
K 02 **Adipose tissue-derived lipids promote a mechanistically distinct pro-inflammatory macrophage phenotype in obese patients and mice.**

13:00 – 14:00 LUNCH

Explorative Lipidomics by Ion Mobility Mass Spectrometry

Thomas O. Eichmann[1,2]

[1] Center for Explorative Lipidomics, BioTechMed-Graz, Graz, Austria
[2] Institute of Molecular Biosciences, University of Graz, Graz, Austria

K 01

Lipids represent a biologically essential family of hydrophobic and amphipathic biomolecules that are vital components of living species. In higher organisms, lipids function as energy reservoir, structural components of membranes, signaling molecules, and bioactive metabolites of the immune system. Moreover, lipids may be the cause or the result of aberrant metabolism and represent possible early markers for disease states and indicators for pathologies such as obesity and the metabolic syndrome, neurodegenerative diseases, severe skin disorders, or cancer. Today, mass spectrometry-based lipidomic techniques can be used to decipher the extremely high structural diversity of lipids that is based on their various backbones and headgroups, and due to high variations in chain length, degree of unsaturation and positional isomerism of constituent acyl- and alkyl-chains. To date, untargeted lipid analysis of complex, biological samples relies on automated and robust lipid feature identification and annotation. Due to great efforts over the past decades, several lipid databases, build on accurate mass and MS/MS fragment information, are accessible for the research community and support fast lipid identification and data processing.

To continue these endeavors, the Center of Explorative Lipidomics (CEL) in Graz focuses on comprehensive lipidomic characterization and annotation of diverse biological sources. Most recently, ion mobility coupled mass spectrometry (IM-MS) is applied, allowing additional lipid separation in the gas phase. IM-MS enables identification of low abundant, rare lipid clusters, and facilitates simultaneous acquisition of orthogonal lipid information (collisional cross section, CCS). Besides accurate mass, CCS constitutes an additional, intrinsic parameter that improves the precision and selectivity of lipid identification.

Examples will be given of how (i) IM-MS can support characterization of lipids, especially of rare classes such as ether lipids or estolides, (ii) CCS supplementation of databases can increase selectivity of automated lipid feature identification, and (iii) new software tools can be integrated for rapid data processing.

The addition of orthogonal parameters, together with biological-probability clustering of lipid entries (organism specific subsets) depicts one possible approach to decrease false-positive discovery rates of database applications in untargeted lipidomic studies. At CEL, CCS-containing databases for diverse model organisms used in lipid research, are currently under construction and evaluation and will be soon available for inter-laboratory cross validation.

Investigating increased lifespan in *C. elegans* DAF-2 mutants by 4D Lipidomics

Aiko Barsch[1],
Sven W. Meyer[1], Ulrike Schweiger-Hufnagel[1], Philipp Schmitt-Kopplin[2], Michael Witting[2]

[1] Bruker Daltonics, Bremen, Germany
[2] Helmholtz Center Munich, Munich, Germany

T 01

The small nematode *Caenorhabdtis elegans* is one of the premier biomedical model organisms and employed in different aspects of basic and applied science such as ageing and longevity research. The *C. elegans daf-2* mutant investigated in this study encodes for the insulin-like growth factor 1 (IGF-1) receptor. DAF-2 is part of the first metabolic pathway discovered to regulate the rate of aging. The mutant worms show extreme changes in animal phenotype, including increased adult size and an increased lifespan. Furthermore, changes in the lipid content were reported in *daf-2* mutants.

Recently, lipidomics, the systematic analysis of all lipids in a given system, joined the *C. elegans* toolbox. Due to several possible headgroups and fatty acids that can be synthesized by *C. elegans* the lipidome is highly complex. High coverage of detected lipids with a corresponding MS/MS spectrum is required for lipid identification. Applying the timsTOF Pro system this is realized by the unique PASEF (Parallel Accumulation Serial Fragmentation) acquisition mode. This PASEF scan mode offers the possibility to generate MS/MS spectra with uncompromised high data quality at high acquisition speeds for lipid profiling. PASEF can generated clean MS/MS spectra by separation of isobaric lipid species co-eluting in the LC domain. Additionally, Trapped Ion Mobility Separation (TIMS) provides reproducible CCS values for increased confidence in lipid identification. Here we present a fully integrated workflow for evaluating 4D-lipidomcis data in one software solution: MetaboScape. Comparing lipid extracts from *C. elegans* wild type and *daf-2* mutants enabled pinpointing of characteristic lipids and their confident identification. Merging information from PASEF MS/MS spectra acquired in positive and negative mode provided complementary information on lipid headgroups and fatty acid side chains. By matching measured CCS values to predicted values generated by the machine learning based CCSPredict routine in MetaboScape increased confidence in lipid assignment even further.

A Lipid-Atlas of the Model Organism *Caenorhabditis elegans*

Michael Witting[1,2],
Victoria Hänel[1], Christian Pendleton[1,2], Bastian Blume[1]

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[2] Chair of Analytical Food Chemistry, TUM School of Life Sciences, Technische Universität München, Maximus-von-Imhof-Forum 2, 85354 Freising

T 02

Lipids play important roles in many different aspects of biology. A major model organism to study lipid metabolism is the small nematode *Caenorhabditis elegans*. Many insights have been generated by the use of GC-MS based analysis of lipid derived fatty acids, but analysis of intact lipid only recently joined the *C. elegans* toolbox. The worm harbors several peculiarities in its lipid metabolomics, e. g. it uses branched chain fatty acids which are produced almost exclusively (99,9 %) by the worm itself. Furthermore, *C. elegans* uses C15:0iso instead of palmitic acid to produce a C17iso sphingoid base.

These and other features make the *C. elegans* lipidome distinct from other species (e.g. human, mice) and lipids that occur in *C. elegans* are only partially covered in lipid databases. Based on a consensus metabolic reconstruction and known lipid biochemistry we have started to generate a stoichiometry model of the worms lipid metabolism and predicting potentially present lipids. Known biochemical reactions were used as templates to produce in silico all possible lipids together with their biosynthesis and degradation pathways. The generated database of lipids will serve as future reference for investigations into the lipidome of the worm. We have started to compare predicted lipids with several different lipid extracts from *C. elegans* measured with UPLCUHR-ToF-MS.

Lipocalin-2 and perilipin-5 have a significant impact on the hepatic lipid composition

Kathrin M. Engel[1],
Anastasia Asimakopoulou[2], Jürgen Schiller[1], Ralf Weiskirchen[2]

[1] Leipzig University, Medical Department, Institute for Medical Physics and Biophysics
[2] RWTH University Hospital Aachen, Institute for Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry

T 03

Lipocalin-2 (LCN2) is a proinflammatory adipokine that is up-regulated in different pathologies, such as obesity, inflammation and different types of cancer. It acts as a secreted protein and plays an important role in the transport of small lipophilic ligands, among them steroids, retinoids, hormones and fatty acids. Recently it has been shown that LCN2 is a key regulator of the hepatic lipid homeostasis which downstream regulates the expression of the lipid droplet-associated protein Perilipin-5 (PLIN5). PLIN5 regulates the storage and release of triglycerides from intracellular lipid droplets and was shown to be up-regulated in liver steatosis. A lack in either LCN2 or PLIN5 impedes the formation of lipid droplets in hepatocytes and leads to fatty liver.

In the present study we investigated the hepatic lipid profiles of wildtype, LCN2 and PLIN5 knockout mice that have been fed either a normal chow or a high fat diet. Analyses were performed by means of combined thin-layer chromatography and electrospray ionization mass spectrometry. We found significant differences in the fatty acyl compositions of lipids between the study groups. LCN2 and PLIN5 seem to influence particularly the levels of linoleic acid and arachidonic acid in the liver. These differences are gender independent. Due to this gender non-specificity the phenotypes of LCN2 and PLIN5 knockout animals can be interpreted robust.
The detailed reasons for the hepato-protective properties of LCN2 and PLIN5 are unknown so far. Our investigations of the LCN2/PLIN5 network could provide deeper insights into liver steatosis, especially in the context of non-alcoholic fatty liver, the dominating liver disease in the Western civilization, for which the available therapeutic approaches are still insufficient.

Mutation-specific changes in fatty liver lipidome profile: An analysis of human non-alcoholic fatty liver by shotgun lipidomics

Olga Vvedenskaya[1],
Oskar Knittelfelder[1], Alessandra Palladini[5], Judith Wodke[3], Jacobo Miranda Ackerman[1], Veera Raghavan Thangapandi[2], Edda Klipp[3], Josch Pauling[4], Jochen Hampe[2], Andrej Shevchenko[1]

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[2] University Hospital Carl Gustav Carus, Dresden
[3] Humboldt University, Berlin
[4] Technical University, Munich
[5] Paul Langerhans Institute, Dresden

T 04

Nonalcoholic fatty liver disease (NAFLD) affects up to 35 % of the world population and, without the proper treatment, NAFLD may lead to the development of cirrhosis or liver cancer. Histological analysis shows that NAFLD manifests as the accumulation of neutral lipid in hepatocytes, however the underlying molecular mechanisms remain unknown. This work reports on the full lipidome quantitative profiling of NAFLD biopsies, as well as the lipidome composition correlated with the mutation risk factors in patients.

Shotgun lipidomics was applied to human liver biopsies; samples were histologically characterized, including the evaluation of NAFLD development stage, and screened for known and suspected NAFLD mutation risk markers, PNPLA3 and MBOAT7 respectively. Meta-data collected from the same patients included BMI, age, disease stage, and hepatitis status, comorbidities and medication.

The MS analysis of the full cohort, comprising 367 liver biopsies, has been recently completed. Variation of the measurements across all the batches is considered acceptable (e. g. QC standard deviation was 15 % for neutral lipids and 24 % for phospholipids). The obtained lipidome underwent post-acquisition filtering, resulting in the dataset of 316 lipids from 19 lipid classes measured in 367 independent liver samples.

The PNPLA3 mutation-based analysis of NAFLD and NASH groups reveals the distinct changes nearly exclusively in TG species in the samples where the homogeneous mutation was present compared to the samples with no mutation in PNPLA3. Interestingly enough, 72 % significantly changed TG species overlap in NAFLD and NASH. The MBOAT7 mutation-based analysis of NAFLD and NASH groups on its turn shows the changes nearly exclusively in PI species in the samples where the homogeneous mutation was present compared to the samples with no mutation in MBOAT7. In the case of this mutation 71 % significantly changed PI species overlap in NAFLD and NASH.

This analysis allows suggesting the presence of different development and manifestation mechanisms of NAFLD and NASH depending on various mutations.

Quantifying the lipidome for respiratory disease: A rapid and comprehensive HILIC-based targeted approach

Lee Gethings[2],
Nyasha Munjoma[2], Giorgis Isaac[1], Robert S. Plumb[1]

[1] Waters Corporation, Milford, MA
[2] Waters Corporation, Wilmslow, UK

T 05

Respiratory linked conditions associated with chronic obstructive pulmonary disease (COPD), asthma, and infection are increasing with significant associated socio-economic costs. Recent reports have shown those costs to exceed 11 billion per year for cases recorded in the UK. COPD, in particular, is a heterogeneous disease that is a major cause of illness and death worldwide. The combination of genetic and lifestyle factors are known to contribute towards increasing the probability of developing the condition. Here, we describe a lipidomics approach to reveal biomolecular factors that may be involved in these pathological processes.

The analyses of plasma samples from three biological states of varying phenotype (control, COPD and asthma patients) were conducted. Sample extraction was performed using a simple protein precipitation with a precooled isopropanol at 4 °C at a ratio of 1:5 (plasma: isopropanol). Lipid analysis was performed using LipidQuan, a hydrophilic interaction chromatography (HILIC) based LC-MS/MS streamlined and integrated lipidomics workflow. The platform consists of highly specific MRM transitions based on the fatty acyl chain fragments that are used for identification and quantification of multiple lipid species. Chromatographic conditions allowed for the separation of individual lipid classes in 8 minutes. Data were processed using TargetLynx and Skyline. Statistical analysis was conducted using SIMCA and additional data visualisation provided by MetaboAnalyst.

Targeted LC-MS data were acquired in positive and negative ion electrospray modes. Samples were randomized and two technical replicates per sample were acquired. Data were acquired using a triple quadrupole MS in MRM mode of acquisition. HILIC chromatography was employed resulting in the separation of lipids by class. Separations were performed in a rapid manner with a turnaround time of 8 minutes, injection to injection. Deuterated standards were used to assess linear response and calibration curves were constructed for each standard. These standards included PC, LPC, LPE and SMs for positive ion mode. Corresponding PC, PG, PE and PIs were used for negative ion mode. For example, SM 18:1 (d9) exhibited a linearity with an R^2=0.9969. Equally, chromatographically reproducibility is showed to ≤ 2 % RSD for over 1500 injections.

Biological significance of the results was established by merging the data from all experiments and performing pathway analysis. Statistical analysis of the data revealed clear separation between the various cohorts. Unsupervised PCA resulted in the separation of healthy controls, COPD and asthma patients. Application of the metadata also revealed significant differences between smoking status, with subsets readily observed within the COPD population. Loadings plot analysis revealed that FFA, LPC, PC and SM classes to be the main contributors to sample type clustering. Additional ANOVA/t-test and hierarchical clustering showed all the lipid classes referenced to be up-regulated with the exception of PCs. A decrease in the level of PCs was observed as significant for subjects associated with smoking. Overall, PCs are a potential marker for oxidative stress (immune activation). Pathway analysis revealed a number of components related to inflammation, oxidative and immunity processes were identified as significant and associated with signalling, metabolic and regulatory pathways.

Adipose tissue-derived lipids promote a mechanistically distinct pro-inflammatory macrophage phenotype in obese patients and mice.

Lev Becker

Ben May Department for Cancer Research, The University of Chicago

K 02

Adipose tissue macrophage (ATM)-driven inflammation plays a key role in obesity and insulin resistance; however, factors activating ATMs are poorly understood. Using a proteomics approach, we show that markers of classical activation are absent on ATMs from obese humans, but readily detectable on airway macrophages of patients with cystic fibrosis, a disease of chronic bacterial infection. Moreover, treating macrophages with lipids derived from obese human or mouse adipose tissue produces a 'metabolically-activated' (MMe) phenotype distinct from classical activation. Markers of metabolic activation are expressed by pro-inflammatory ATMs in obese humans/mice and are positively correlated with adiposity and insulin resistance. Here, we will discuss *i*) the mechanisms by which MMe macrophages regulate obesity and its associated diseases, *ii*) the unique mechanism by which lipids promote inflammatory cytokine expression in MMe macrophages, and *iii*) opportunities to leverage metabolomics to identify adipose tissue-derived lipids that support the MMe phenotype.

Monday | **November 11**

14:00 – 15:00 **KEYNOTE 3**

Shane R. Ellis, Maastricht University (Maastricht, Netherlands)

K 03 **A deeper view of localized lipid biochemistry using advanced mass spectrometry imaging approaches.**

15:00 – 15:45 **SESSION 2 A** | Chair: Robert Ahrends

Michal Holčapek

T 06 **Quality Assurance and Quality Control in the Lipidomic Quantitation Using Lipid Class Separation – Mass Spectrometry**

Harald Köfeler

T 07 **Lipid Data Analyzer: A novel LC-MS based lipidomics software tool**

15:45 – 16:00 **COFFEE BREAK**

16:00 – 17:00 **SESSION 2 B** | Chair: Robert Ahrends

Catherine G. Vasilopoulou

T 08 **Trapped Ion Mobility Spectrometry (TIMS) and PASEF acquisition method enable in-depth 4D lipidomics**

PRICE Patrick Helmer

T 09 **Analysis of oxidized cardiolipins by heart-cutting HPLC coupled to ESIHRMS**

PRICE Johanna Striesow

T 10 **Oxidative lipidomics of blood**

17:00 – 18:00 **KEYNOTE**

Hülya Bayir, University of Pittsburgh (Pittsburgh, US)

K 04 **Redox Lipidomics: Clinical applications for mechanistic understanding of disease pathogenesis, biomarker discovery and treatment.**

18:00 – 20:00 **POSTER SESSION**

20:00 **DINNER**

COME TOGETHER / JAZZ Matthäus Winntzki: piano | John Hughes: double bass | Björn Lücker: drum set

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A deeper view of localized lipid biochemistry using advanced mass spectrometry imaging approaches

Shane R. Ellis

M4I, The Maastricht Multimodal Molecular Imaging Institute, Maastricht University

K 03

Lipids are an underappreciated and extremely complex family of molecules playing important roles in many cellular functions. Mass spectrometry imaging (MSI) is a powerful approach enabling localized changes in lipid metabolism to be visualized on the micrometer level throughout tissues. However, significant limitations with respect to sensitivity and lipid identification capabilities means MSI has largely failed to translate the distributions of ions having distinct m/z values to those of defined molecular species, meaning insight into the biochemical origins and implications of MSI data have been lacking. In this talk I will present recent development made in MSI to bridge this gap between images and molecules. In particular new ionization methods to improve the range of lipids that can be detected in MSI experiments, parallelized MS/MS to automatically identify lipids observed in an MSI experiment and the use of gas-phase chemistry to resolve and unambiguously identify lipid isomers will be presented. Combined, these methods open the door for detailed studies of lipid biochemistry within tissues and provide an avenue to associate lipid distributions with localized enzymatic activities.

Quality Assurance and Quality Control in the Lipidomic Quantitation Using Lipid Class Separation – Mass Spectrometry

Michal Holčapek,
Denise Wolrab, Michaela Chocholoušková, Robert Jirásko, Ondrej Peterka

University of Pardubice, Department of Analytical Chemistry, Pardubice, Czech Republicn.pl

T 06

Many lipidomic studies report potential disease biomarkers, but there are often difficulties to reproduce these finding in other laboratories or even to translate into the routine clinical use. Reasons behind these problems are rather complex, and it is important to identify key aspects involved in the reproducibility issues of the lipidomic quantitation among different laboratories, different analytical approaches, and during longer period of time. Various factors may play a role, such as the lack of full method validation in line with recommendations of authoritative organizations, such as FDA and EMA, the missing use of quality control samples, problems during pre-analytical phase, etc.

We will present examples with the recommended way of analytical validation and the use of quality control samples for high-throughput and large-scale lipidomic quantitation using 2 approaches: hydrophilic interaction liquid chromatography mass spectrometry (HILIC/MS) and ultrahigh-performance supercritical fluid chromatography – mass spectrometry (UHPSFC/MS). The first step is careful optimization of the whole methodology including preanalytical phase, sample extraction, MS analysis, data processing, and statistical evaluation. The typical steps included in the analytical method validation will be presented and illustrated on examples of HILIC/MS and UHPSFC/MS.

The optimized and validated methods are then applied for the reference material SRM 1950 human plasma from NIST, and concentrations are compared with published data in the literature[1, 2]. The final step is the application to real clinical cohorts.

This work was supported by project No. 18-12204S (Czech Science Foundation).

1 O. Quehenberger et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. J. Lipid Res., 51:3299–3305, 2010.
2 J. A. Bowden et al. Harmonizing Lipidomics: NIST Interlaboratory Comparison Exercise for Lipidomics using Standard Reference Material 1950 Metabolites in Frozen Human Plasma. J. Lipid Res., 58(12):2275– 2288, 2017.

Lipid Data Analyzer: A novel LC-MS based lipidomics software tool

Harald C. Köfeler[1],
Jürgen Hartler[2], Martin Hardt[4], Dominik Schwudke[3], Benjamin W. Neuman[2], Stephan Pleschka[1], John Ziebuhr[1]

[1] Medizinische Universität Graz, Austria
[2] Technische Universität Graz, Austria

T 07

Lipid Data Analyzer (LDA) is a decision rule set based software, which enables automated and reliable annotation of lipid species and their molecular structures in high-throughput fashion from chromatography coupled tandem mass spectrometry data. The key strengths of this software compared to conventional spectral library approaches are: *i*) reliability; *ii*) ability of identifying novel lipid molecular species; *iii*) easy extendibility; iv) capability of unambiguously detecting coeluting species. The instrument independence of LDA was proven in many mass spectrometric experiments, running at various collision energies on various Orbitraps, Q-Exactive, Q-TOF and QTrap instruments. In a benchmark to other software, LDA proved to be superior in terms of positive predictive value and sensitivity.

Trapped Ion Mobility Spectrometry (TIMS) and PASEF acquisition method enable in-depth 4D lipidomics

Catherine G. Vasilopoulou[1],
Karolina Sulek[2], Andreas-David Brunner[1], Aiko Barsch[1], Sven Meyer[3], Ulrike Schweiger-Hufnagel[3], Ningombam Sanjib Meitei[4], Matthias Mann[1,2], Florian Meier[1]

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[3] Bruker Daltonik GmbH, Bremen, Germany;
[4] PREMIER Biosoft, Indore, India

T 08

Lipids form a highly diverse group of biomolecules fulfilling central biological functions, ranging from structural components to intercellular signaling. Yet, a comprehensive characterization of the lipidome from limited starting material, for example in tissue biopsies, remains very challenging. Here, we develop a high-sensitivity lipidomics workflow based on nanoflow liquid chromatography and trapped ion mobility spectrometry (TIMS). Taking advantage of the PASEF principle[1], that synchronizes precursor selection with TIMS we fragmented on average nine precursors in each 100 ms TIMS scans, while maintaining the full mobility resolution of co-eluting isomers. The very high acquisition speed of about 100 Hz allowed us to obtain MS/MS spectra of the vast majority of detected isotope patterns for automated lipid identification. We analyzed lipid extracts from human plasma (SRM 1950), mouse liver and HeLa cells via nanoflow LC coupled to a high resolution TIMS-QTOF mass spectrometer (Bruker timsTOF Pro). Raw data were processed with MetaboScape (Bruker) and MS/MS spectra were annotated with SimLipid (PREMIER Biosoft). As compared with conventional TIMS-MS/MS, we acquired ten times more MS/MS scans with PASEF, which translated into about four times more lipid identifications. The PASEF speed allowed us to reduce the nanoLC analysis time from 90 to 30 min, while still covering over 90 % of the lipids identified with the longer gradient. Our single-extraction workflow surpasses the plasma lipid coverage of extensive multi-step protocols in common lipid classes and achieves attomole sensitivity. The ion mobility dimension further allows separation of isomeric species and provides a high-precision measure of lipid collisional cross sections (CCS) with median CV<0.2 %. In a single experiment, we compiled a CCS library of over 2000 lipids and show high correlation with literature as well as predictions based on machine learning (R2>0.98). Our study establishes PASEF in lipid analysis and paves the way for sensitive, ion mobility-enhanced lipidomics in four dimensions.

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Analysis of oxidized cardiolipins by heart-cutting HPLC coupled to ESI-HRMS

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T 09 PRICE

Cardiolipins (CL) are a special class of phospholipids (PL). Exclusively located in bacteria or in mitochondria of eukaryotic cells, this lipid class is essential for energy metabolism and cell respiration. Due to their four fatty acid residues, CL have a special structure compared to other PL classes. CL contribute significantly to the structure of mitochondria and are essential for the stability of the protein complexes of the respiratory chain. Oxidative stress, for example by reactive oxygen species in mitochondria, can result in various oxidation products and leads to dysfunction of the respiratory chain. Among other effects, this may result in apoptosis and is considered as one cause of cardiovascular and neurological diseases such as Alzheimer's and Parkinson's disease.

Due to their complex structure consisting of four partially different fatty acid residues with variations in chain length and degree of saturation, CL are very diverse. This diversity is increased by different oxidation products. Since CL are only present in mitochondria, the concentration in lipid extracts is low compared to other membrane PL such as phosphatidylcholine or non-polar lipids such as triacylglycerides. These PL classes can affect the chromatography as well as the electrospray ionization by ion suppression. For this reason, the investigation of CL and their oxidation products is challenging and requires sensitive and selective methods.

The hyphenation of two chromatographic techniques offers the possibility to separate interfering matrix components such as PL or triacylglycerides. First, the individual PL classes have been separated by their head group using hydrophilic interaction liquid chromatography (HILIC) in the first dimension. Non-polar lipid classes such as triacylglycerides or cholesterol and esters thereof experience insufficient retention on the HILIC column and were removed. By means of a heart-cut setup, the CL fraction was transferred to a reversed phase column in second dimension. There, CL species were separated according to their chain length and degree of unsaturation. For this purpose, a fast HILIC method was developed, a transfer window for CL and their oxidation products was determined, and the heart-cut setup optimized. In order to demonstrate the efficiency of this method, a lipid extract artificially oxidized by means of Fenton reaction was characterized. The identification of different CL species was carried out by means of accurate mass and MS/MS fragmentation.

Oxidative lipidomics of blood

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T 10 PRICE

Since discovery of the promising and powerful effects of cold atmospheric plasma (CAP) in wound closure, antimicrobial activity and the control of inflammatory diseases, research in the field of plasma medicine has focused on identification of reactive oxygen and nitrogen species (RONS) released by CAP and its possible targets in biological systems, to reveal possible redox signalling pathways[1].

Recent studies focusing on CAP-assisted blood coagulation could show that plasma triggers the natural haemostasis mechanisms by an activation of platelets. Furthermore, it was shown in a murine incision model that a CAP treatment is comparable to cauterization techniques used in the clinics to seal blood vessels with the benefit of not damaging the tissue, opening the door for another medical application of CAP[2, 3]. However, the possible biological targets and biochemical mechanisms involved have yet to be fully understood. Since blood coagulation can be linked to an oxidation of phospholipid-esterified eicosanoids either through enzymatic or non-enzymatic oxidation reactions, these biomolecules could play an important role in a CAPmediated haemostasis[4].

In this study, whole blood from healthy donors was treated with CAP. Immediately after treatment, lipids where extracted using the acidified Bligh and Dyer technique. According to this protocol, polar and neutral lipids should be extracted. The lipid extract was spiked with an internal standard, separated on a reversedphase liquid chromatography column and analysed on a high-resolution mass spectrometer equipped with an Orbitrap mass analyzer. Lipid species as well as lipid oxidation products were identified using LipidSearch software. Results show, that lipid oxidation is highest after 5 min of treatment by using Argon/Oxygenas feed gas. Oxidative modifications occurred mostly on triacylglycerols and phosphatidylcholines by an addition of oxygen or by truncations in the fatty acid chains and an addition of an aldehyde or carboxy-group with arachidonic, linoleic and eicosapentaenoic acid as targets.

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Redox Lipidomics: Clinical applications for mechanistic understanding of disease pathogenesis, biomarker discovery and treatment

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K 04

Lipids are central to cellular structure and function especially in the central nervous system. Among different lipid classes, phospholipids play essential structural roles and act as important signaling molecules in health and disease. Lipid peroxidation has long been established as a key player in the pathophysiology of a number of diseases such as traumatic or ischemic brain injury and neurodegenerative disorders. Most studies in this regard focused on end products of lipid oxidation limiting mechanistic understanding and discovery and development of targeted therapies. With recent developments in liquid chromatography mass spectrometry based redox lipidomics, identification of a wide variety of enzymatically generated lipid oxidation products both in clinical as well as animal injury models have become possible. Such lipid mediators have been found to play important roles in injury, inflammation, and recovery in disease states such as sepsis or head trauma. This lecture will focus on application of redox lipidomics approaches in identification and quantification of phospholipid oxidation products in complex biological samples such as brain tissue and plasma; their mechanisms of production and signaling roles in programmed cell death and inflammatory response; their roles as markers of injury and predictors of outcome; and therapeutic approaches targeting their production. We will also discuss possible methodologic improvements that can offer a deeper insight into the region-specific distribution and subcellular localization of phospholipid oxidation.

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P 13		Comprehensive mathematical modeling of sphingolipid metabolism by integration of lipidomics and proteomics data	Canan Has
P 14	PRICE	Evaluating a quantification approach for the analysis of phosphatidylinositol phosphates	Yam Fung Hilaire Cheung
P 15		How to automate boring lipidomic extraction	Justine Bertrand-Michel
P 16		Mass spectrometry based lipid profiling of *Mycobacterium tuberculosis*	Franziska Waldow
P 17		Comparative study of SMILES Generation Algorithms of LIPID MAPS, Swiss Lipids and LIFS as Basis of Lipidome Homology Determination	Fadi Al Machot
P 18		Evaluation of micro Pillar Array Columns (µPAC™) Combined with High Resolution Mass Spectrometry for Lipidomics	Christof Mitterer
P 19		Validation & Beta-Test of a Novel Standardized Kit for Comprehensive Targeted Profiling	Ulf Sommer
P 20		Monitoring of lipid metabolic perturbations during *M. tuberculosis* infection in human sputum	Anke Bollen
P 21	PRICE	Lung lipidome analysis: a comparative study in several animal models	Daniel Krause
P 22		Insource fragmentation caused at the S-Lens of Q Exactive type of instruments	Dominik Schwudke
P 23		Identification of endocannabinoid-like lipids by nano-liquid chromatography high resolution mass spectrometry in mouse organs	Hans-Frieder Schött
P 24		Inactivation of bacteria by γ-irradiation to characterize the interaction of synthetic anti-lipopolysaccharides peptides with membranes.	Wilmar Correa

Shotgun Lipidomics Combined with Laser Capture Microdissection: A Tool To Analyze Histological Zones in Cryosections of Tissues

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P 01

Shotgun lipidomics provides a quantitative snapshot of the lipidome composition of cells, tissues, or model organisms; however, it does not elucidate the spatial distribution of lipids. Here we demonstrate that shotgun lipidomics could quantify low-picomole amounts of lipids isolated by laser capture microdissection (LCM) of liver cryosections. We identified metabolically distinct periportal (pp) and pericentral (pc) zones by immunostaining of 20 µm thick cryosections of a healthy mouse liver. LCM was used to ablate, catapult, and collect the tissue material from 10 to 20 individual zones covering a total area of 0.3 to 0.5 mm^2 containing ca. 500 cells. Top-down shotgun profiling quantified more than 200 lipid species from 17 lipid classes including glycero- and glycerophospholipids, sphingolipids, cholesterol esters, and cholesterol. Shotgun LCM revealed the overall commonality of the full lipidome composition of pp and pc zones along with significant ($p \leq 0.001$) difference in the relative abundance of 13 lipid species. Follow-up proteomics analyses of pellets recovered from an aqueous phase saved after the lipid extraction identified 13 known and 7 new protein markers exclusively present in pp or in pc zones and independently validated the specificity of their visualization, isolation, and histological assignment.

Mass spectrometric analysis of cell metabolism following parasitic infection

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P 02 PRICE

Parasites and resulting diseases pose health and economical threats to humans and livestock. Some parasites of the *Apicomplexa* phylum (such as *Neospora caninum*, *Eimeria bovis* or *Cryptosporidium parvum*) have not been studied extensively in a biochemical context. For this purpose, mass spectrometry (MS) and MS imaging (MSI) were used, coupled to high performance liquid chromatography (HPLC) or matrix-assisted laser desorption/ionisation (MALDI), respectively. The aim of the study is to identify molecular biomarkers for parasitic infection of host cells and, if possible, to clarify their function. With MALDI MS(I), infected and non-infected cell pellet samples were investigatedin order to detect possible markers. A Q Exactive™ HF orbital trapping mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) in combination with an AP-SMALDI10® imaging ion source (TransMIT GmbH, Gießen, Germany) was used for this purpose (Mass resolution R = 240,000 @ m/z 200; pixel size: 5 µm). Cell pellets consisted of bovine umbilical vein endothelial cells (BUVEC) that are often used as a highly immunoreactive model system for cattle infection. In comparison, immortal cell lines were used as an additional, simplified infection model system. Monolayers of both cell types were available for MSI analysis. This allowed for depicting marker compounds in parasite-infected single cells in comparison to control cells. To find potential biomarkers, the software Mirion (TransMIT GmbH, Gießen, Germany) in combination with the Perseus software platform (MPI of Biochemistry, Martinsried, Germany) was used.[1] HPLC-MS/MS (Dionex UltiMate 3000 RSLC-System, Thermo Fisher Scientific, Dreieich, Germany) experiments were employed for structural identification of the detected molecular markers. Ions in purified and chromatographically pre-separated fractions of cell pellet extracts were fragmented. For the preliminary identification of the detected molecular markers the software LipidMatch (SECIM, Gainesville, USA) was used.[2] It was found that the lipid class of phosphatidylcholines in particular was strongly represented. To date, *Neospora caninum* samples were investigated with MALDI-MS and HPLC-MS. The MS imaging experiments of cell monolayers are still ongoing, but preliminary markers were already detected and assigned. Furthermore, investigations of *Cryptosporidium parvum* just started.

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Analysis of *Mycobacterium tuberculosis* – induced alterations in the lipid metabolism of primary human macrophages

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P 03 **PRICE**

Cellular metabolism is critical for normal cell function. Alterations and disruptions in e.g. lipid homeostasis are found in several chronic inflammatory pathologies including tuberculosis (TB). During *Mycobacterium tuberculosis* (Mtb) infection, the formation of lipid laden, "foamy" macrophages is linked to disease progression and granuloma necrosis. Alongside with that, host-derived fatty acids, which are stored in intracellular storage organelles called lipid droplets (LDs), were described as Mtbs primary carbon source for intracellular growth and survival. However, the metabolic mechanisms causing macrophages to adopt a foamy phenotype and promote Mtb growth are not well understood. In the present study we used several approaches to shed light on the lipid metabolic network in primary human macrophages during Mtb infection. Utilization of fluorescently labeled fatty acids indicated that the degradation of fatty acids is significantly reduced during Mtb infection, while the uptake and overall amount of neutral lipids remains unchanged. Furthermore, Mtb infection causes alterations in mitochondrial metabolism as indicated by a reduction in mitochondrial mass and a concomitant increase in mitochondrial activity. In agreement with that, extracellular flux analyses of primary human macrophages revealed a dose-dependent increase in the basal oxygen consumption rate (OCR) at late time points, in contrast to a decreased basal OCR at early time points of infection. In parallel, several other parameters such as proton leak, ATP production and non-mitochondrial respiration are time- and dose-dependently altered indicating a shift in oxidative metabolism in the course of TB infection. Targeted inhibition of critical lipid metabolic enzymes such as the acetyl-CoA-carboxylase (ACC) 2 and the diacylglycerol O-acyltransferase (DGAT) 1 and 2 led to a rewiring of macrophage metabolism and affected Mtb growth in human macrophages. Additionally, as LDs are considered as key players in macrophage lipid metabolism, we isolated LDs from primary human macrophages to characterize LD size, composition and heterogeneity among human donors. Collectively, our data indicate that modulation of macrophage lipid metabolism might be an approach for future therapeutic intervention, but further studies are required to better understand the complexity of the macrophage metabolic network.

Structural elucidation of lipid A in Gram-negative bacteria using LC-MS/MS

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P 04 PRICE

The lipopolysaccharide layer is a main component of the outer membrane from gram-negative bacteria. It consists of three regions: an O-polysaccharide, which extends outwards from the cell surface, an oligosaccharide core, and the membrane-anchored lipid A moiety. Lipid A is the part that can be sensed by humans and animals to detect the presence of Gram-negative bacteria in their tissues and plays a key role in thepathogenesis of bacterial infections.

Lipid A features a ß(1,6)-linked glucosamine backbone, having fatty acids substituted at the 2, 3, 2, and 3 positions and zero, one or two polar groups like phosphate groups linked to the 1 and 4 positions. The toxicity of lipid A is heavily influenced by its primary structure, speaking of the number and length of the linked fatty acids, as well as the level of phosphorylation. A great degree of structural diversity is not only observed between distinct bacteria but also within individual species. These dynamic modifications help the bacteria to survive under various conditions, such as different temperatures or growing media compositions. Therefore several lipid A structures can be observed in a single organism. The environmental factors can be manipulated to engineer specific lipid A molecules and use these for the development of therapeutics such as vaccine adjuvants.

In this work we present a new method to distinguish and characterize various lipid a species originating from different Gram-negative bacteria. Cells of *Escherichia coli* (*E. coli*), *Pseudomonas putida* (*P. putida*) and *Pseudomonas taiwanensis* (*P. taiwanensis*) have been extracted and analysed by LC-MS/MS. The lipid A species were identified using high-resolution mass spectrometry and tandem-MS experiments. Eight lipid A species could be identified in the *E. coli* extract. They vary in their bound fatty acids and their degree of phosphorylation. Four of these structures were confirmed via MS/MS fragmentation. In *P. taiwanensis* 17 different lipid A molecules have been detected, with ten of these also occurring in *P. putida*. 13 and six, respectively, of these structures were observed for the first time. Detailed structural elucidation will be carried out in future research amongst others by tandem-MS.

In-depth characterization of phosphatidylethanolamines from *Pseudomonas* bacteria

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P 05 PRICE

Rhamnolipids are biosurfactants consisting of rhamnose molecules linked to fatty acyls, which can be produced by *Pseudomonas aeruginosa* and *Burkholderia glumae* bacteria. However, these bacteria have pathogen properties, which hinders their application. It was shown that rhamnolipids can also be synthesized by *Pseudomonas putida* (*P. putida*), which are non-pathogen bacteria.

Studies show that the fluidity of cell membranes can be influenced by the presence of rhamnolipids. Membranes comprise a lipid bilayer mainly consisting of phospholipids, such as phosphatidylglycerol or phosphatidylethanolamines. Their fluidity is influenced by the lipids structure, i.e., double bonds result in a more fluid membrane. This work aims to analyze lipid changes induced in *P. putida* during rhamnolipid production.

P. putida lipid extracts were analyzed by a two-dimensional heart-cut liquid chromatography method. Hydrophilic interaction liquid chromatography (HILIC) is used in the first dimension to separate lipid classes according to their head groups. The heart-cut setup transfers specific lipid classes to a second dimension via sample loop. By reversed-phase liquid chromatography (RPLC) in the second dimension, the lipid class is further separated into individual lipid species, reducing ion suppression and giving the possibility to differentiate lipid isomers, i.e., *cis* and *trans* isomers. Lipids are identified using high-resolution mass spectrometry and MS/MS experiments. A special focus was set on phosphatidylethanolamine (PE) species.

Differences can be detected in PE lipid levels in the bacteria lipid extracts harvested after 3 h. Tendencies are observed towards less unsaturated fatty acids during rhamnolipid production. Lipid extract of rhamnolipid producing *P. putida* obtained after 3 h growth time shows elevated levels of PE species, suggesting adjustment to the fluidizing effect of rhamnolipids on lipid membranes. Separation of different lipid isomers is observed, some consisting of different PE species with varying fatty acyls bound to the lipids, others indicating *cis* and *trans*-isomers or methyl-branched lipid species. Results show equalization of PE lipid changes after 24 h growth time.

LipidCreator: A workbench to probe the lipidomic landscape

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P 06

Lipids are imbedded in biology; they form cells and organelles, mediate information flow, protect cells and tissues from a hostile environment and serve as energy building blocks. Targeted lipidomics focuses on a reproducible, quantitative analysis of a subset of lipids of interest. Unfortunately, software development to screen lipids lacks behind, especially for targeted lipidomics.

Here we introduce LipidCreator, a lipid building block-based workbench and knowledge base for the automated generation of targeted lipidomics MS assays and *in-silico* spectral libraries. Assay generation can be conducted with a generic user interface (GUI) or at the command line, covering lipids of the following categories: sphingolipids (SL), glycerolipids (GL), glycerophospholipids (PL), cholesterol and its derivates (Ch, ChE), as well as fatty acids, including mediators (LM). LipidCreator can calculate masses for lipid species and their derived fragment ions, covering over 60 lipid classes and a lipid array of 10^{12} lipid molecules with the consensus nomenclature recommended by the lipidomics standards initiative. Using the batch-processing mode, LipidCreator computes precursor-product ion pairs at a rate of 60,000 pairs per second on a standard notebook (i5-4310M CPU @ 2.70GHz with 8GB main memory). Besides the computation of fragment ions, another critical feature to generate an *in-silico* spectral library is the ability to determine the relative intensities of fragment ions at different defined collision energies (CE). We therefore trained non-linear regression models on empirical data from standard measurements of lipid mediators on two different MS instrument types.

LipidCreator further contains a visual inspection level for fragments and a lipid nomenclature translator supporting LIPID MAPS formulations. It can be integrated into KNIME and Galaxy workflows as a native node via its command line interface on Linux and Windows. LipidCreator can work standalone but it is also fully integrable into Skyline and its small molecule support, allowing vendor-independent assay usage, data visualization and quality control of MS and MS/MS data.

How Cold Atmospheric Plasma Affects Red Blood Cell Lipid Bilayer

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P 07

Cold atmospheric plasma (CAP) is a promising tool for several biomedical applications including blood coagulation, wound healing and cancer treatment due to its potential to create a broad diversity of reactive oxygen and nitrogen (RONS) species[1]. However, the related mechanisms are not entirely known. So studying the interaction of CAP with biological membranes as one of the most vital and functional components of the cell representing a dynamic barrier and providing transport and regulatory tasks is of great importance[2]. When in contact with CAP, oxidation of the lipid constituents can be assumed[3]. It is therefore essential to study the behavior of the oxidized cell membrane and its subsequent impact on RONS penetration rates. Due to the rather complicated structure, anisotropy and inhomogeneity, the study of the physical and electrochemical properties of biomembranes are difficult. Therefore, a model lipid bilayer has been transferred onto a gold electrode surface using the combined LangmuirBlodgett/LangmuirSchaefer deposition to mimic the structure and properties of the red blood cell (RBC) membrane. The distribution of the lipids in the RBC membrane is highly asymmetric. The majority of sphingomyelin (SM) and phosphatidylcholine (PC) are localized in the outer layer, while phosphatidylethanolamine (PE) and phosphatidylserine (PS) are predominantly in the cytoplasmic half of the bilayer[4]. The model lipid bilayer was prepared in a way to have similar lipid composition with the RBC membrane. Then, the lipid bilayer supported gold electrode was treated with the kINPen IND (kINPen, neoplas tools) with Argon as feed gas at a flux of 3.0 slm. The changes in the lipid bilayer functionality due to the CAP generated RONS was investigated using electrochemical techniques. The obtained results show that in the presence of CAP, the performance of the extracellular and the cytoplasmic sides of the lipid bilayer are entirely different so that the cytoplasmic side is more stable against RONS. To have a better understanding of the chemical modifications of the CAP treated lipids, they were analyzed by direct-infusion high-resolution tandem mass spectrometry (DI-MS) and liquid chromatography-tandem MS (RP-LC/MS).

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Embedding Lipidomics into the "omics" Landscape

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P 08 PRICE

Metabolic diseases are common in modern industrialized societies. They are often associated with unhealthy lifestyles and are a huge burden for the health care system. Especially chronic diseases cause great suffering for the affected patients. Many of the defects can be related to lipid metabolism. Well-known representatives are Crohns disease or the non-alcoholic liver disease (NAFLD). Globally, 55 % of type 2 diabetes mellitus patients are affected by NAFLD and a total prevalence estimated at 24 % (Younossi et al. 2018). Since a variety of diseases exists, an easy classification is not possible and a reliable diagnosis of e.g. NAFLD still requires an invasive liver biopsy.

The goal of precision medicine is to provide optimal personalized treatments for patients. To achieve this, detailed knowledge about the phenotype of a disease for every patient is necessary. Not only in a qualitative, but also in a quantitative fashion. A basis for this are automated quantitative analysis tools of patient data, which classify patients diseases into subtypes and therefore allow predictions for appropriate treatment strategies.

Analysis of lipid data is a crucial step for improving diagnoses for patients. Current challenges for the analysis of such data in the bioinformatics pipeline are standardized statistical routines for quality control and a functional analysis combined with integration into omics fields (Pauling and Klipp, 2016). The goal of this project is to develop automated solutions and standards for subtyping of patients based on lipidomic data from mass spectrometry measurements. This includes analysis tools to integrate lipidomic with proteomic data to extract important features and get a mechanistic understanding of the diseases and make prognosis predictions for patients. Subtyping of patients and extraction of parts of the cellular metabolic/signaling machinery that play an important role for the disease will be based on machine learning approaches in combination with network enrichment tools like the KeyPathwayMiner (Albcaraz et al. 2014). Adaptations and development of new tools will be necessary to make the lipidomic data accessible. Various online lipid databases will be utilized to profit from their knowledge for analysis and predictions.

As a first step, patient lipidomic data will be analyzed to extract similarities between patients and detect potential subtypes of diseases, which can potentially later be predicted from specific biomarkers. This will be followed by the development and implementation of data mining tools to establish a link between lipidomics and other omics data and connect them to patient-specific clinical anamneses.

Improved lipid annotation depth using automatically generated inclusion and exclusion lists on an Orbitrap-based mass spectrometer.

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David Peake, Reiko Kiyonami

Thermo Fisher Scientific

P 09

Lipid profiling provides valuable information to identify disease states and other physiological changes. A common approach for lipidomics profiling is to identify lipid species by their MS/MS spectra prior to extraction of precursor information for relative quantitation. With this approach, collecting MS/MS data on every sample relevant compound is crucial for confident lipid species annotation. The novel intelligent data acquisition software (AcquireX) on the Tribrid Orbitrap mass spectrometer excludes background ions from the MS/MS acquisition and prioritizes MS/MS acquisition on sample relevant compounds automatically, resulting in deeper lipidome coverage. Here we present the lipid identification coverage for lipid extract samples were being improved by using AcquireX on a Thermo Scientific Orbitrap ID-X Tribrid mass spectrometer.

Bovine liver and heart total lipid extracts (Avanti Polar Lipids) were used as model and separated on a Thermo Scientific™ Accucore™ C30 column (2.1x150mm, 2.7µm). LC-MS analyses were performed on a Thermo Scientific Vanquish UHPLC systemcoupled with an Orbitrap ID-X mass spectrometer. Mass spectral data were acquired in positive and negative ion modes at 120K resolution (FWHM @ m/z 200) and data dependent HCD MS^2 and product ion- or neutral loss-triggered CID MS^2 and MS^3 experiments were performed to characterize the eluting lipids. AcquireX DeepScan mode was used to automatically generate background exclusion and compound inclusion lists that were updated iteratively for replicate injections. The raw data was analyzed using LipidSearch 4.2 software to identify lipid molecular species.

Bovine liver and heart total lipid extracts were chosen to demonstrate the utility of the workflow. Sample LCMS data was acquired in triplicate for each type of extract with data-dependent acquisition, where AcquireX was used to update the inclusion and exclusion lists between injections, in order to reduce redundant data collection and trigger more lipids for fragmentation. Data was analyzed with LipidSearch by first identifying lipid annotations on the basis of MS^n fragmentation data compared to the subset of relevant lipid sub-classes in the LipidSearch database. Data were then aligned across the different samples and both polarities. Preliminary results indicate that using the AcquireX DeepScan feature on the Orbitrap ID-X, a significantly larger number of lipids (≥ 40 %) are initially detected based on the ac-

quired fragmentation data and the overall confidence of the annotations was improved relative to 'traditional' DDA approaches.

In addition, the use of neutral loss-triggered CID-MS^3 and product ion-triggered CID-MS^2 improved the confidence in the lipid annotations. For example, the use of fatty acid neutral loss-triggered MS3 fragmentation with triglycerides allowed to distinguish multiple co-eluting isomers with different fatty acid chain lengths based on their respective MS^3 data. This study demonstrates the application of an automated background-exclusion and compound -inclusion generation workflow for global lipidome profiling, leading to a greater number and greater confidence of lipid annotations.

Discovering potential diabetic lipid biomarkers using HRAM LC-MS/MS approach on a high field Hybrid Quadrupole-Orbitrap Mass Spectrometer

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Thermo Fisher Scientific

P 10

Lipids are known to play a key role in cell, tissue and organ physiology and have been implicated in many diseases such as cancer and diabetes. Lipids may also play a role as potential markers for the presence or absence of certain disease states. The identification of such unique biomarkers may aid in detecting potential risk of disease in certain individuals. Although recent advances in HPLC-MS platforms have allowed the rapid and sensitive detection of a variety of lipid species with minimal sample preparation, many challenges remain. The diversity in the structural and physical/chemical properties of the lipidome requires the combination of HPLC separation technique with a high resolution, accurate mass spectrometer for accurate lipid molecular ion determination. Moreover, the molecular weight information only may not be sufficient for identifying each isomer of individual lipid species. MS/MS information is further required for unambiguous identification of each individual lipid species in biological samples.

The new released Thermo Scientific Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer features an ultra-high-field Orbitrap analyzer which doubles its speed and resolution compared to first generation of Orbitrap analyzer. The ultra-high resolution (up to 240,000) of the Q Exactive HF MS allows accurate mass measurement with less than 3 ppm accuracy and the faster scan speed (up to 18 Hz) of the Q Exactive HF MS results in higher number of precursor ions triggered for MS/MS, allowing more lipid identification in a single HPLC/MS/MS run with improved productivity. The Q Exactive HF MS also offers several high resolution accurate mass approaches (Full MS; SIM: selected ion monitoring; PRM: parallel reaction monitoring) for highly sensitive and highly selective quantification of individual lipid species of interest.

In this study, a Q Exactive HF MS was used to carry out a large scale lipid profile analysis of two phenotypes of the rat (ZDF vs. lean wild type). Per each type of the rat model, three different lots of rat plasmas were used. The lipids were extracted from the plasmas using solvents of Chloroform, Methanol and water. A Thermo Scientific Dionex UltiMate 3000 Rapid Separation LC (RSLC) system was used for lipid separation. Mobile phase A was 60:40 Acetonitrile / Water and mobile phase B was 90:10 IPA / Acetonitrile; both A and B contained 10mM ammonium formate and 0.1 % formic

acid. The column was an Accucore C18 (Thermo Scientific, 2.1 x 150mm, 2.6µm) column operated at 55°C with flow rate of 260 µL/min.

The Q Exactive HF mass spectrometer was operated using data dependent LC MS/MS method in both positive mode and negative mode, respectively. Each full MS scan (120,000 R at 200m/z) was followed by 15 MS/MS (30,000 R at 200 m/z) scans. The cycle time was 2.4 seconds providing sufficient scans across the chromatographic peak profile for accurate relative quantification using the HR/AM precursor ion while simultaneously acquiring dd-MS/MS spectra for lipid identification. Lipid Search software was used for lipid identification and quantification. The Q Exactive HF MS system delivers high mass accuracy and resolution combined with faster scan speed, enabling faster and deeper lipidome coverage. Approximately more than 1000 lipid species were simultaneously identified and quantified with high confidence and great analytical precision from the two types of rat plasma. Moreover, significant concentration increases of triglycerides and phospholipids were observed from the ZDF rat plasmas.

A multi-omics approach to screen for modified hippocampal lipid signaling pathways triggered by lifestyle conditions

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In mammalian brain, information retrieval, storage and processing take place in synapses. The synaptic cleft, as central part of the pre- and post-synaptic junction, is the subcellular location where cell-to-cell communication and signal transduction is mediated. Lipid metabolism and signaling are essential components for this neuronal synaptic plasticity. Simultaneous multiomics extraction and analysis are highly informative technics to probe the interplay of proteins and lipids in neuronal signal transduction. In this study on hand, the molecular inventory of the cleft is accessed and a novel mechanism of reduced endocannabinoid signalingelicited by environmental stimuli was identified. Endocannabinoid signaling was linked to increased surface expression of AMPAR, which likely regulates alterations in synaptic plasticity of mice exposed to an enriched environment.

A multi-molecular dissection of diseased hearts from mice links dominant caveolinopathy to a metabolic shift associated with dilatative cardiomyopathy

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Current evidence from the medical fields such as cancer, cardiovascular research and immunology suggest that metabolic rewiring is orchestrated by the concerted interplay of multiple processes modulated by lipids, metabolites and proteins, acting in juxtaposition with central metabolic pathways. Despite significant therapeutic advances, various cardiovascular diseases (including myocardial ischemia or infarction and inherited or acquired cardiomyopathies) merge most often into heart failure leading to premature death. Dysregulation of metabolism was seen as a minor secondary process within numerous cardiovascular diseases but with the current knowledge, we now have to accept that abnormalities of myocardial metabolism are associated with heart failure and that the metabolism itself is controlling numerous disease steps. From a molecular perspective, there is no unified, complete mechanism neither explaining why the cardiac dysfunction results into failure nor explaining how exactly several cardiac conditions biochemically manifest and progress. Multiomics analysis has the potential to provide deeper insights into the underlying mechanisms and to offer a better understanding of the molecular interplay within different molecular levels leading to heart failure. Here the previously established SIMPLEX multiomics strategy was applied to examine the molecular basis of an inherited dilative cardiomyopathy utilizing cardiac tissue derived from a well-established transgenic mouse model carrying a dominant mutation (P104L) in the Caveolin-3 (CAV3) protein. Employing SIMPLEX were able to recapitulate several past findings in caveolininopathy. However, applying this multiomics approach to the heart of P104L-CAV3 transgenic mice allowed us for the first time to draw a direct line of evidence from the CAV3 deficiency towards the global dysregulation of main shunts of the energy metabolism including glycolysis, lipolysis and the citric acid cycle. All in all we not only demonstrated that an multiomics strategy can close gaps of single sided strategies but also revealed that CAV3-related pathophysiology also includes a staged metabolic rewiring classifying the cardiomyopathic phenotype also as a metabolic disease, not only as structural one.

Comprehensive mathematical modeling of sphingolipid metabolism by integration of lipidomics and proteomics data

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Sphingolipids play important role in cell membrane composition and lipid raft formation. In addition to that, they exert a vital role in regulating multiple cellular functions including signaling, apoptosis, inflammation. Sphingolipids show antagonistic activities. While ceramide and sphingosine are proapoptotic to benefit growth arrest and apoptosis, ceramide-1-phosphate and sphingosine-1-phosphate are known to promote cell proliferation, transformation and inflammation. The metabolism of sphingolipids, including the all enzymes and lipids involved, has been extensively studied. This metabolic pathway delineates an integrated system linking multiple synthesis and catabolism pathways by centering ceramide. Three main pathways that compose sphingolipid metabolism are de novo synthesis or the hydrolysis of sphingomyelin or recycling of gangliosides, i.e. sphingosine, also called salvage pathway. The alteration in sphingolipid metabolism could result in pathogenicity of several diseases including neurodegenerative diseases, metabolic diseases. Hence, integration of quantitative lipid and protein data along with biochemical reaction kinetics to model sphingolipid metabolism would be crucial to shed a light on sphingolipid related disease mechanisms.

Here, we propose a comprehensive and extended model of sphingolipid metabolism in mouse macrophage cells. Contrary to the previous studies, we incorporated the de novo synthesis of ceramide and the cross talk between de novo synthesis and the salvage pathway. The rate constants were estimated through parameter estimation via COPASI and the resulting model was fit two independent experimental data for all species. In silico knockouts of CerS and SMS enzymes were introduced and the results were compared with the literature. The model we present here will further be applied to unravel behavior of sphingolipid metabolism in disease models such as Alzheimer and insulin-resistance.

Evaluating a quantification approach for the analysis of phosphatidylinositol phosphates

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Phosphatidylinositol phosphates (PIPs) play crucial roles in numerous signal transduction pathways. Two classes of cell surface receptor-regulated enzymes, phospholipase C gamma (PLCG) and phosphoinositide 3-kinase (PI3K), are essential in PIPs metabolism. PLCG hydrolyses PI(4,5)P2 to yield diacylglycerol, an activator of protein kinase C (PKC), and inositol (1,4,5) trisphosphate, which releases calcium ions from the endoplasmic reticulum. PI3K phosphorylates PI(4,5)P2 to produce PI(3,4,5)P3, which recruits platelet signaling proteins such as Akt and PDK1 via the PH domain and activates them. The rapid development of liquid chromatography-mass spectrometry (LC-MS) methods have popularized its usage in PIPs analysis, and numerous LC-MS methods have been developed to separate and quantify PIPs. Nevertheless, the separation and analysis of PIPs positional isomers remains a challenge to the field. In this study, we deacylated the PIPs to increase the PIPs polarity and facilitates its separation using ion chromatography (IC). We also optimized the IC gradient and successfully resolve the PIPs isomers, and achieved a comprehensive analysis of individual PIP isomers. The use of IC instead of RPLC for PIPs separation eliminated the need of adding any ion-pairing reagents to the sample, which would contaminate the MS ion source and affects its performance. Moreover, the developed method successfully identify and absolutely quantify the PIPs species in platelets, which evidently demonstrated the power of our method in PIPs analysis. We believe that the developed method should greatly facilitate the elucidation of PIPs-associated signal transduction pathways.

How to automate boring lipidomic extraction

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MetaToul-Lipidomic Facility

P 15

Lipids are ubiquitous biomolecules essential to all life, found in every cellular type, ranging from the human body and vegetal organisms, down to bacteria. They have many different functions in cell structuration, energy storage and signaling so they are natural biomarkers for different diseases like cancer, cardiovascular disease, neurodegenerative disease, lung disease, their study and quantification are then crucial. Mass spectrometry (MS) coupled with liquid chromatography or gas chromatography is mainly used for global and specific analysis of lipids. But before their analysis, there is an important and time consuming step of sample preparation. Due to their amphiphilic properties, there are usually two types of extractions: liquid-liquid extraction (LLE) and solid phase extraction (SPE). LLE and SPE are two very long protocols which are tedious when there are massive numbers of samples. They can also be the source of many errors, considering the experimenter-dependant possible repeated artefacts. To circumvent this point and increase the analytical service delivery, a TECAN (Fluent 780) robot has been acquired by MetaToul-Lipidomic facility to automate the sample preparation. Due to the specificity of lipid extraction, the robot needs a lot of optimization. This presentation will show part of adaptation we had to perform to validate fatty acid, neutral lipid and phospholipid profiling. We compared the use of needles and plastic tips with optimization of solvent sampling: which implies new adjustment accuracy and precision for each of them, modification of speed of dispense and stiring. First lipidomic's results obtained with a complete automated sample preparation will be presented for liver and plasma sample.

Mass spectrometry based lipid profiling of *Mycobacterium tuberculosis*

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Mycobacteria are the cause of several human diseases such as tuberculosis (TB) and leprosy. It is estimated that approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb). It is assumed that the cell envelope of Mycobacteria plays a key role in pathogenesis and resistance towards antibiotics. The mycobacterial envelope has a complex and unusual structure with a very high lipid and carbohydrate content. Lipids of the cell envelope constitute up to 60 % of the cell dry mass of *Mtb*. Furthermore, mycobacterial lipids play important roles in directing host-pathogen interactions, are required for cell growth and are involved in pathogenesis. Until now, it remains elusive if the lipid composition itself plays a role as virulence factor. Due to the structural diversity of mycobacterial lipids and the number of complex structures that are unique to *Mtb*, an overall characterization of the lipid composition is a significant technical challenge. Different methods for lipid profiling, such as traditional TLC-based methods, direct infusion-based as well as chromatography-based mass spectrometry methods are reported in the literature. However, these methods require prior sample separation, are time consuming or are not feasible for quantification.

Here, we show first results of a nanoESI-MS/MS method, which allows simultaneous detection, identification and quantification of membrane phospholipids and complex glycolipids of the mycobacterial cell envelope within a single analysis. Therefore, shotgun lipid*omics* was performed using a Q Excative Plus with a Triversa Nanomate as ion source. Lipid identification and quantification was performed using LipidXplorer.

Comparative study of SMILES Generation Algorithms of LIPID MAPS, Swiss Lipids and LIFS as Basis of Lipidome Homology Determination

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Cellular lipidomes contain signatures for cell identity, disease status and developmental stage but also represent the integral status of genetic and epigenetics of an organism. This results in systematic structural differences in lipidomes of which the functional impact is widely unknown. We introduced the LUX score as a metric to determine lipidome homologies[1] to enable systematic associations between structural deviation and function.

At the centre of the lipidome homology determination is the generation of Template SMILES that are computed according to a dedicated database model that contains building blocks for each lipid class, e.g., fatty acids, head groups, and sphingosines. For all Template SMILES, a structural position that is most conserved and can be considered as a reference point for all lipids of a class. As a result, the generated SMILES have a unified and formal chemical space.

Here we present a new approach to generate Template SMILES that is implemented in the LUX score browser. As benchmark test for evaluating different SMILES generation processes, we used the new SMILES generator, LIPID MAPS[2] and Swiss Lipids[3] databases and applied them on lipidome data sets of yeast[4], fruit fly[5] and human lung. The LUX score results using our SMILES generator showed best consistency compared to LIPID MAPS and Swiss Lipids. We further can show that the chemical space for the generator is more compact and structural related lipids are better clustered.

In our future work, we will integrate correlation analyses based on lipid quantities and LUX score metric using non-linear optimizatio

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Evaluation of micro Pillar Array Columns (µPAC™) Combined with High Resolution Mass Spectrometry for Lipidomics

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As an alternative to conventional packed bed nano LC columns, PharmaFluidics offers micromachined chip columns known as micro Pillar Array Columns (µPAC™). The high permeability and low 'on-column' dispersion obtained by the perfect order of the separation bed makes µPAC™ based chromatography unique in its kind. The peak dispersion originating from heterogeneous flow paths in the separation bed is eliminated and therefore components remain much more concentrated during separation resulting in unprecedented separation performance[1]. The freestanding nature of the pillars also leads to much lower backpressure allowing a high operational flow rate flexibility with exceptional peak capacities[2].

State-of-the-art µPAC™ columns coated with octadecyl are applicable for a challenging application such as lipidomics. Here, the performance is illustrated with the analysis of human blood plasma lipids.

Using a 200 cm long µPAC™ column in combination with high resolution mass spectrometry, an enormous complexity is revealed and a high lipidome coverage can be obtained. All major lipid classes are detected and their locations are shown on the chromatograms. Next to the inter-class separation, the methodology provides intra-class separation based on the number of carbons and the degree of saturation in the fatty acid side chains. Furthermore, isomeric lipids can be resolved[3]. Taken together, this approach holds great potential in lipidomics studies.

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Validation & Beta-Test of a Novel Standardized Kit for Comprehensive Targeted Profiling

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Mass spectrometry is the key analytical technique for comprehensive lipidomics analysis. Reliable analytical results and improved inter-laboratory comparability, automation, and standardization of the metabolomics workflow are of utmost importance. Here, we present the newly developed quantitative MxP® Quant 500 kit-based assay for multiplexed MS/MS analysis of 630 metabolites/lipids from 26 analyte classes in only 10 µL sample volume. Among these, 13 classes comprising 535 analytes can be counted as lipids. The assay allows standardized analysis in a variety of biological sample matrices (e.g. blood, feces, tissue) and species, including the microbiome.

The ready-to-use assay combines UHPLC- and FIA-MS/MS into a single workflow. Automated data analysis of analytes and internal standards analyzed in 96-well format (blanks, QCs, standards, and samples) was performed with Biocrates' MetIDQ™ software, which controls the entire workflow from sample registration to data processing and reporting. For beta-testing 14 samples (NIST SRM 1950, 6 human plasma samples, 2 human serum samples, lipemic human plasma, mouse plasma, rat plasma, mouse liver, and human feces) were evaluated on SCIEX 5500 LC-MS systems.

The assay was successfully validated following EMA/FDA guidelines. The analytical accuracy and precision of the assay were safeguarded by three levels of QC samples. We will present the analytical workflow and performance parameters, including intra/inter-batch accuracies and precision, and accuracy data of reference materials. MetIDQ™ software enabled data processing including quality assessment and the combination of all results. Beta-test data across 8 independent data sets across the 14 samples will be presented showing excellent accuracies and precision results, the basis for longitudinal robustness and inter-laboratory comparability. About 400 of the 534 lipids are detected > LOD in normal human, rat, or mouse plasma.

Monitoring of lipid metabolic perturbations during *M. tuberculosis* infection in human sputum

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P 20

Tuberculosis (TB), the most prevalent bacterial infection worldwide, is caused by *Mycobacterium tuberculosis* (*Mtb*). Despite successful antibiotic treatment, patients can suffer from severe lung pathology and pulmonary insufficiency. We hypothesize, that lipid metabolism

I) can reflect the inflammatory level of host responses to *Mtb*.

II) can be utilized for point of care diagnostics,

III) can represent targets for host-directed therapies (HDT), which support antibiotic treatment and limit immunopathology.

We employed two analytical approaches to investigate perturbation of the lipid metabolism in Mtb infected neutrophils, using a Q Exactive Plus mass spectrometer (Thermo Scientific™, Bremen, Germany).

1) liquid chromatography (LC-MS/MS) applying parallel reaction monitoring used for identification and quantification of polyunsaturated fatty acids (PUFAs) and their derivative lipid mediators (LM);

2) shotgun lipidomics focusing on glycerolipids, glycerophospholipids, sphingolipids, cardiolipins and cholesterol derivatives.

Analysis of sputum from four different TB patients before and during therapy allowed quantification of 21 LMs from total lipid extracts by LC-MS/MS. Since sputum is a complex matrix with a high variability between in samples of mixed qualities, this study lacks the normalization of the LM concentration to the protein content. Therefore, a clear correlation to the status of the therapy was not possible.

Subsequently, we will analyze sputum from 10 TB patients before and under treatment and sampled on a weekly basis until culture conversion. To keep sample treatment as uniform as possible, all samples will immediately be homogenized, diluted in methanol containing butyl-hydroxy-toluol and stored at -80 °C to block metabolic activity and inactivate *Mtb*. Increasing the number of patients will compensate for the missing normalization. Ultimately, we want correlate individual LM concentrations and treatment outcome to develop a personalized HDT to limit long-term sequelae and pulmonary insufficiency.

Lung lipidome analysis: a comparative study in several animal models

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Animal models are used to investigate complex mechanisms of lung disease progression in a controlled environment. The model acts as an abstraction of the human system but does not regard the differences in physiology and anatomy of the different organs or lungs in each species. So far, differences between species are not reflected in the lipidome studies of organs (muscle, liver, kidney or heart) with the exception of the brain[1], proposing a core set of lipids that are present even in phylogenetic distant species. Until today, no in-depth study on the lung lipidome across species was performed and how differences between animal model and human might affect functional interpretations.

In a first proof of concept study we showed that the lipid metabolism of the human lung is influenced by various traits like age, sex and BMI.[2] However, animal models like pigs and mice are used to understand fundamental processes in disease progression and we determine the overall similarity between lung tissue lipidomes. In this study, we investigate differences between the lung lipidomes of mice, pigs, sheep and humans. The project aims to catalogue the lipidomes of multiple species and associate them with clinical parameters and histology.

To profile the lung lipidomes, a shotgun approach was applied using a Q-Exactive Plus (Thermo, Bremen, Germany) coupled with a Triversa Nanomate (Advion, Ithaka, USA) as nano-ESI source and autosampler. We quantified approximately 400 lipid species from 16 lipid classes in glycerolipid, glycerophospholipid, sphingolipid and sterol lipid categories. From this data set, we compare how aging of the lung is reflected in the lipidome of pigs and humans, look at effects of a weight gain mouse model in association to high BMI in humans and determine overall lipidome homology between organisms.

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Insource fragmentation caused at the S-Lens of Q Exactive type of instruments lead to lipid profile artifacts

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Lipid identification applying shotgun lipidomics is highly dependent on correct assignment of chemical sum composition to precursor m/z values and its correct assignment of the corresponding MS/MS experiment. Correct lipid identification using the Molecular Fragmentation Query Language (MFQL) in LipidXplorer is depending on this uniquely associated data structures. In the analysis of complex lipid extracts using spectral stitching, we observed an increased number of unusual lipid lds and a systematic shift in the overall lipidome composition. Thus we systematically tested the conditions for formation of insource fragments using the nano-ESI source Triversa Nanomate (Advion, Ithaca, US) and Q Exactive Mass spectrometer (Thermo Scientific, Bremen, Germany). Lipid standards and complex extracts were dissolved in $CHCl_3$/MeOH/isopropanol (1/2/4, v/v/v) containing 3.7 mM ammonium acetate.

In general, insource fragmentation in shotgun experiments is known for lipids comprising low energy leaving groups like H_2O (-18 Da) for sphingolipids and loss of acetate plus methyl-group for phosphatdiylcholines (-74 Da). We analysed as model compound PC 14:1 / 17:0 in the negative ion mode. At optimum ionization condition applying a source temperature of 100C and a mass range (*m/z* 300 – 1200) approximately 3 % of the insource fragment was observed. Only slight intensification of the insource fragmentation was observed in dependence of increased source temperature. The largest impact had the choice of the applied mass range, nearly tenfold increase was observed in MS1 when the *m/z* range was changed to 725 – 875. At the same time an even lower degree of insource fragmentation was observed when the precursor *m/z* 776.5 was selected and an collision energy of NCE 1 was applied, which indicated that the transfer from the quadrupole / c-Trap and collision cell does not cause the observe fragmentation. However, precursor selection at the neutral loss fragment *m/z* 702.5 with applying NCE 1 resulted in 25 % of the initial precursor intensity at *m/z* 776.5. Further MS/MS analyses at NCE 35 proved the same fatty acid composition of the standard with 14:1 / 17:0. Additional tests changing transfer parameters indicate clearly that the automated adjustment of the s-lens ion transfer led to increased insource fragmentation. As first result of this finding, we suggest for the community to abandon spectral stitching approaches as long as the user has not control about the s-lens transfer. Furthermore, we suggest the application of post processing routines that check the ratio of precursor intensities and fragment ions. Specifically for assignments of PC – PE and PS – PA in complex samples such filter should be applied to reduce number of wrong assignments.

Identification of endocannabinoid-like lipids by nano-liquid chromatography high resolution mass spectrometry in mouse organs

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P 23

The endocannabinoid system (eCBS) is a lipid mediated signalling pathway involved in the regulation of different processes within the brain, gut and immune system. Their endogenic ligands and metabolic enzymes have been implicated in a wide variety of diseases states in cognition, pain and heart diseases.[1-4] Until today the best characterized members of the eCBS signalling pathway are N- arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) which are the ligands to the major eCBS G protein-coupled receptors CB1 and CB2 as well as their biosynthetic and degradative enzymes.[5]

Generally, endocannabinoids are N-or O-derivatives of polyunsaturated fatty acids acting as cannabinoid receptor agonists. During the last years of research other endogenous endocannabinoid-like (eCB- like) N-acyl ethanolamines and monoacyl glycerols as well as N-acyl amino acids (NAAA) and n-acyl dopamine's have been described.[6,7]

Until today no comprehensive evaluation of eCB-like lipids in mice organs is available. We introduce an identification strategy of eCB-like lipids consistent of fatty acids with C16, C18, C20, C22 and C24 acyl chains as well as their prolongation and desaturation products. By utilization of nano-liquid chromatography high resolution mass spectrometry we evaluate the critical analytical parameters for eCB-like identification. We challenge our identification strategy to identify eCB-like in mice liver, serum, brain and subcellular brain regions which will give the opportunity for a quantitative approach for the investigation of circulating eCB-like lipids.

1 Immunology 144 (2015) 352–364.
2 Gastroenterology 151 (2016) 252–266.
3 International journal of molecular sciences 19 (2018)
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7 Cannabis Cannabinoid Res. 2 (2017) 183–196.

Inactivation of bacteria by γ-irradiation to characterize the interaction of synthetic anti-lipopolysaccharides peptides with membranes

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P 24

The rise of bacterial resistance against therapeutic drugs is leading to a concomitant reduction of treatment options[1]. Therefore, the development of bioactive molecules with a novel mechanism of action has a critical priority. This need is particularly urgent in the case of drugs targeting Gram-negative bacteria[1]. In this context, antimicrobial peptides (AMP) are among the most promising candidates. To evaluate their mechanism of action, there is a growing need for methods that allow studying molecular mechanisms in microbial systems, particularly bacterial membrane systems in all their complexity. We use ionizing radiation at low doses and sodium azide to generate bacterial ghosts (non-metabolically active bacteria), in which the metabolic activity of the bacteria and cell division processes cease. These procedures allow the direct analysis of the binding process of AMP on the bacterial outer membrane by isothermal titration calorimetry (ITC) to get valuable thermodynamic information. Our approach gives new insights to disclose the role of the bacterial membrane potential ($\Delta\Psi$) as a possible modulating parameter of the antimicrobial peptide binding and activity.

We applied this method to evaluate the anti-inflammatory activity of anti-lipopolysaccharides peptides (SALP). The suppression of inflammation signals in human in vitro systems (isolated human mononuclear cells and whole blood assays) is connected to an extremely high covering of the bacterial cell envelope (peptide/bacteria surface area ~ 7). This peptide accumulation on the outer membrane causes a strong reorganization of the lipid packing of the inner membrane with the loss of the phase transition[2]. We show a direct proof of the biological relevance of peptide binding to LPS not only in free form but as a constituent of the bacterial outer membrane to avoid the hyperproduction of pro-inflammatory cytokines.

This way, we provide an approach to bridge the gap between molecular and cellular investigations. Other studies can benefit from this strategy by minimizing biosafety problems while reducing damage and modification to bacterial envelopes.

1 Tacconelli, E. et al. The Lancet Infectious Diseases 18, 318-327(2018).
2 Correa, W. et al. The FEBS journal, 286,1576-1593 (2019).

Tuesday | **November 12**

9:00 – 10:00 **KEYNOTE**
Karsten Hiller, Technische Universität Braunschweig (Braunschweig, Germany)
K 05 **Metabolic crosstalk between mammalian cells and *Clostridium difficile* bacteria.**

10:00 – 10:15 COFFEE BREAK

10:15 – 11:15 **SESSION 3** | Chair: Nils Hoffmann

PRICE Verena Ertl
T 11 **Development and application of a high resolution mass spectrometry method to identify and quantify faecal lipid species**

PRICE Harald Schoeny
T 12 **How to solve the limits in absolute quantification on reversed phase LC-MS-based lipidomics approaches? A novel flow injection/ reversed phase LC-MS workflow with LILY as ISTD**

Yulia Popkova
T 13 **Differences in the lipid patterns during maturation of 3T3-L1 adipocytes investigated by thin-layer chromatography, gas chromatography and mass spectrometric approaches**

11:15 – 11:30 **LIPIDOMICS FORUM AWARDS for best Talk and Poster** Ronny Herzog, Dominik Schwudke

11:30 – 12:00 **Foundation of the International Lipidomics Society (ILS)** Kim Ekroos, Robert Ahrends
- Aims and mission statement
- Introduction of the society structure
- Overview of the different working groups
- Presentation of the current board and their responsibilities

12:00 – 13:00 LUNCH

13:00 – 14:30 **PANEL DISCUSSION**
What is next?
Future steps of the ILS and the involvement of you!

Metabolic crosstalk between mammalian cells and *Clostridium difficile* bacteria

Karsten Hiller[1,2],
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K 05

Lipid identification applying shotgun lipidomics is highly dependent on correct assignment of chemical sum composition to precursor m/z values and its correct assignment of the corresponding MS/MS experiment. Correct lipid identification using the Molecular Fragmentation Query Language (MFQL) in LipidXplorer is depending on this uniquely associated data structures. In the analysis of complex lipid extracts using spectral stitching, we observed an increased number of unusual lipid lds and a systematic shift in the overall lipidome composition. Thus we systematically tested the conditions for formation of insource fragments using the nano-ESI source Triversa Nanomate (Advion, Ithaca, US) and Q Exactive Mass spectrometer (Thermo Scientific, Bremen, Germany). Lipid standards and complex extracts were dissolved in $CHCl_3$/MeOH/isopropanol (1/2/4, v/v/v) containing 3.7 mM ammonium acetate.

C. difficile is an anaerobic pathogen in the human intestine that can cause severe complications in patients under antibiotic treatment. More than 20,000 patients die every year as a direct consequence of a *C. difficile* infection in the Unites States. Current knowledge indicates that these bacteria damage the integrity of the intestinal epithelial barrier mainly by the secretion of toxins (TcdA and TcdB) as a major virulence factor and subsequent inflammatory processes.

We were interested to study the metabolic crosstalk during the infection and performed co-culture experiments of the pathogen with mammalian host cells. We could reveal that that the presence of the bacteria induced a reprogramming of cellular metabolism and that this reprogramming is mediated by bacterial metabolites or small peptides. First results of stable-isotope driven metabolic profiling indicate that the secretion of this compound induces changes in mitochondrial and cellular metabolism of the host cells to finally secret a metabolite into the growth medium that might serve as a carbon source or as an electron sink for the bacteria.

Development and application of a high resolution mass spectrometry method to identify and quantify faecal lipid species

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T 11 PRICE

It is now generally accepted that gastrointestinal system in particular the intestinal microbiome plays an important role in human health and disease. Faecal materials reflect the microbial activity and the analysis of remaining unabsorbed metabolites including lipid species and provide an estimate of metabolic interaction between gut microbiota and host. To identify subtle metabolic variations induced by diet alterations and to characterize the metabolic impact of variations of the gut microbiota, metabolic profiling is becoming increasingly popular. Here we applied flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS) to identify and quantify lipid species and their abundance in faeces.

Faecal homogenates were subjected to total lipid extraction according to the protocol of Bligh and Dyer (B/D). Analysis of crude lipid extracts was performed by FIA-HRMS. A high heterogeneity was observed in faecal sample from different subjects. First experiments showed high amounts of triglycerides and diglycerides in the majority of samples. Species profiles included highly unsaturated species which could be confirmed in MS/MS spectra. Therefore, we validated a FIA-HRMS method for quantification of triglyceride and diglyceride species in human faeces. In order to guarantee sample stability, we are currently optimizing the preanalytical conditions. However, due to the complexity of these samples, additional separation techniques like hydrophilic interaction liquid chromatography will be coupled to MS (HILIC/HRMS) in a next step to increase the specificity and sensitivity for the identification and quantification of minor lipid species.

In summary, FIA-HRMS offers a high throughput method to analyse and quantify lipid species profiles of faecal samples. The application of these methods in various samples should provide a comprehensive picture of the faecal lipidome and improve the understanding of the role of the microbiome in human health.

How to solve the limits in absolute quantification on reversed phase LC-MS based lipidomics approaches – A novel flow injection-reversed phase LC-MS workflow with LILY as ISTD

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T 12 PRICE

"Lipidomics needs more standardization" the title of the recently published comment sums up the current situation in lipidomics[1]. Quantification integrating internal standards (ISTD) has become the method of choice; however the exact application remains a topic of discussion. Several rules were proposed: first of all the internal standard should show a high structural similarity (isotopically labeled analogues in the ideal case) to the target analyte and should not be present in the samples; it should be added prior to lipid extraction; at least one internal standard per lipid class (as defined by LIPID MAPS classification) is desirable; and finally, the applied MS method should allow simultaneous ionization with the analyte[2]. This latter requirement can be met by shotgun-lipidomics and HILIC/NP separations, but in reversed phase (RP) chromatography the lipid species within one lipid class are separated leading to the fact that one ISTD per lipid class is not sufficient. Therefore, absolute accurate quantification on reversed phase LC-MS-based lipidomics approaches is only enabled by compound-specific or at least retention time matched ISTDs (Level 1 and level 2 quantification after the definition of LSI). To the best of our knowledge, this was not applied to a higher number of analytes.

Lipidome isotope labeling of yeast (LILY)[3] has the potential to fulfill this pre-request as the whole lipidome of a eukaryotic organism is fully 13C-labeled. 250 lipid species from 19 classes (Cer, CL, Co, DG, HexCer, IPC, MG, LPC, LPE, PA, PC, PE, PG, PI, PS, SE, SPH, ST, TG) are available but their concentration is still unknown, as variations during the fermentation and different storage times change the lipidome. A novel flow injection/ reversed phase LC-MS workflow will be proposed including an intraday- quantification of LILY by a small number of unlabeled commercially available standards via flow injection and, in a second step, the use of the fully quantified 250 LILY lipids on a sample of interest via RP-LC-MS. This method benefit of the lower cost of LILY compared to other isotopically labeled ISTD, the higher number of available ISTDs, an intraday correction of the LILY concentration and the simultaneous use of the RPC for identification (potential isomer separation) and quantification.

1 Lipidomics Standards Initiative Consortium. Lipidomics needs more standardization. Nature Metabolism, 1:745–747, 2019
2 Wang M., Wang C., and Han X. Selection of internal standards for accurate quantification of complex lipid species in biological extracts by electrospray ionization mass spectrometry – What, how and why? Mass Spectrom Rev., 36(6):693–714, 2017
3 Rampler E. Coman C., Hermann G., Sickmann A., Ahrends R., and Koellensperger G. LILY-Lipidome Isotope Labeling of Yeast: In vivo synthesis of 13C labeled reference lipids for quantification by mass spectrometry. Analyst, 142:1891–1899, 2017

Differences in the lipid patterns during maturation of 3T3-L1 adipocytes investigated by thin-layer chromatography, gas chromatography and mass spectrometric approaches

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T 13

Populations of industrialized countries have registered a dramatically increasing prevalence in obesity since many years. Despite continuous research, mechanisms involved in the storage of chemical energy and utilization in adipocytes are still under investigation. Adipocytes have the task to store excessive energy in the form of triacylglycerols (TG) and it is already well-known that the fatty acyl (FA) composition of TG is largely determined by the composition of the diet. In contrast to TG, the composition of adipocyte phospholipids was less comprehensively investigated[1,2].

In this study the compositions of the most abundant phospholipid classes of 3T3-L1 undifferentiated (preadipocytes) and differentiated cells (adipocytes) were determined. The lipid fractions were isolated by normal phase high performance thin-layer chromatography (HPTLC) and subsequently analyzed by electrospray ionization mass spectrometry (ESI-MS). Additionally, the FA compositions were determined by gas chromatography (GC). The positions of the FA residues were further confirmed by phospholipase A2 digestion of isolated phospholipid classes. It will be shown that undifferentiated 3T3-L1 and mature adipocytes differ extremely regarding their compositions[3,4]. This goes along with an increase in odd chain fatty acids.

In addition to the biological background, we also addressed the question which analytical method (ESI, HPTLC and GC) is most suitable to assess the lipid composition of complex samples.

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2 Crown SB, Marze N, and Antoniewicz MR. Catabolism of branched chain amino acids contributes significantly to synthesis of odd-chain and even-chain fatty acids in 3T3-L1 adipocytes. PLoS One, 10(12):e0145850, 2015
3 Wang J, Wang C, and Han X. Tutorial on lipidomics. Anal Chim Acta, 1061:28–41, 2019
4 Jurowski K, Kochan K, Walczak J, Baranska M, Piekoszewski W, and Buszewski B. Analytical techniques in lipidomics: state of the art. Crit Rev Anal Chem, 47(5):418–37, 2017

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