LIPIDOMICS
FORUM

15. - 17. November 2015

VENUE
Research Center Borstel
Parkallee 1-40
23845   B O R S T E L
Germany

Coordination: Jutta Passarger

ORGANIZERS
Robert Ahrends │ ISAS
Nicolas Gisch │ RCB
Dominik Schwudke │ RCB

AbstractBook

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Sunday | November 15

18:00

OPENING KEYNOTE
Kai Simons
T 01 **From Lipid Rafts to Clinical Lipidomics**
Max Planck Institute for Molecular Cell Biology and Genetics and Lipotype GmbH, Dresden, Germany;
E-Mail: simons@mpi-cbg.de

19:00

CONCERT
Come together with Jazz
Juhn Hughes (b) Björn Lücker (dr) Matthäus Winnitzki (p)

DINNER

Monday | November 16

9:00 - 9:45

TUTORIAL
Robert Ahrends
T 02 **LC-MS in Lipid Research**
Leibniz-Institut für Analytische Wissenschaften - ISAS - e. V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany;
E-Mail: robert.ahrends@isas.de

9:45 - 10:00

COFFEE BREAK

10:00 - 10:45

KEYNOTE
Martin Giera
T 03 **A Role for Adrenic Acid in the Resolution Phase of Inflammation**
Center for Proteomics and Metabolomics, Leiden University Medical Center, Albinusdreef 2, 2300RC Leiden,
The Netherlands; m.a.giera@lumc.nl

10:45 - 12:15

SESSION 1 | Chair: Chakravarthy Marella

10:45 `PRICE` Annika Ostermann
T 04 **Targeted Metabolomics of Eicosanoids and other Oxylipins formed in the Arachidonic Acid Cascade**
Institut für Lebensmitteltoxikologie und Chemische Analytik, Tierärztliche Hochschule Hannover, Germany;
E-Mail: annika.ostermann@tiho-hannover.de

11:05 Mesut Bilgin
T 05 **High Resolution LC-MS/MS Screening for Endocannabinoids Discovery**
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany;
Department of Cell Death and Metabolism, Danish Cancer Society Research Center (DCRC), DK-2100 Copenhagen,
Denmark; E-Mail: mesutb@cancer.dk

11:25 Alaa Othman
T 06 **1-deoxysphingolipids: Novel Biomarkers and Therapeutic Targets in Type 2 Diabetes Mellitus**
Core Facility "Bio-analysis and Mass Spectrometry", Center of Brain Behavior and Metabolism (CBBM),
University of Lübeck, Germany; E-Mail: alaa.othman@pharma.uni-luebeck.de

11:45 `PRICE` Katharina Wozny
T 07 **Approach for Untargeted Analyses of Lipidomes by Using LC-ESI-Orbitrap-MS**
Heidelberg University Biochemistry Center, 69120, Heidelberg, Germany;
E-Mail: katharina.wozny@bzh.uni-heidelberg.de

12:15 - 13:30

LUNCH BREAK

13:30 - 15:15	**SESSION 2**	Chair: Matthias Krajewski

13:30

T 08   Klaus Dreisewerd

MALDI-2: Sensitive MS Imaging through Laser-induced Generation of Secondary MALDI-like Ionization Processes

Institute for Hygiene, University of Münster, Germany; E-Mail: dreisew@uni-muenster.de

14:00   **PRICE**   Johanna von Gerichten

T 09   **Targeted and Untargeted Mass Spectrometry Imaging of Lipids in the Brain**

Instrumental Analytics and Bioanalytics, Mannheim University of Applied Sciences, Mannheim, Germany; E-Mail: j.vongerichten@hs-mannheim.de,

14:20   **PRICE**   Fabian Eiersbrock

T 10   **Viscous Ionic Liquid Matrices for MALDI-MS Imaging of Brain Lipids with High Lateral Resolution**

Institute for Hygiene, University of Münster, Germany; E-Mail: f_eier01@wwu.de

14:40   **PRICE**   Yulia Popkova

T 11   **Analysis of the Lipid Composition of (Adipose) Tissues by Spectroscopic Methods**

University of Leipzig, Medical Faculty, Institute of Medical Physics and Biophysics, Germany; E-Mail: yulia.popkova@medizin.uni-leipzig.de

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15:15 - 16:00   **COFFEE BREAK**

16:00 - 16:45   **KEYNOTE**

Matej Orešič

T 12   **Metabolic Disease Signatures Translated to Underlying Mechanisms – a Lipidomics Approach**

Steno Diabetes Center, Gentofte, DK-2820, Denmark; E-Mail: mtjo@steno.dk

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16:45 - 17:30   **TUTORIAL**

Dominik Schwudke

T 13   **Shotgun Lipidomics – Opportunities and Limits**

Division of Bioanalytical Chemistry - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: dschwudke@fz-borstel.de

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17:30 - 19:30   **POSTER SESSION**

PRICE   Julius Brandenburg

P 01   **Wnt6 affects Macrophage Lipid Homeostasis and Susceptibility to Mycobacterium tuberculosis Infection**

Division of Microbial Interface Biology - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: jbrandenburg@fz-borstel.de

PRICE   Cristina Coman

P 02   **SIMPLEX: A Combinatorial Multimolecular Omics Approach for Systems Biology**

Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany; E-Mail: cristina.coman@isas.de

PRICE   Niklas Danne-Rasche

P 03   **Development of a nanoLC-MS Method for Lipidomics**

Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany; E-Mail: Niklas.DanneRasche@isas.de

PRICE   Lars F. Eggers

P 04   **The Human Lung Lipidome: A Potential Connection of Histopathological Phenotypes and Development of Cancer or Emphysema**

Division of Bioanalytical Chemistry - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: leggers@fz-borstel.de

PRICE   Annabelle Fülöp

P 05   **Mass Spectrometry Imaging of PEGylated Liposomes in Mice**

Center for Applied Research in Applied Biomedical Mass Spectrometry (ABIMAS) Mannheim University of Applied Sciences, Paul-Wittsack-Straße 10, 68163 Mannheim, Germany; E-Mail: a.fueloep@hs-mannheim.de

Martin Giera

P 06 Differential Mobility Separation of Leukotrienes and Protectins
Center for Proteomics and Metabolomics, Leiden University Medical Center, Albinusdreef 2, 2300RC Leiden, The Netherlands; E-Mail: m.a.giera@lumc.nl

Nicolas Gisch

P 07 Lipid Profiling of *Mycobacterium tuberculosis* East African Indian Strains by ^{1}H,^{13}C -Heteronuclear Single Quantum Coherence-NMR
Division of Bioanalytical Chemistry - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: ngisch@fz-borstel.de

PRICE Matthias Krajewski

P 08 *Mycobacterium tuberculosis* induced Changes of Oxidized Polyunsaturated Fatty Acid Metabolism analyzed by Liquid Chromatography-Tandem Mass Spectrometry
Division of Bioanalytical Chemistry - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: mkrajewski@fz-borstel.de

PRICE Chakravarthy Marella

P 09 LUX Score: Homology Metric for Global Comparison of Lipidomes
Division of Bioanalytical Chemistry - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: cmarella@fz-borstel.de

PRICE Christian Marsching

P 10 Imaging Mass Spectrometry: Quantitative Aspects in Lipid Analysis
Center for Applied Research „Applied Biomedical Mass Spectrometry" (ABIMAS), Mannheim, Germany; E-Mail: c.marsching@hs-mannheim.de

PRICE Dinh Lien Chi Nguyen

P 11 Systems Biology of the Unfolded Protein Response in Glioma
Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany; Germany; E-Mail: chi.nguyen@isas.de

PRICE Bing Peng

P 12 Adaptation of Skyline for Targeted Lipidomics
Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany; E-Mail: bing.peng@isas.de

PRICE Victoria Sohst

P 13 Fetal Calf Serum (FCS) Compensates the Lipid Profile of Acid Sphingomyelinase (aSMase) Deficient Bone Marrow Derived Macrophages (BMDM)
Division of Microbial Interface Biology - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: vsohst@fz-borstel.de

PRICE Franziska Waldow

P 14 In-depth Analysis of the Membrane Phospholipid Composition of *E. coli* Strains with Genetically Engineered Lipopolysaccharide Structure
Division of Bioanalytical Chemistry - Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany; E-Mail: fwaldow@fz-borstel.de

PRICE Karina Lindner

P 15 Simultaneous Analysis of Lipids, mRNA and Protein in Murine Tracheal Airway Epithelial Cells
Institute for Anatomy, University Lübeck, Germany; E-Mail: lindner@anat.uni-luebeck.de

PRICE Sarah Hofmann

P 16 HCV Replication Alters the Cellular Lipid Profile and is itself Influenced by Fatty Acids
Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany; E-Mail: sarah.hofmann@hpi.uni-hamburg.de

PRICE Chris Bielow

P 17 An Automated Software Pipeline for Shotgun Lipidomics using Direct-Infusion, High-Resolution Mass Spectrometry
Integrative Proteomics and Metabolomics Platform, Berlin Institute of Health (BIH), Robert-Rössle-Straße 10, 13125 Berlin, Germany; E-Mail: chris.bielow@mdc-berlin.de

PRICE Laura Kutzner

P 18 **Differentiated Analysis of the Fatty Acid Pattern in Food Supplements**
Institut für Lebensmitteltoxikologie und Chemische Analytik, Tierärztliche Hochschule Hannover, Germany;
E-Mail: annika.ostermann@tiho-hannover.de

PRICE Andrej Meusel

P 19 **Analysis of Lipid Mixtures by Continuous Wavelet Transformed ^{1}H NMR**
Institute of Medical Physics and Biophysics, Haertelstrasse 16-18, University of Leipzig, Germany;
E-Mail: Andrej.Meusel@medizin.uni-leipzig.de

PRICE Katharina Rund

P 20 **Oxylipin and Fatty Acid Pattern following Feeding of Mice with n3-Polyunsaturated Fatty Acids**
Institut für Lebensmitteltoxikologie und Chemische Analytik, Tierärztliche Hochschule Hannover, Germany;
E-Mail: katharina.rund@tiho-hannover.de

PRICE Jenny Schröter

P 21 **Unexpected Products of the HOCl-Induced Oxidation of Oleic Acid: a Study using TLC-ESI MS,**
MALDI MS and NMR Spectroscopy
University of Leipzig, Medical Faculty, Institute of Medical Physics and Biophysics, Germany;
E-Mail: Jenny.Schroeter@medizin.uni-leipzig.de

PRICE Katja Steckhan

P 22 **Matching Thin-Layer Chromatography, Overlay Immunodetection,**
and Desorption Electrospray Ionisation (DESI) – Mass Spectrometry for Glycosphingolipid Analysis
Institute for Hygiene, University of Münster, 48149 Münster, Germany; E-Mail: k_stec01@wwu.de

PRICE Christian Vosse

P 23 **Lipid-Profiling by LC-ESI-MS/MS**
Institute of Inorganic and Analytical Chemistry, Westfälische Wilhelms-Universität Münster, Germany;
E-Mail: christianvosse@uni-muenster.de

PRICE Nico Heise

P 24 **Probing the Effect of ROS on Properties of Mitochondria and Liposomes made of Synthetic**
and Natural Cardiolipins
University of Greifswald, Institute of Biochemistry, Felix Hausdorff-Straße 4, D-17489, Greifswald, Germany;
E-Mail: nico.heise@uni-greifswald.de

Tuula Penate Medina

P 25 **Site Specific Release of Liposomal Nanotheranostic Agents through Enzymatic Sensitizing of Liposomes**
by Phopholipases in Combination by Remote Activation by Alternating Magnetic Field
Section Biomedical Imaging, Department of Diagnostic Radiology and Neuroradiology,
University Hospital Schleswig-Holstein, Campus Kiel, Germany; E-Mail: oula.penate@rad.uni-kiel.de

Steven H. L. Verhelst

P 26 **Chemically Cleavable Linkers for Target Identification**
Leibniz-Institut für Analytische Wissenschaften - ISAS - e. V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany;
E-Mail: steven.verhelst@isas.de

Elena Sokol

P 27 **Rapid Phospholipid Characterization Using a Novel Intelligent**
Workflow on a Tribrid Orbitrap Mass Spectrometer
Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA; E-Mail: elena.sokol@thermofisher.com

| 19:30 - 21:30 | **DINNER AND GET TOGETHER** |
| | **Musical Guest: Harkov** |

Tuesday | November 17

9:00 - 9:45	**TUTORIAL**
	Ronny Herzog
T 14	**Lipid Identification with LipidXplorer – a Tutorial**
	Lipotype GmbH, Tatzberg 47, 01307 Dresden, Germany; E-Mail: herzog@lipotype.com

| 9:45 - 10:00 | **COFFEE BREAK** |

10:00 - 10:45	**KEYNOTE**
	Gerhard Liebisch
T 15	**Quantitative and Metabolic Profiling of Lipid Species**
	Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany; E-Mail: gerhard.liebisch@ukr.de

| 10:45 - 12:15 | **SESSION 3** | Chair: Lars Florian Eggers |

10:45	David A. Peake
T 16	**Acquisition and Processing of a High Resolution LC-MS-MS Dataset for the NIST Inter-laboratory Comparison Exercise for Lipidomics Measurements in Human Serum/Plasma**
	Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA; E-Mail: david.peake@thermofisher.com

11:15	Rita Derua
T 17	**Analysis and Quantification of Cardiolipins on a Hybrid Triple Quadrupole/ion Trap (4000 QTRAP) Mass Spectrometer**
	Laboratory of Protein Phosphorylation and Proteomics, KU Leuven, Belgium; E-Mail: rita.derua@med.kuleuven.be

11:35	**PRICE** Ariane Nimptsch, Kathrin M. Engel
T 18	**The Maturation of Sperm is Accompanied by Changes of the (Phospho-) Lipid Composition – A MALDI -TOF MS Investigation of Murine Epididymal Spermatozoa**
	Universität Leipzig, Medical Department – Institute of Medical Physics and Biophysics, Germany; E-Mail: ariane.nimptsch@medizin.uni-leipzig.de & kathrin.engel@medizin.uni-leipzig.de

| 11:55 | **PRICE** Sarita Hebbar |
| T 19 | **Linking *Drosophila* Crumbs and Lipids in Photoreceptor Cell Morphology** |
| | Max-Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany; E-Mail: hebbar@mpi-cbg.de |

12:15 - 13:00	**KEYNOTE**
	Andrej Shevchenko
T 20	**Lipid Diet Rules the Life of Flies**
	MPI of Molecular Cell Biology and Genetics, 01307 Dresden, Germany; E-Mail: shevchenko@mpi-cbg.de

| 13:00 - 13:30 | **CLOSING SESSION / PRICES FOR BEST POSTERS AND TALK** |

| 13:30 | **LUNCH AND FAREWELL** |

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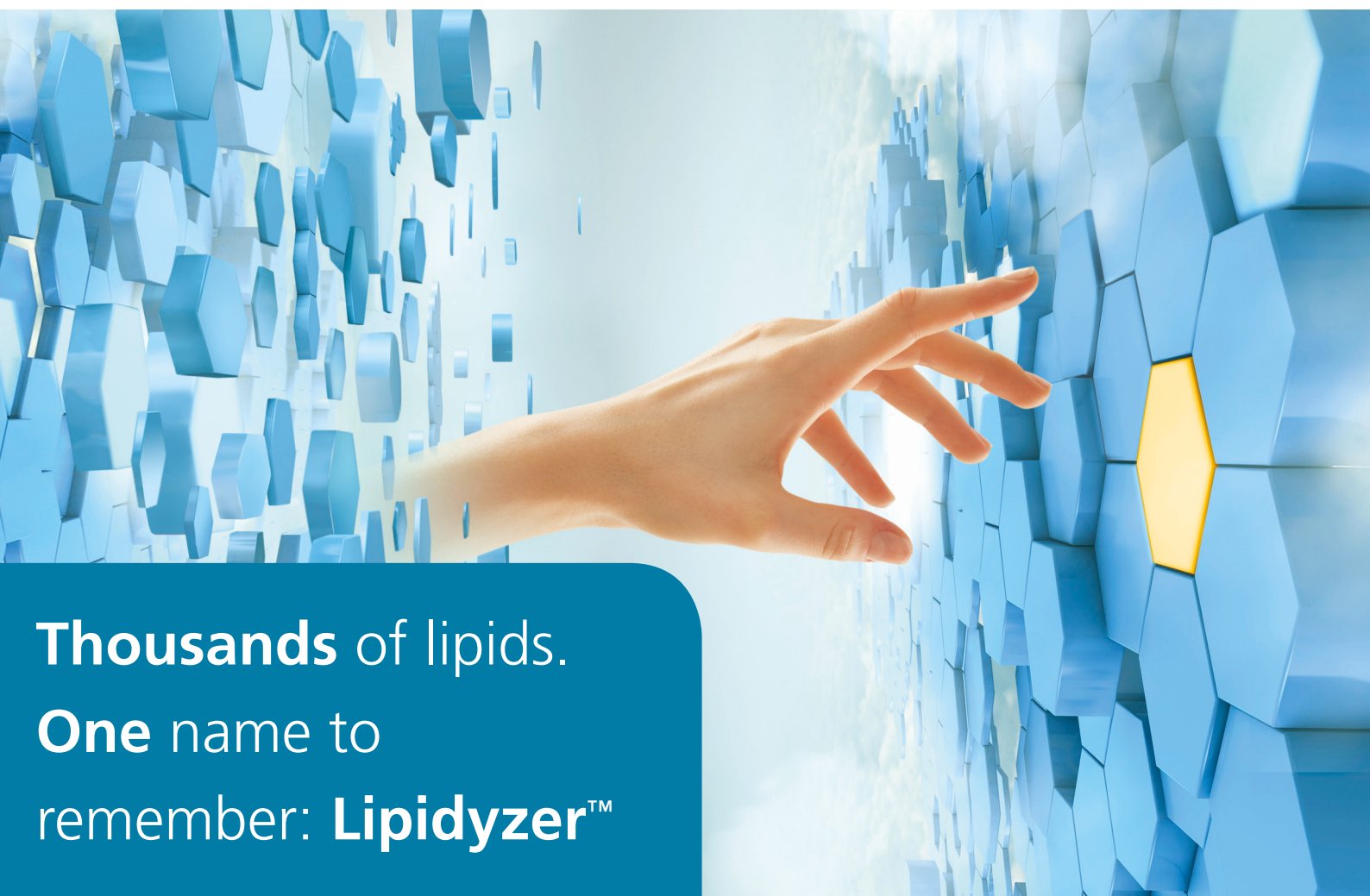
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Talks

From Lipid Rafts to Clinical Lipidomics

Kai Simons[1]

[1] Max Planck Institute for Molecular Cell Biology and Genetics and Lipotype GmbH, Dresden, Germany;
E-Mail: simons@mpi-cbg.de

T 01

Lipids are important building blocks of life. They have many different biological functions. Their main function is to form the matrix of our cell membranes where they support a variety of functions essential for life. This 2-dimensional fluid matrix has evolved incredible material properties. One is the capability to sub-compartmentalize the fluid into dynamic cholesterol-stabilized sphingolipid-protein assemblies that function in membrane trafficking, signalling and other membrane functions. This capability is a property of cell membranes that are poised close to a phase transition, enabling coalescence into subdomains. For this to occur, the lipid and the protein composition has to be tightly regulated. We have developed a shotgun mass spectrometry platform that now can analyse hundreds of lipids in only a few minutes with absolute quantification. This effective routine of shotgun lipidomics became possible by introducing a unique workflow that combined improved extraction protocols with cutting edge mass spectrometry and novel software. The technology is highly reproducible, achieving an average coefficient of variations of $<10\%$ (intra-day), approx. 10% (inter-day) and approx. 15% (inter-site) for most lipid species. The platform is easily transferable allowing the direct comparison of data acquired in different acquisition sites. We have used this technology to analyze blood cells and plasma with the aim to establish multi-parametric lipid signatures that are of diagnostic value.

LC-MS in Lipid Research

Robert Ahrends[1]

[1] Leibniz-Institut für Analytische Wissenschaften - ISAS - e. V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany;
  E-Mail: robert.ahrends@isas.de

T 02

Liquid chromatography-mass spectrometry (LC-MS)-based lipidomics has been a subject of vivid developments over the last decade. This tutorial will give a brief introduction into LC/MS based lipidomics. Thereby, different chromatographic techniques will be evaluated regarding their selectivity and performance. We will discuss the advantages and the drawbacks of each technique for the LC dependent lipid separation. Furthermore, an overview of currently applied MS methods for LC based lipid analysis will be given and different techniques will be compared to each other. Additionally, various software solutions for targeted and non-targeted LC/MS data analysis will be presented. We hope that the workshop will lead to a fruitful discussion and help to overcome current bottlenecks in LC/MS based lipidomics.

A Role for Adrenic Acid in the Resolution Phase of Inflammation

Martin Giera[1]

Hulda S Jónasdóttir[1], Hilde Brouwers[2], Dick-Paul Kloos[1], Marije Kuipers[1], André M Deelder[1], Tom WJ Huizinga[2], Jona Freysdottir[3], Ingibjörg Hardardottir[3], Joan Claria[4], Cristina López-Vicario[4], René Toes[2], Andreea Ioan-Facsinay[2]

[1] Center for Proteomics and Metabolomics, Leiden University Medical Center, Albinusdreef 2, 2300RC Leiden, The Netherlands; E-Mail: m.a.giera@lumc.nl
[2] Departement of Rheumatology, Leiden University Medical Center, Albinusdreef 2, 2300RC Leiden, The Netherlands
[3] Faculty of Medicine, School of Health Sciences, University of Iceland, Vatnsmýrarvegi 16, 101 Reykjavik, Iceland
[4] Department of Biochemistry and Molecular Genetics and Department of Physiological Sciences I, University of Barcelona, Barcelona, Spain

T 03

The resolution phase of inflammation is crucial to prevent acute inflammation from becoming chronic. It has become evident during recent years that resolution is an active process that involves several cells and molecules. Especially a novel class of specialized lipids recently emerged as potent pro-resolving mediators (SPM). Nonetheless, the molecular identity and interplay of these lipid SPM are not fully understood and novel molecules continue to be defined as important in this process. Therefore, we extensively characterized the inflammatory response in the murine zymosan induced peritonitis model using several mass spectrometry based platforms. Interestingly, we found that the n-6 polyunsaturated fatty acid adrenic acid (AdA) as well as its cyclooxygenase and lipoxygenase metabolites accumulated in the peritoneal exudate cells during the resolution phase of inflammation, suggesting a pro-resolving function of AdA. To address this possibility, we investigated the effect of AdA on immune cells. By using an LC-MS/MS based screening platform, we show that low micromolar concentrations of AdA block the formation of the potent neutrophil chemo attractant leukotriene B4 (LTB$_4$) and its pathway marker 5-HETE, without affecting cell viability. Further investigation revealed that AdA does not block calcium influx upon ionophore stimulation, but rather blocks the release of arachidonic acid (AA) from phospholipids, suggesting an inhibitory effect on cytosolic phospholipase A2, which catalyzes this process. These findings are specific for neutrophils, as no inhibition of LTB$_4$ production was observed in M1 macrophages. In conclusion, our data indicate that AdA selectively blocks the release of AA in neutrophils, influencing the AA cascade and thereby unveiling a novel pathway that could promote the resolution of inflammation.

Targeted Metabolomics of Eicosanoids and other Oxylipins formed in the Arachidonic Acid Cascade

Annika Ostermann [1]

Ina Willenberg[1], Nils Helge Schebb[1, 2]

[1] Institut für Lebensmitteltoxikologie und Chemische Analytik, Tierärztliche Hochschule Hannover, Germany;
E-Mail: annika.ostermann@tiho-hannover.de
[2] Institut für Lebensmittelchemie, Universität Wuppertal, Germany

T 04 PRICE

Lipid mediators play an important role in biology. Particularly eicosanoids (C20) and oxidative products of other long chain polyunsaturated fatty acids (PUFA) regulate a large variety of cellular and physiological functions.

In mammals, these oxylipins are formed enzymatically via three pathways: i) constitutively expressed cyclooxygenase 1 (COX-1) and inducible COX-2, ii) 5-, 12- and 15-lipoxygenases (LOX) as well as iii) cytochrome P450 monooxygenases (CYP) and non-enzymatically by (aut)oxidation. The initially formed products can be further converted by several other enzymes, for example by microsomal prostaglandin E synthase (mPGES) or by soluble epoxide hydrolase (sEH) leading to a pleiotrop of oxylipins formed in the arachidonic acid (AA) cascade.

Quantification of eicosanoids and other oxylipins in biological samples is crucial for a better understanding of the biology of these lipid mediators. Moreover, a robust and reliable quantification is necessary to monitor the effects of pharmaceutical intervention and diet on the arachidonic acid (AA) cascade, one of today's most relevant drug targets. Low (sub-nanomolar) concentrations and a large number of structurally similar analytes, including regioisomers, require high chromatographic resolution and selective as well as sensitive MS analysis. Currently, reversed phase LC in combination with detection on sensitive triple quadrupole instruments, operating in selected reaction monitoring (SRM) mode, is dominantly used for quantitative oxylipin analysis.

In the talk the challenges for quantitative analysis are summarized and the application of LC-qqq-MS methods for the investigation of the effects of n3-PUFA and inhibitors of enzymes of the AA cascade are presented.

Ostermann, A. I.; Willenberg, I.; Schebb, N. H., Comparison of sample preparation methods for the quantitative analysis of eicosanoids and other oxylipins in plasma by means of LC-MS/MS. Anal Bioanal Chem 2015, 407, (5), 1403-14.

Willenberg, I.; Ostermann, A. I.; Schebb, N. H., Targeted metabolomics of the arachidonic acid cascade: current state and challenges of LC-MS analysis of oxylipins. Anal Bioanal Chem 2015, 407, (10), 2675-83.

High Resolution LC-MS/MS Screening for Endocannabinoids Discovery

Mesut Bilgin[1, 2]
Juergen Grassler[3], Petra Born[1], Suzanne Eaton[1], Andrej Shevchenko[1]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[2] Department of Cell Death and Metabolism, Danish Cancer Society Research Center (DCRC), DK-2100 Copenhagen, Denmark;
  E-Mail: mesutb@cancer.dk
[3] Department and Outpatient Department of Medicine III, Medical Faculty Carl Gustav Carus, Technische Universitaet Dresden, Dresden, Germany

T 05

Endocannabinoids (EC) are physiological ligands of CB1 and CB2 cannabinoid receptors and important signalling molecules in the central and peripheral nervous system. Five structurally distinct molecular classes of EC are recognized: they comprise fatty acid or fatty alcohol moieties conjugated to ethanolamine, glycerol or dopamine via amide, ester or ether bonds. While a large pool of structurally related molecules is produced in vivo, their molecular composition and biological function is poorly characterized. Particularly, it remains unknown if other polar moieties could be conjugated with fatty acids / alcohols in vivo giving rise to novel EC classes.

Here we report on a currently most comprehensive screen covering >2000 known and putative structures of EC-related compounds. Compounds identification relied upon structural similarity between different EC classes that translated into commonality of their fragmentation pathways. To detect specific fragments and match them to plausible precursor we applied high-resolution LC-MS survey scan followed by LC-MS/MS all-ions fragmentation scan on a Q Exactive mass spectrometer. The method does not rely on an inclusion list of masses of plausible precursors and the sensitivity is at the level of a few picograms per injection into a LC-MS/MS system. In a rat kidney extract three species of N-acylaspartates (NAAsp) – a novel class of endogenous EC-related compounds, along with other 66 known and 63 new species of 17 known classes of EC-related compounds, including bona fide EC such as anandamide and 2-arachidonoyl glycerol. Newly discovered NAAsp's are not ligands of CB1 and CB2 cannabinoid receptors, but in vitro assays showed that they inhibited Hedgehog signalling pathway with the same efficiency as anandamide. Altogether, the screen provided a valuable resource for discovery novel EC-related compounds.

1-deoxysphingolipids:
Novel Biomarkers and Therapeutic Targets in Type 2 Diabetes Mellitus

Alaa Othman[1, 2, 3]
Arnold von Eckardstein[2, 3], Thorsten Hornemann[2, 3]

[1] Core Facility "Bio-analysis and Mass Spectrometry", Center of Brain Behavior and Metabolism (CBBM), University of Lübeck, Germany; E-Mail: alaa.othman@pharma.uni-luebeck.de
[2] Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Germany
[3] Institute for Clinical Chemistry, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

T 06

Sphingolipid synthesis is typically initiated by the conjugation of L-serine and palmitoyl-CoA – a reaction catalyzed by the serine-palmitoyltransferase (SPT). SPT can metabolize other acyl-CoAs, and other amino acids such as L-alanine or glycine. Of special interest are the sphingolipids resulting from the conjugation of alanine with palmitoyl-CoA leading to a novel class of sphingolipids called 1-deoxysphingolipids (1-deoxySLs) which were initially discovered in hereditary sensory neuropathy type I (HSNI). In HSN1, several missense mutations in SPT increase dSLs generation and eventually lead to the neuropathy.

Using LC/MS after chemical hydrolysis and derivatization, we compared the plasma sphingolipids profile in healthy humans and patients with type II diabetes mellitus (T2DM) and the metabolic syndrome (MetS) in several case-control, interventional and prospective clinical studies (n = 2000). We also measured the sphingolipid content in plasma and tissues of several animal models of metabolic diseases and deciphered several pathomechanisms of 1-deoxySLs in several in vitro models. 1-deoxySLs were significantly elevated in patients with MetS or T2DM. Multivariate analysis (OPLS-SA) showed that 1-deoxySLs are important contributors to the MetS state model, just next to triglycerides and above many of the conventional indicators of MetS. Partial correlation analysis revealed that 1-deoxySLs independently correlated to glucose and triglycerides. In a prospective study, 1-deoxySLs were independent predictors for the development of T2DM. Interestingly, fenofibrate (a PPARa agonist and a lipid lowering drug) lowered significantly the plasma 1-deoxySLs while extended-release niacin and statins did not. This suggests a potential mechanistic link between PPARa and 1-deoxySLs. Moreover, after screening several animal models for sphingolipids backbone profiles, streptozotocin (STZ) rat model showed elevated 1-deoxySLs in plasma and liver and not in other tissues suggesting a specific role of the liver in 1-deoxySLs metabolism. Interestingly, serine-enriched diet significantly decreased the plasma 1-deoxySLs in STZ rats and improved the neuropathy phenotype.

Taken together, our findings strongly argue for the potential use of 1-deoxySLs as a new class of biomarkers in the metabolic syndrome and type 2 diabetes mellitus and offer a potential therapeutic strategy to decrease their plasma levels which could help prevent diabetic neuropathy.

Approach for Untargeted Analyses of Lipidomes by Using LC-ESI-Orbitrap-MS

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T 07 **PRICE**

In the present talk an automated untargeted approach based on full MS data is described. Due to the highly complex lipid patterns analyzed, a chromatographic separation prior to MS analysis turned out to be indispensable even in the case of the very high resolution instrument employed. Thus, mass spectrometric overlaps and ion suppression could be almost completely avoided. Due to the use of full MS data, the analysis is not impaired by the intensity cut-off needed for precursor ion selection. Hence, it is possible to reliably detect and identify lipid species of very low abundance lipid classes.

The approach was used for the comparison of the lipidomes of alkaloid-treated and non-treated *E.coli* cells and allowed for fast identification of as many as 500 different lipid signals. The lipids identified were glycerolipid species and species of different glycerophospholipid classes. In addition, about the same number of non-lipid signals was found for both the treated and the untreated sample.

The quantitative comparison of the alkaloid-treated and the non-treated sample revealed 11 signals with significantly increased relative abundance in the alkaloid-treated sample. Of these, four signals are due to lipid molecules.

MALDI-2: Sensitive MS Imaging through Laser-induced Generation of Secondary MALDI-like Ionization Processes

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T 08

In MALDI, only a fraction of the desorbed molecules is also ionized. This problem is aggravated in highly-resolved MALDI imaging (MALDI-MSI) where only small amounts of material are available and because of ion suppression effects. Here we used a postionization laser to initiate secondary MALDI-like ionization processes in the gas phase. A MALDI Synapt G2-S (Waters) was used as mass spectrometer. The ion source was modified for (i) operation at elevated N2 buffer gas pressure (up to 4 mbar) [2], (ii) for achieving an effective focal laser spot size of 5 μm diameter, and (iii) for adopting the beam of an optical parametric oscillator laser (OPO, versaScan, GWU Lasertechnik, λ=213–2550 nm, τ~6 ns). Positive and negative ion mode mass spectra were recorded with 2,5-dihydroxybenzoic acid (DHB) and norharmane matrices, respectively. In the positive ion mode, in particular lipid species that form $[M+H]^+$ ions benefitted strongly from the postionization step [e.g., cholesterol, phosphatidylethanolamines (PE), -serines (PS) and galactosylceramides (GalCer)]. Numerous species were detected from mouse brain with up to 2 orders of magnitude higher signal intensities tissue than with conventional MALDI-MSI; about 2-3 more lipid species could tentatively be assigned. Ion suppression by abundant phosphatidylcholines (PC) is strongly reduced. In addition, liposoluble vitamins A, D3 and E, and K2 could be imaged using MALDI-2. In the negative ion mode, strongly elevated $[M-H]^-$ ion signals of numerous membrane lipids (including PE and PS) were generated. Key parameters for a high MALDI-2 ion yield are (i) a sufficient buffer gas pressure in the ion source (2-3 mbar), which differentiates the method from conventional single and multi-photon ionization performed under high vacuum, (ii) a photon energy exceeding the two-photon ionization threshold of the matrix (~310 nm for DHB), and (iii) a suitable pulse delay and laser pulse energy range. Our findings suggest that the MALDI-2 ionization process is driven by photoionization of matrix molecules and subsequent gas phase proton transfer reactions similar to those discussed for conventional MALDI-MS. Given the high signal intensities which we obtained by MALDI-2- MSI from only 5 μm-wide pixels we hypothesize that the technology could in the future enable sensitive MS imaging of lipids with even higher lateral resolution in the 1-2 μm range.

1   Soltwisch J, Kettling H, Vens-Cappell S, Wiegelmann M, Müthing J, Dreisewerd K (2015) Science 348:211-215

2   Kettling H, Vens-Cappel S, Soltwisch J, Pirkl A, Haier J, Müthing J, Dreisewerd K (2014) Anal Chem 86:7798-7805

Targeted and Untargeted Mass Spectrometry Imaging of Lipids in the Brain

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T 09 | PRICE

Mass spectrometry imaging (MSI) recently became a common tool to show the spatial distribution of distinct lipids in biological tissues [1]. With the potential to identify most disturbed cell populations of a tissue it can substantially contribute in understanding the biology of diseases [2].

MALDI-TOF (matrix assisted laser desorption/ionization-time of flight) is so far the most commonly used MSI technique due to its wide mass range and relatively high spatial resolution. Generating simple MS[1] data it may be considered untargeted MSI. With the purpose to generate targeted, specific, and fast MSI from tissue sections, we set up DESI MS[2]I using a triple quadrupole instrument. In DESI (desorption electrospray ionization) molecules are ionized under ambient conditions via a charged spray solution and desorbed ion droplets are transferred from the tissue into the MS [3]. Coupling to a triple quadrupole facilitates the use of tandem MS scan modes such as precursor ion scanning, neutral loss scanning and multiple reaction monitoring (MRM). These modes generate additional signal specificity and may enhance sensitivity significantly. Here we compare the two different MSI techniques, DESI MS/MS and MALDI TOF and provide sample preparation tools for DESI MS[2]I, which help to reduce matrix effects [4].

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2   Aichler, M. and A. Walch, *MALDI Imaging mass spectrometry: current frontiers and perspectives in pathology research and practice. Lab Invest*, 2015. 95(4): p. 422-31.

3   Takats, Z., J. M. Wiseman, and R. G. Cooks, *Ambient mass spectrometry using desorption electrospray ionization (DESI): instrumentation, mechanisms and applications in forensics, chemistry, and biology. J Mass Spectrom*, 2005. 40(10): p. 1261-75.

4   Wang, H. Y., C. B. Liu, and H. W. Wu, *A simple desalting method for direct MALDI mass spectrometry profiling of tissue lipids. J Lipid Res*, 2011. 52(4): p. 840-9.

Viscous Ionic Liquid Matrices for MALDI-MS Imaging of Brain Lipids with High Lateral Resolution

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T 10   PRICE

Analytical sensitivity and lateral resolution are key factors in MALDI-MS imaging and are critically determined by the physico-chemical properties of the matrix-solvent system and the morphology of the coating. A few studies have previously reported the use of viscous room temperature ionic liquid (RTIL) matrices for MALDI-MSI of phospho- and glycolipids [1, 2]. Step sizes of 50 and 75 µm, respectively, were used in those works, which limited predictions about the possible occurrence of analyte diffusion below these values. Here we synthesized a total of 180 RTILs and identified 7 as particularly suited for MALDI-MSI of lipids. Using these 7 compounds we analyzed mouse brain lipids at a step size of 15 µm. RTILs were synthesized using a proton exchange reaction and (typically) using a classical UV-absorbing MALDI matrix as the acidic component. To screen the principle suitability of the generated RTILs, a modified QStar-type mass spectrometer (AB Sciex) was used with lipid standards. A modified MALDI Synapt G2-S mass spectrometer (Waters) served for high-resolving MSI experiments of the 7 selected RTILs. An effective focal spot size (area of material ablation) of about 12 µm in diameter was achieved with the employed N_2 laser. Exact values depended somewhat on laser fluence and matrix. Matrix coatings were produced with an airbrush sprayer. All mass spectra were recorded in the positive ion mode. All selected RTILs provided a high analytical sensitivity. However, the obtained signal intensities for individual phospho- or glycolipids were found to be matrix-dependent to some extent. Presumably, the RTILs contribute to the analyte extraction from the tissue. Importantly, none of the tested RTILs showed analyte diffusion beyond the applied 15 µm-step size of the MSI analysis. Comparison with H&E stained tissue slices showed that mouse cerebellum structures were precisely imaged by the MS data. A very uniform lipid signal response was obtained across the coated tissue as was determined with liver homogenate. The described RTILs compromise a powerful alternative to conventional crystalline MALDI-MSI matrix preparations. In particular, they offer an easy sample preparation protocol and particularly uniform sample coatings.

1   K. Shrivas et al., Anal. Chem. 82 (2010) 8800-8806

2   K. Chan et al., Anal. Chim. Acta 639 (2009) 57-61

3   J. Soltwisch. et al., Science 348 (2015) 211-214

Analysis of the Lipid Composition of (Adipose) Tissues by Spectroscopic Methods

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T 11   PRICE

During the last decades many studies focused on the investigation of obesity-related (bio)markers such as adipocyte-derived peptides, but minor interest has been paid to the investigation of the lipid (especially triacylglycerols (TAGs)) and fatty acyl compositions of adipose tissues.

In this study we combined the strengths of NMR and MS to investigate the diet impact on the lipid compositions of different mice adipose fat depots. Beside the localization of the adipose tissue (brown, visceral and subcutaneous) compositional differences in dependence of the diets (high fat (HF) vs. standard (SD)) were of particular interest. Extracts of supplied diets and of different adipose tissues were first analyzed by MALDI MS. Qualitative compositions were additionally approved by HPTLC analysis. Quantitative fatty acyl compositional analysis was performed by means of GC and NMR spectroscopy.

Both diets consist almost exclusively of triacylglycerols (TAG), beside minor amounts of phosphatidylcholines (in particular PC 16:0/18:2 (m/z 758.6 and 780.6) and PC 16:0/20:4 (m/z 782.6 and 804.6)) in the „standard" food. However, the overall fatty acyl compositions of the TAGs in both diets differ significantly: the SD is characterized by the presence of TAGs with longer (particularly C18), unsaturated fatty acyl residues (e.g. m/z 901.7 - TAG 54:6), whereas the high fat diet contains nearly exclusively shorter, saturated fatty acyl residues (e.g. m/z 661.5 - TAG 36:0).

The lipid composition of the supplied diet has an extreme impact on the lipid composition of the adipose tissues. There are considerable differences in dependence on the diet and only moderate differences in dependence on the adipose tissue type. All spectra are dominated by the presence of TAGs while there are also, particularly in the brown fat, some PC species. The observed TAG fatty acyl patterns resemble closely the composition of the „standard" diet. In contrast, the adipose tissue extracts at conditions of the HF diet, are dominated by shorter, saturated fatty acyl residues which are also predominately present in the HF diet.

Metabolic Disease Signatures Translated to Underlying Mechanisms – a Lipidomics Approach

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T 12

There is a need for early markers to track progression from a state of normal glucose tolerance through pre-diabetes (impaired fasting glucose and/or impaired glucose tolerance) to type 2 diabetes (T2D). Several diabetes risk models and scores have been developed as prognostic tools. However, these are mainly based on established risk factors of T2D and they lack the specificity required for clinical practice.

We applied global lipid profiling based on ultra-high performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight (QTOF) mass spectrometry (MS) at baseline and at 5-year follow-up plasma samples of well phenotyped male participants (107 T2D progressors, 216 matched normoglycemic controls; dataset 1) from the longitudinal study METSIM (METabolic Syndrome In Men) to identify lipidomic profiles of progression to T2D, and to develop a lipid-based predictive model for T2D. The selection of progressors was based on fastest progression to T2D and greater glucose AUC at follow-up. Age-matched controls were selected as non-progressors.

A total of 277 plasma lipids were analyzed. A persistent lipid signature characterized by higher levels of triacylglycerols and diacyl-phospholipids as well as lower levels of alkylacyl phosphatidylcholines and lysophosphatidylcholine acyl C18:2 in cases vs. controls, was associated with progression to diabetes. A lipid-based model comprising five lipids was developed to predict incident diabetes [AUC = 0.80, 95 % CI = (0.772, 0.826)]. The combination of lipids with the FINDRISC model and the fasting plasma glucose (FPG) model significantly improved the prediction of both clinical models.

The lipid-based model was validated in a representative sample of the adult male population (n=631). In FPG-matched subset of the progressors and non-progressors, the model remained predictive of diabetes.

Our study indicates that a lipid signature characteristic of diabetes is present years before the diagnosis and predicts progression to diabetes independently of other risk factors. The lipid signature predictive of diabetes is similar as previously observed in prediction of non-alcoholic fatty liver disease. The lipid signature may therefore in part reflect contribution of fatty liver to diabetes progression.

Shotgun Lipidomics – Opportunities and Limits

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T 13

Under Shotgun Lipidomics are all mass spectrometric approaches summarized that utilize direct infusion experiments and electrospray ionization (ESI). We will discuss how different mass spectrometric strategies like precursor ion scan (PIS), neutral loss scan (NLS), multiple reaction monitoring (MRM) and data dependent acquisition (DDA) are employed to address lipidomics questions. In principle all common types of mass spectrometers are applicable to perform such experiments. However, parameters like mass accuracy, resolution and dynamic range have to be considered to design and interpret such experiments correctly. Finally, we will specifically review the high-throughput capabilities of shotgun approaches for biomedical research.

Lipid Identification with LipidXplorer – a Tutorial

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T 14

LipidXplorer is an open-source software kit that supports the identification and quantification of molecular species of any lipid class detected by shotgun experiments performed on any mass spectrometry platform. It features batch processing of multiple acquisitions by high-resolution mass mapping, precursor and neutral-loss scanning and MS/MS detection. From the few existing tools for shotgun lipidomics, LipidXplorer sticks out with an unusual approach: It does not rely on a database of reference spectra. Because due to the nature of shotgun lipidomics, m/z values of different lipids easily overlap and hamper the clear identification from a reference spectrum. In LipidXplorer instead, lipid identification routines are user defined using a query language. The molecular fragmentation query language (MFQL) enables identification of any small molecule. Although MFQL is a quite simple language, the learning curve might still be steep, in particular for non-programmers. This tutorial gives a step-by-step introduction in writing MFQL queries and using LipidXplorer as well as some hints for experienced users.

Quantitative and Metabolic Profiling of Lipid Species

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T 15

The molecular composition of lipids has great influence on biological functions by modulation of membrane fluidity and curvature affecting signaling processes as well as activity of membrane bound enzymes.

Electrospray tandem mass spectrometry (ESI-MS/MS) offers an excellent platform to quantify lipid species with high sample throughput. Major glycerophospholipid and sphingolipid classes are accessible by direct flow injection of crude lipid extracts. Whereas low abundant or isobaric species require frequently liquid chromatographic separation coupled to tandem mass spectrometry (LC-MS/MS). Lipid species quantitation is applicable for biomarker search in large clinical studies as well as basic research in a variety of sample materials including plasma, lipoprotein fractions, cells and tissues. Moreover, these methods provide insight into the dynamics of the lipid species metabolism by administration of stable isotope labeled precursors or lipid species. For example major pathways of the glycerophospholipid metabolism may be profiled using D_9-choline, D_4-ethanolamine and $^{13}C_3$-serine.

Taken together, mass spectrometry offers a powerful tool box to discover novel lipid biomarker in the blood compartment and to unravel mechanisms underlying the regulation of the lipid metabolism.

Acquisition and Processing of a High Resolution LC-MS-MS Dataset for the NIST Inter-laboratory Comparison Exercise for Lipidomics Measurements in Human Serum/Plasma

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T 16

Application of lipidomics profiling to disease phenotype analysis is a rapidly growing aspect of translational medical research. Identification of lipids by discovery lipidomics requires sophisticated software with an extensive lipid database. We present here some of the challenges of processing data from NIST plasma and serum extracts using LipidSearch software. New algorithms were introduced specifically to reduce false positives, improve peak integration and to help automate the data review.

Sample preparation and analysis of lipid extracts obtained from NIST human plasma (SRM 1950) and serum (SRM 2378-1, 2378-2 and 2378-3) was described previously [1]. Human plasma and serum lipid extracts were separated via a C30, 1.9μm prototype column using a Dionex 3000 RSLC chromatograph and LC-MS-MS was performed using a Thermo Scientific™ Q Exactive™ HF Orbitrap mass spectrometer.

Datasets from lipid plasma/serum extracts were processed using LipidSearch 4.1 software which incorporates new peak quality, signal-to-noise and alignment algorithms designed to accurately identify and merge annotated peaks and improved data filtering tools for reviewing the lipid results. Lipid identification was performed by searching a database of precursors and fragment ion accurate masses predicted for each lipid adduct form in the database. LC/MS-MS spectra were searched against predicted fragment ions for potential lipid species within ±5 ppm precursor and product ion mass tolerance. Each potential lipid ID is ranked by mass tolerance, match to predicted fragmentation and the fraction of total MS-MS peak intensity.

The number of lipid species identified in each LC-dd-MS^2 experiment were aligned within a chromatographic window and both positive and negative ion data were merged into the results table. This approach provides lipid annotation that reflects the appropriate level of MS^2 fragment ions from the complete dataset giving higher confidence in lipid identifications.

Results were filtered by main adduct ion, match score, ID quality, signal-to-noise and peak area coefficient of variation; manual integration was performed if necessary prior to estimating concentration relative to an internal standard for each lipid class. These results demonstrate that in a 60 min LC-MS run it is possible to identify and quantify approximately 1,000 lipid species from human plasma/serum.

1   Large scale lipid profiling of a human serum lipidome using a high resolution accurate LC/MS/MS Approach, Reiko Kiyonami[1], David A Peake[1], Xiaodong Liu[2] and Yingying Huang[1] presented at 2015 ASMS in St. Louis, USA

Analysis and Quantification of Cardiolipins on a Hybrid Triple Quadrupole/ion Trap (4000 QTRAP) Mass Spectrometer

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T 17

Cardiolipins (CL) are complex mitochondrial-specific phospholipids that contribute to the regulation of multiple mitochondrial signaling functions and bioenergetics in mammalian cells. Here, we present a method to screen and quantify CL molecular species from lipid extracts of isolated mitochondria of cultured cells on a 4000 QTRAP hybrid triple quadrupole/ion trap mass spectrometer equipped with a robotic nano-electrospray source (Advion Triversa NanoMate) for infusion.

CL can be quantified as the ^{13}C (m+1) isotopomers of deprotonated doubly charged molecular ions. The isotopomer peaks $[M-2H+1]^{2-}$ or $[M-2H+3]^{2-}$ of doubly charged CL species are very unique, whereas $[M-2H]^{2-}$ and $[M-2H+2]^{2-}$ may be overlapping with molecular ions in other lipid classes. Therefore, specific CL quantification is performed by comparison of the de-isotoped peak intensity of the $[M-2H+1]^{2-}$ peak with the de-isotoped peak intensity of the $[M-2H+1]^{2-}$ peak of internal standards [1].

Based on this principle, we have developed a CL screening and quantification method using the enhanced resolution (ER) (250 amu/s) ion trap scanning mode of a 4000 QTRAP MS. This method produces baseline resolved $[M-2H+1]^{2-}$ peaks of CL molecular species, enabling us to execute quantifications down to the fmol/µl level.

In order to accelerate data analysis, a software application was written to extract CL ^{13}C (m+1) isotopomer data from MS peak lists. The method was checked by diluting commercial bovine heart CL (Sigma) in a total cellular lipid extract, thereby reproducing the naturally occurring background of a mitochondrial extract (CL are representing 5-20 mol% of the mitochondrial lipids). We have shown the linearity of our assay down to below 0.1 mol% of CL versus total lipids. Using this method, we were able to monitor the effect of Soraphen A, a lipogenic inhibitor, on the mitochondrial CL composition of LNCaP cells. This demonstrates that the method can be applied to quantify CL in biological mitochondrial extracts and to fingerprint their expression profiles after different cellular treatments.

1   Han, X., et al. Journal of Lipid Research (2006), 47, 864-79.

The Maturation of Sperm is Accompanied by Changes of the (Phospho-) Lipid Composition –
A MALDI -TOF MS Investigation of Murine Epididymal Spermatozoa

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T 18   PRICE

After leaving the testis and upon the passage through the epididymis, spermatozoa undergo important steps of maturation to increase their motility and fertilizing ability. These changes include modifications of the phospholipid composition, which is assumed to be particularly important for the achievement of motility and fertilizing capacity.

The epididymis consists of caput, corpus and cauda, through which spermatozoa are transported and undergo distinct maturation stages. Using MALDI-TOF MS, we established three striking differences in the lipid composition of murine spermatozoa from the different epididymal regions: compared to the caput sperm, sperm from the cauda are characterized by a higher content of unsaturated lipids, an enhanced plasmalogen and lyso(phospho)lipid content. For both parts of the epididymis positive ion mass spectra with intense phosphatidylcholine (PC) as well as sphingomyelin (SM) signals could be recorded. The discrimination of differently maturated spermatozoa based on their lipid mass spectra is, thus, possible.

The most abundant signals in the spectra of the caput sperm extract can be assigned to PC 18:0/20:4, PC 18:0/18:1 and PC 18:0/18:2. In the cauda, however, PC 18:0/22:5, PC 18:0/22:6, PC 18:0/18:2, PC 16:0/22:5 and PC 16:0/18:1 predominate. Some peaks in the MS^1 spectra cannot be unequivocally assigned: e.g., the H$^+$ adduct of PC 16:0/20:4 and the Na$^+$ adduct of PC 16:0/18:1 result in isobaric ions. This problem can be easily overcome by saturating the DHB matrix with Cs$^+$. This leads to a significant mass shift (132.9 amu) that makes assignments simple.

These data suggest that spermatozoa membranes become more fluid during epididymal maturation due to the higher content of double bonds, which is also expressed by an increase of the PC (18:0/22:5 and 18:0/22:6) / PC (18:0/18:1 and 18:0/20:4) ratio from caput to cauda. This ratio increased from 0.1 to 3.61. These changes of the fatty acyl composition are also reflected by changes of sperm motility, which increases (as well as further biochemical parameters) upon transition from caput to cauda. Finally, the fertilizing ability of sperm also correlates with the observed changes in the lipid composition. It is suggested that the lipid composition of sperm may be a useful fertility marker.

Linking *Drosophila* Crumbs and Lipids in Photoreceptor Cell Morphology

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T 19 **PRICE**

Our research focuses on the transmembrane protein Crumbs, a regulator of apico-basal epithelial polarity, whose localization is extremely specific and polarized. We are interested in identifying if, and how, Crumbs influences membrane lipid components. Crumbs is an evolutionary conserved protein first identified for its role in *Drosophila* embryonic development. It is also relevant in the development and maintenance of the photoreceptor cells (PRCs) of the retina in flies. This role of Crumbs is remarkably conserved, because mutations in *crumbs* (flies) and *CRB1* (human orthologue), result in retinal degeneration. In PRCs, Crumbs is localized to the sub-apical region of the plasma membrane (stalk), juxtaposed between the adherens junctions and the photosensitive apical membrane (rhabdomeres). Altered Crumbs level, by genetic manipulations, results in abnormal morphology, specifically in the size ratio of rhabdomeric/stalk membrane domains. Decreased level of Crumbs correlates with bulky rhabdomeres/reduced stalk membrane and vice-versa. This suggests that *crumbs* regulates the size of the rhabdomeric/stalk membrane domain. To begin identifying the interaction of Crumbs and membrane lipids in this regulation, we applied an unbiased lipidomics approach to screen different *Drosophila* mutants wherein the ratio of rhabdomeric/stalk membranes in PRCs is disrupted. Our lipidomics analyses on dissected eyes covered 14 lipid classes across which 180 lipid species were quantified. We observed a specific and strong correlation between increased sphingolipid hydroxylation and the phenotype of *crumbs* mutants (bulky rhabdomeres/reduced stalk membrane). The two-fold increase in hydroxylated species is most evident in Ceramide Phosphorylethanolamine (CerPE), the most abundant membrane sphingolipid in these tissues. Furthermore, we identified that hydroxylation on the fatty acid component of CerPE contributes to this increase in *crumbs* mutants. This result was validated by qRT-PCR that showed increased transcript levels of *fa2h* in *crumbs* mutants; the enzyme responsible for sphingolipid hydroxylation. Following the results of our lipidomics screening approach, we genetically manipulated *fa2h* and consequently sphingolipid hydroxylation. We observe that increased sphingolipid hydroxylation mildly phenocopies aspects of the *crumbs* phenotype including bulky rhabdomeres/reduced stalk membrane. Overall, we have identified increased sphingolipid hydroxylation as a component coupling Crumbs and altered rhabdomeric/stalk membrane ratio. Future studies are aimed at understanding the mechanisms underlying this influence of Crumbs on lipids.

Lipid Diet Rules the Life of Flies

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T 20

Drosophila can develop on a variety of foods, although being a sterol auxotroph, flies require dietary sterols to build lipid membranes and to produce the molting hormones ecdysteroids. Flies can use yeast and plant sterols and also cholesterol, but how animals ensure the timely production of desired hormone(s) that support their sustained development from embryos to adulthood? Diet alters the lipid composition of Drosophila in a tissue-, but also lipid class-dependent manner and a central question remains if these multifaceted changes constitute a part of the adaptation response mechanism that maintains the integrity and functionality of the membrane proteome. And finally, if the lipidome and proteome could interplay, even if not being directly linked via shared elements of biosynthetic pathways? We addressed these and other important issues of the dietary impact by the comparative study of the lipidome, proteome and ecdysteroidome of developing flies [1, 2].

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Posters

Wnt6 affects Macrophage Lipid Homeostasis and Susceptibility to *Mycobacterium tuberculosis* Infection

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The Wnt signaling pathway, an ancient and highly conserved signaling network, has been shown to exert immuno-regulatory functions in inflammatory and infectious disease settings including tuberculosis. We recently showed that Wnt6 is expressed in foamy-like macrophages in the *M. tuberculosis*-infected murine lung and that Wnt6 shifts macrophage polarization towards a M2-like phenotype. In the study presented here, the effect of Wnt6 on macrophage lipid metabolism was studied in fatty acid supplemented primary cells derived from *Wnt6*$^{+/+}$ and *Wnt6*$^{-/-}$ mice by immunofluorescence- and mass spectrometry analyses, as well as by real-time oxygen consumption measurements. Gene expression of lipid metabolic enzymes was analyzed by microarray and qRT-PCR based approaches in *M. tuberculosis*-infected macrophages. To study the impact of Wnt6 on the growth of *M. tuberculosis* within macrophages, the intracellular bacterial burden was monitored in primary cells derived from *Wnt6*$^{+/+}$ and *Wnt6*$^{-/-}$ mice over a period of 7 days. The obtained data indicate that Wnt6 affects *M. tuberculosis* growth by shaping immune and metabolic responses of macrophages.

SIMPLEX: A Combinatorial Multimolecular Omics Approach for Systems Biology

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P 02 PRICE

Interconnected molecular networks are at the heart of signaling pathways that mediate adaptive plasticity of eukaryotic cells. To gain deeper insights into the underlying molecular mechanisms, a comprehensive and representative analysis demands a deep and parallel coverage of a broad spectrum of molecular species. Therefore, we introduce SIMPLEX (Simultaneous Metabolite, Protein, Lipid EXtraction procedure), a novel strategy for the quantitative investigation of lipids, metabolites and proteins. Compared to unimolecular workflows, SIMPLEX offers a fundamental turn in study design, since multiple molecular classes can be accessed in parallel from one sample with equal efficiency and reproducibility. Application of this method in mass spectrometry based workflows allowed the simultaneous quantification of 360 lipids, 75 metabolites and 3327 proteins from 10^6 cells. The versatility of this method is shown in a model system for adipogenesis – PPARG signaling in mesenchymal stem cells – where we explored with SIMPLEX cross-talk within and between all three molecular classes and identified novel potential molecular entry points for interventions, indicating that SIMPLEX provides a superior strategy compared to conventional workflows.

Development of a nanoLC-MS Method for Lipidomics

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Introduction:

Lipids are key players of cellular systems that are particularly known for their roles in structural compartmentalization and energy storage. Furthermore, they are also able to fulfill signaling functions in biological systems at marginal concentrations. In order to identify low abundant lipids such as mediators, analytical methods need to be developed to allow an accurate characterization and quantification of these biomolecules with high sensitivity and specificity.

Methods:

In comparison to conventional LC-MS analyses, nanoLC-MS systems promise significant higher measurement sensitivity, reduced sample consumption, and decreased solvent usage. For this reason, we aim to develop a nanoLC-ESI-MS method for lipid analyses.

Preliminary data:

Different column materials, flow rates and temperatures were compared to investigate their impact on capillary reverse phase chromatography.

We further aim to benchmark the developed nanoLC-MS method against a corresponding narrowbore LC-MS method in terms of sensitivity, dynamic range and number of lipid identifications in a complex biological extract.

The Human Lung Lipidome: A Potential Connection of Histopathological Phenotypes and Development of Cancer or Emphysema

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P 04 **PRICE**

The lung lipidome shows specific features related to respiratory functions. Constantly lipids are secreted and recycled to stabilize the alveoli, and to protect them from environmental stresses. Together with specialized proteins, lipids form a surface-active layer, the pulmonary surfactant, which stabilizes the alveoli and has additionally immune-protective functions. The alveolar morphology and those of their cellular components is associated to the lipid metabolism in an unknown way and needs further research. Here we present a pilot study including 45 human lung tissue lipidomes of 26 lung cancer patients. Each lung tissue sample was characterized, to be tumor or tumor-free, and was divided, firstly, to enable histopathological characterizations, and secondly, to perform shotgun lipidomics. With our current analytical method we are quantifying approximately 350 lipid species from 11 classes.

To gain insight in lipid metabolic alterations, lipidome data were set into relation to patient data and histopathological scores. We found that lipidomes from tumor-free and tumor tissue-biopsies can clearly be distinguished. By applying principle component analysis (PCA), hierarchical clustering (HC) and partial least squares regression (PLSR), we were able to draw correlations between lipidome data and histological characterizations. Pulmonary surfactant related lipid species, mainly saturated phosphatidylcholines (PC) and phosphatidylglycerols, are potential indicators for tumor-free tissues. In contrast to these findings, tumor tissues are characterized by higher amounts of unsaturated PCs and neutral lipids like triacylglycerol as well as free cholesterol and cholesteryl esters; especially when necrosis was observed. Beyond these findings, we found indications for different lipidomes of adenocarcinomas and squamous-cell carcinomas. Tumor-free tissues were further analyzed in context of association to the pulmonary emphysema marking the destruction of alveoli, which is often observed in chronic obstructive pulmonary disease (COPD). Here we found indications that specific lipidome changes are associated with emphysema grade. Our data show that histopathological characterization and lipidomics have a great potential in functional studies.

Mass Spectrometry Imaging of PEGylated Liposomes in Mice

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Phospholipid are main components of cellular membranes and have therefore an excellent biocompatibility. This make them most appropriate as important pharmaceutical excipients and main component of liposomal drug carriers. In particular, PEGylated carriers have been shown to extend blood-circulation time while reducing uptake by the mononuclear phagocyte system. Usually, the in-vivo distribution of liposomal drug carriers is monitored by the use of radio- or fluorescence-labels in the liposomes.

Here we outline Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) imaging as method for the detection of liposomal drug carriers in mouse tissue. Therefore, a single phosphatidylglycerol species (DPPG) and the polyethylene glycerol head group-modified lipid (PEG_{36}-DSPE) (Lipoid GmbH, Ludwigshafen) were incorporated in the coat of liposomal drug carriers, while indocyanine green (ICG), a well-established fluorophore in clinical applications, was integrated inside the carrier. After injection of liposomes to mice, tissue was measured by MALDI MS imaging. Thereby, it was demonstrated that all evaluated liposomal components could be measured in the same tissue slice. While DPPG and ICG were detected in the negative ion reflector mode, PEG_{36} DSPE was measured in the positive ion reflector mode using the same MALDI matrix. After evaluation of liposomal distribution, hemoglobin was measured in the same tissue slice by MALDI MS to determine the localization of the liposome either in the tissue or the circulating blood.

The results characterize MALDI MS imaging as useful tool for analysis of PEGylated liposomes, regarding not only the distribution of various liposomal components but also the localization of liposomal carriers in tissue.

Differential Mobility Separation of Leukotrienes and Protectins

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P 06

Differential ion mobility spectrometry, termed Selexlon® is capable of separating ions based on their mobility in an oscillating electrical field with asymmetric wave form. Thus, it is "orthogonal" to chromatography and (tandem) mass spectrometry. Particularly bioactive lipids of the eicosanoid and docosanoid classes present very high structure activity relationships. Moreover does the geometry of specific eicosanoids and docosanoids also reflect their biochemical origin. Taken together, the unambiguous characterization of closely related isomers of the eicosanoid and docosanoid classes are of fundamental importance to the understanding of their origin and function in numerous biological processes. We here show that using Selexlon®-technology coupled to microLC-MS/MS is capable of separating at least five closely related leukotrienes partially co-eluting and (almost) unresolvable using LC-MS/MS only. We applied the developed technology to the separation of LTB_4 and its co-eluting isomer 5S,12S-diHETE in murine peritoneal cells. LTB_4 was present only in peritoneal cells from mice injected with zymosan, while its isomer 5S,12S-diHETE was present only in resident peritoneal cells from mice administered PBS. Additionally we show that Selexlon®-technology can also be applied to the analysis of docosanoids, separating PD1 and PDX (10S,17S-diHDHA) two isomeric protectins about which much confusion has been present in recent literature.

In conclusion, we showed that differential mobility is a powerful tool for adding an additional dimension of selectivity to chromatography for difficult separations of isomeric eicosanoids and docosanoids even for compounds present only at very low levels in biological samples.

Lipid Profiling of *Mycobacterium tuberculosis* East African Indian Strains by ^{1}H,[13]C -Heteronuclear Single Quantum Coherence-NMR

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P 07

The cell envelope of *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB) infection, consists of a peptidoglycan-linked arabinogalactan, which is esterified by long-chain mycolic acids. Various complex lipids like trehalose dimycolates, phosphatidyl-*myo*-inositol mannosides, phenolic glycolipids (PGLs) and phthiocerol dimycocerosates (PDIMs) are associated with these mycolic acids, thus forming an effective outer envelope. The lipid composition differs among mycobacterial species and some of the lipids have proven potential for biomarker use in tracing the evolution of TB. Especially PDIMs and PGL-tb (identified mostly in *M. tuberculosis* Beijing isolates) have been associated with mycobacterial hyper-virulence and evasion of the host immune system.

In our study, we investigated the lipid composition of *M. tuberculosis* East African Indian (EAI) strains responsible for TB infection in the Eastern part of Africa and West-Asia. Based on whole genome sequencing data of 191 *M. tuberculosis* EAI strains, we selected 11 clinical isolates representing the uncommonly high genetic diversity of this lineage. For our NMR-based analysis of their lipid composition we applied and fine-tuned the powerful methodology of two-dimensional ^{1}H,[13]C-Heteronuclear Single Quantum Coherence (HSQC)-NMR lipid profiling. For this, mycobacterial lipids were extracted from partially ^{13}C-labeled cells with chloroform-methanol. The lipid profiles of the investigated strains showed a correlation between their genetic background and the expressed lipid pattern. Major differences have been observed especially within the group of PGLs, which can be explained only in part by a specific single nucleotide polymorphism in Rv2962c, a gene encoding for a glycosyltransferase which is involved in PGL biosynthesis. Interestingly, isolates belonging to specific branches within the EAI lineage maintain a certain PGL pattern, which ranges from mycoside B to phenolphthiocerol dimycocerosates to the complete absence of PGLs, although many isolates contain a complete and functional PGL biosynthesis cluster. Growth analysis of these strains in human macrophages, the natural host cell of the TB pathogen, revealed significant differences between single isolates. However, this did not match with an identified PGL phenotype. Thus, neither the presence or absence nor the type of PGL molecules appear to determine the growth rate of M. *tuberculosis* EAI strains in human macrophages.

Mycobacterium Tuberculosis induced Changes of Oxidized Polyunsaturated Fatty Acid Metabolism analyzed by Liquid Chromatography-Tandem Mass Spectrometry

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Oxidized species of arachidonic, eicosapentanoic and docosahexanoic acid derived by lipoxygenases and cyclooxygenases play a crucial role in signalling during microbial infections. Lipid mediators like prostaglandins and leukotrienes act in a pro-inflammatory manner, whereas resolvins and lipoxins have anti-inflammatory and pro-resolving properties. All lipid mediator types are thought to exert their regulatory action by binding to G protein-coupled receptors to control a multitude of aspects of tissue inflammation.

Only the quantitation of these oxidized derivatives by liquid chromatography – tandem mass spectrometry (LC-MS[2]) enables a comprehensive insight into the regulation of immune cell activities. We established a LC-MS[2] platform to characterize the lipid mediator alterations in *Mycobacterium tuberculosis* (M.tb.) infection. Wild-type mice (*C57BL/6*) were used to perform a time course experiment for 100 days after aerosol M.tb. infection. A LC-gradient was developed to directly analyse total lipid extracts with LC-MS[2] avoiding time consuming SPE purification steps. In contrast to many triple quadruple applications we performed micro-LC coupled with QqTOF (Q-TOF Ultima™) utilizing the superior resolution and mass accuracy.

We quantified 24 known molecular species during the time course experiment from which 22 were significantly increased at 70 days post M.tb. infection. M.tb. perturbed the host's oxidized PUFA metabolism interfering in the quantities of di- and mono-hydroxylated PUFAs and prostaglandins, respectively. Seven oxidized PUFAs were significantly increased during M.tb. infection, namely thromboxane B2, prostaglandin E2 and D2, 11,12-epoxy-eicosatrienoic acid, 15-hydroxy-eicosatetraenoic acid, leukotriene B4 and 10-hydroxy-docosahexaenoic acid. Our findings might help to understand how M.tb evades the host's immune system. In future we plan to utilize the LC-MS[2] platform for a systematic analysis of infection-induced inflammation with selected pathogens.

LUX Score: Homology Metric for Global Comparison of Lipidomes

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Lipids play an important role in maintaining cellular homeostasis. Many diseases including, cardiovascular disease, metabolic syndrome and diabetes have altered lipid metabolism. To gain insight into fundamental processes for disease progression animal models are employed. Mass spectrometry based lipidomics enables characterization of lipidomes in model organisms as well as humans. However, data analysis tools for comparing lipidomes across species are basically not existent. Here, we propose to establish a homology metric for lipidomes based on structural similarity between constituent lipids. For example, sphingomyelin, a major component of cell membrane in higher mammals is absent in model organisms like drosophila and yeast. Instead, drosophila comprises a structural relative, Ceramide Phosphotidylethanolamaine. Cholesterol is preferred sterol in mammals and a structural analogue, Ergosterol, is predominant in yeast and drosophila. To facilitate global comparison of lipidomes, comprising structurally related lipids, we developed the Lipidome jUXtaposition (LUX) Score.

Computational workflow to determine LUX Scores for estimating lipidome homologies involves multiple steps. In the first step, LIPIDMAPS Structure Drawing tools are employed to generate structures for all lipids identified by mass spectrometry. Next, lipid structures are converted into Simplified Molecular-Input Line-Entry System (SMILES) strings. Levenshtein Distance is used to determine structural similarity between lipid SMILES. Finally, average Levenshtein Distance between unique lipids between a pair of lipidomes is returned as LUX Score. In summary, LUX Score describes 'extent of structural overlap' between lipidomes as a numerical value between 0 and 1.

Lipidomes of yeast elongase mutants (Ejsing et. al. 2009) were used to evaluate the LUX score. Hierarchical clustering of mutant strains using LUX Score grouped BY4741, Elongase1 in one cluster and Elongase2, Elongase3 mutants in second cluster. This clustering pattern confirms phenotype observation of mutants reported earlier (Marella et al. 2015). Now we applied the LUX Score to compare lipidomes of human lung tissue, with- and without- carcinoma and in this case also, LUX score clearly separates tumor and alveolar tissues. To extend the capacity and scope of this approach, we combined the structural similarity metric with lipids quantities and observed superior clustering of lipid profiles in yeast, drosophila-larvae and human lung lipidomes compared to purely quantity based methods.

Imaging Mass Spectrometry:
Quantitative Aspects in Lipid Analysis

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Owing to its capability of discriminating subtle mass-altering structural differences such as double bonds or elongated acyl chains, MALDI-based imaging MS (IMS) has emerged as a powerful technique for analysis of lipid distribution in tissue at moderate spatial resolution of about 50 μm. However, it is still unknown if MS^1-signals and ion intensity images correlate with the corresponding apparent lipid concentrations. Analyzing renal sulfated glycosphingolipids, sulfatides, we validate for the first time IMS-signal identities using corresponding sulfatide-deficient kidneys. To evaluate the extent of signal quenching effects interfering with lipid quantification, we surgically dissected the three major renal regions (papillae, medulla, and cortex) and systematically compared MALDI IMS of renal sulfatides with quantitative analyses of corresponding lipid extracts by on-target MALDI TOF-MS and by ultra-performance LC-ESI-(triple-quadrupole)tandem MS. Our results demonstrate a generally strong correlation ($R^2 > 0.9$) between the local relative sulfatide signal intensity in MALDI IMS and absolute sulfatide quantities determined by the other two methods. However, high concentrations of sulfatides in the papillae and medulla result in an up to 4-fold signal suppression. In conclusion, our study suggests that MALDI IMS is useful for semi-quantitative dissection of relative local changes of sulfatides and possibly other lipids in tissue.

Systems Biology of the Unfolded Protein Response in Glioma

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Diffuse gliomas are one of the most common types of human brain tumor and among the most deadly of all human cancers. Depending on the tumor stage, they can be extremely invasive and resistant to different kinds of cancer therapies. Recent studies reveal that the unfolded protein response (UPR) network might be responsible for the tumor proliferation and therapeutic resistance. Depending on the ER stress level, the UPR network can be either protective, where the ER homeostasis is reestablished, or apoptotic, where it leads to cell death. Due to this reason, the UPR network has become a potential target for the treatment of cancer with the aim to shift the balance to the UPR apoptotic site to induce cancer cell death. However, the cellular signals and the way they integrate with each other to trigger cell decision to either cell death or survival are unknown. Moreover, it has already been suggested in various studies that on the one hand, the alteration in membrane lipid composition can induce UPR and on the other hand, UPR can affect the lipid metabolism of the cell. In this project, we aimed to gain deeper insights into the UPR network in gliomas and investigate its equilibrium stage using quantitative proteomics and lipidomics assays. For this purpose, first, global proteomics experiments will be utilized to create a library of proteins, which are altered in response to ER stress. Subsequently, targeted proteomics experiments will be developed for the quantitative analysis of the different UPR branches. In parallel, quantitative lipidomics analysis will be carried out to investigate the effect of membrane lipid composition on UPR and how UPR can alter the cellular lipid metabolism. The final aim of this project is, to define novel therapeutic targets contributing to the development of clinical therapies against gliomas.

Adaptation of Skyline for Targeted Lipidomics

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In response to the urgent-need for analysis software that is capable of handling data from targeted high-throughput lipidomics experiments, we here present a systematic workflow for the straightforward method design and analysis of selected reaction monitoring data in lipidomics based on lipid building blocks. Skyline is a powerful software primarily designed for proteomics applications where it is widely-used. We adapted this tool to a 'Plug and Play' system for lipid research. This extension offers the unique capability to assemble targeted mass spectrometry methods for complex lipids easily by making use of their building blocks. With simple yet tailored modifications, targeted methods to analyze main lipid classes such as glycerophospholipids, sphingolipids, glycerolipids, cholesteryl-esters and cholesterol can be quickly introduced into Skyline for easy application by end users without special bioinformatics skills. To illustrate the benefits of our novel strategy, we used Skyline to quantify sphingolipids in mesenchymal stem cells. We demonstrate a simple method building procedure for sphingolipids screening, collision energy optimization and absolute quantification of sphingolipids. In total, 72 sphingolipids were identified and absolutely quantified at the fatty acid scan species level by utilizing Skyline for data interpretation and visualization.

Fetal Calf Serum (FCS) Compensates the Lipid Profile of Acid Sphingomyelinase (aSMase) Deficient Bone Marrow Derived Macrophages (BMDM)

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Intracellular membrane trafficking is regulated by the interaction of signaling molecules like membrane lipids and proteins. Acid sphingomyelinase (aSMase) hydrolyzes the lipid mediator sphingomyelin (SM) to ceramide in response to cellular stress. Consequently, the lack of intracellular lipid signaling enzymes results in alterations of the lipid profile. In this study, we characterized the lipidome of *in vitro* generated gene deficient aSMase bone marrow derived macrophages (BMDM), freshly isolated bone marrow stem cells (BMSC) and peritoneal exudates cells (PEC). We found a significant phenotype with regard to SM species in aSMase KO BMSC and PEC. However this was not observed in *in vitro* generated BMDM.

These results prompted us to develop a fetal calf serum (FCS)-free macrophage generation protocol. Several FCS-free media were tested for the development of BMDM as shown by FACS analysis. In contrast to BMDM generated in FCS-containing media we showed that aSMase KO BMDM generated in FCS-free media resemble the phenotype of BMSC and PEC. These data show that FCS-derived lipids present in cell culture media have a strong compensatory effect on the SM levels in macrophage deficient in aSMase. This strongly suggests the use of FCS-free cell culture systems to study lipid metabolism *in vitro*.

In-depth Analysis of the Membrane Phospholipid Composition of E. coli Strains with Genetically Engineered Lipopolysaccharide Structure

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Lipopolysaccharides (LPS) of Gram-negative bacteria activate the innate immune system response in mammalian cells. A heterodimer of the Toll-like receptor 4 (TLR4) and myeloid differentiation protein 2 (MD-2) is formed upon binding of a common pattern in structurally diverse LPS molecules, the lipid A. To induce dimerization of the TLR4/MD-2/LPS complex and activation of the TLR4/MD-2 signaling pathway, *Escherichia* coli LPS/ lipid A has to be penta- to hepta-acylated. *E. coli* strains of the KPM (Kdo pathway mutant) series have a genetically engineered LPS. This has been accomplished by a drastic modification of LPS biosynthesis through the stepwise incorporation of finally seven genetic deletions (Δ*gutQ*, Δ*kdsD*, Δ*lpxL*, Δ*lpxM*, Δ*pagP*, Δ*lpxP* and Δ*eptA*) in strain KPM404. This leads to the absence of the carbohydrate decorations and the lack of secondary acyl chains that are usually transferred to the lipid A moiety. A primarily introduced compensating mutation in the *msbA* gene enables the viability of KPM cells in the presence of the so-called "lipid IV_A", a tetra-acylated precursor of lipid A biosynthesis, which does not cause an endotoxic response in human cells.

Here, we investigated the alteration in the phospholipids (PL) and the fatty acid composition in membranes of E. *coli* KPM strains in response to the changes of the LPS structure. For that, selected KPM strains were grown under identical conditions to an optical density of about 0.8, and the PL were extracted by the Bligh-Dyer method. The in-depth analysis was done by 1) ^{31}P NMR spectroscopy for quantification of the phospholipid classes, 2) GC/MS for analysis and quantification of the fatty acid composition, and 3) shotgun lipidomics to study the lipid species on the molecular level. The radical alteration of the LPS structure showed no significant changes in the PL composition of the mutant strains in comparison to the BL21 (DE3) wild-type strain, but significant changes in the fatty acid composition, i.e. the ratio of saturated fatty acid/unsaturated fatty acid/ cyclopropane fatty acid content. In MS/MS-experiments, we observed a decrease in the content of both saturated and unsaturated fatty acids in the mutant strains in PG, PE and *lyso*-PE species. In contrast, we determined a significant increase in the incorporation of cyclopropane fatty acids into the PL species of the KPM strains.

Simultaneous Analysis of Lipids, mRNA and Protein in Murine Tracheal Airway Epithelial Cells

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Airway epithelial cells play a central role in various airway diseases such as asthma and COPD. Changes in airway epithelial cells during disease result in RNA expression, protein synthesis and lipid composition. Research is usually focused on one of these molecule classes due to a limitation in sample size that can be derived from murine airways.

We therefore developed a method for simultaneous extraction of lipids, RNA and protein that allows the analysis of small quantities of airway epithelial cells derived from murine tracheae.

Explanted mouse tracheae were cut in two pieces of equal size and one piece was stimulated 30 min, 1h and 2h with LPS in HEPES buffered Ringer solution at 37° C. The other part of the trachea remained in in HEPES buffered Ringer solution without LPS and served as internal control. The epithelial cells of both pieces were isolated individually with a sterile swab which was subsequently snap-frozen.

Lipids were isolated according to Bligh and Dyer[1] and analyzed with a high resolution mass spectrometer[2]. The mRNA and proteins were isolated from the remaining aqueous fraction with a column-based kit system. The proteins remained in the flow-through. The mRNA expression of chemokines and cytokine (Mip-2, KC, Mcp-1, IL-6) were investigated with qRT-PCR. Proteins in the flow through were precipitated with trichloroacetic acid, washed with acetone and collected in SDS based lysis buffer. Western blot analysis was used to quantify the phosphorylation of the transcription factor NFkB. To determine the influence of the lipid extraction on the analysis of RNA and Proteins, individual samples were used exclusively for protein and mRNA analysis, respectively. Lipid extraction caused a loss of approximately 40 % mRNA and combined lipid and mRNA extraction resulted in loss of approximately 80 % of proteins. However, LPS-induced increase of cytokine and chemokine mRNA expression and increased NFkB phosphorylation was readily detectable in all samples with prior lipid extraction. We found the same mRNA expression profile and the same increase of NFkB phosphorylation in samples processed with and without lipid extraction. In the lipid analysis more than 280 lipid species from 10 lipid classes were detectable in each sample. LPS exposure up to 2 hours showed stable lipid class profiles and only slight changes in lipid species profiles. Our results demonstrate that changes in lipids, mRNA and proteins can be readily analyzed in small sample amounts from murine airway epithelial cells.

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2   Zehethofer N, Bermbach S, Hagner S, Garn H, Müller J, Goldmann T, Lindner B, Schwudke D, König P (2015)
    Lipid Analysis of Airway Epithelial Cells for Studying Respiratory Diseases. Chromatographia. 78(5-6):403-413.

HCV Replication Alters the Cellular Lipid Profile and is itself Influenced by Fatty Acids

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Hepatitis C virus (HCV) infection has long been associated with abnormalities in lipid metabolism, most notably the occurrence of steatosis. In addition, lipids and lipid storage organelles (lipid droplets) have been shown to play crucial roles in different steps of the viral life cycle. Here, we performed lipidomic studies of HCV-infected and uninfected hepatoma cells. Huh7.5 cells were infected with an HCV reporter virus (Jc1[NS5A/B-EGFP]) and 6 - 10 days post infection, when infection rates were above 80 %, lipids were extracted and subjected to mass spectrometry. Our results indicate that HCV infection induces an alteration of the ratio between neutral and membrane lipids. In addition, we observed that HCV infection caused changes in the relative abundance of different lipid species. HCV-infected cells had a higher relative abundance of phosphatidylcholines (PC) and triglycerides (TG) with longer fatty acids than control cells. The analysis of free fatty acids revealed a significant increase of arachidonic, eicosapentaenoic and docosahexaenoic acid caused by HCV infection. Importantly, inhibition of the PUFA synthesis pathway decreased virus replication. We also analyzed the impact of different fatty acids on viral RNA replication and assembly and release of progeny virions and could detect striking differences between fatty acids depending on chain length and saturation. Taken together our results suggest that HCV alters the lipid metabolism of the host cell to enhance its replication.

An Automated Software Pipeline for Shotgun Lipidomics using Direct-Infusion, High-Resolution Mass Spectrometry

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In the last ten years, lipidomics has attracted increasing attention as a research tool in a wide range of disciplines including physiology, lipid biochemistry, clinical biomarker discovery and pathology. Lipid metabolism is found to be critically aberrant in several different human diseases such as diabetes, obesity, atherosclerosis and Alzheimer's disease. All these characteristics make lipids profiling an essential tool not only for investigation of many pathological processes but also in identifying potential biomarkers for establishing preventive or therapeutic approaches for human health. Here we present a direct-infusion mass spectrometry approach (shotgun lipidomics) in order to identify and quantify at least ten lipid species classes using a two-step extraction procedure, enabling both lipidiomics as well as polar metabolite analysis via GC-MS. We developed a new automated data analysis pipeline, which allows for the fast and robust quantification and identification of lipid species from high-resolution MS data.

The software is based on the open-source C++ library OpenMS and R scripts, and supports automated ion-mode and adduct detection, isotope- assembly and correction, non-linear mass calibration, an in-house lipid database combining LipidMaps and HMDB for mass-based identification. Quality control plots are created for all major processing steps for each sample, allowing the operator to quickly judge data quality. Spike-in internal standards serve as abundance normalization for their respective lipid class. Additionally, a new robot-based sample preparation method is introduced, which allows standardized sample handling, ensures rapid sample processing, and minimizes potential variations in pipetting or weighing. Furthermore, due to use of glass vials, we show that common background signal (e.g. from tris(ditert-butylphenyl) phosphate) are significantly reduced.

To study the effects of lifestyle factors, we investigate the lipidome changes in a mouse model considering four tissue types (WAT, serum, muscle and liver) under normal vs. high-fat diet.

Differentiated Analysis of the Fatty Acid Pattern in Food Supplements

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The dietary intake of n3 long chain polyunsaturated fatty acids (LC n3-PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with several beneficial health effects, e.g. on cardiovascular diseases or on inflammation. In order to understand the underlying mechanisms, investigation of the fate of LC n3-PUFA upon absorption, e.g. their distribution in the organism and incorporation in lipids such as polar lipids and triglycerides is crucial. Moreover, the nutriokinetics of LC n3-PUFA bound as ethyl esters, triglycerides or polar lipids differ. Thus, proper chemical characterization of dietary supplements with respect to the PUFA pattern in the different fractions is crucial.

On the poster, an efficient protocol for the separation of lipid classes by solid phase extraction followed by gas chromatography with flame ionization detection (GC-FID) is presented. Using standards of individual fractions, the separation efficacy is characterized. Moreover the results from an application on n3-PUFA dietary supplements are presented.

Ostermann, A. I.; Muller, M.; Willenberg, I.; Schebb, N. H., Determining the fatty acid composition in plasma and tissues as fatty acid methyl esters using gas chromatography - a comparison of different derivatization and extraction procedures. Prostaglandins, leukotrienes, and essential fatty acids 2014, 91, (6), 235-41.

Analysis of Lipid Mixtures by Continuous Wavelet Transformed ^{1}H NMR

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[1]H nuclear magnetic resonance (NMR) is a convenient method for investigating lipid compositions of tissues or mixtures in a quantitative manner. As ^{1}H is the most sensitively detectable NMR nucleus, even diluted samples can be analyzed in a comparably short time. Suppression effects as observed in mass spectrometry do not play a role. However, due to the relatively small signal range in ^{1}H-NMR spectra severe signal overlap occurs in complex samples and, thus, the quality of ^{1}H NMR spectra and to be able to separate overlapping peaks, sophisticated spectral processing techniques can be used. Finding "hidden" peaks in crowded regions can be achieved by continuous wavelet transformation (CWT). Here the spectrum is convolved with a wavelet of different widths resulting in a baseline-flattened, denoised spectrum solely containing information about the position of peaks. Peak patterns obtained in this way can be easily compared with reference spectra of the compounds of interest stored in a database. Spectral fitting is now performed in an iterative process with different wavelet widths. Using this approach otherwise hidden compositional information can be extracted to distinguish between very similar molecules. Using fatty acid mixtures of defined compositions the power of this approach will be verified. Finally, „real world" fatty tissue samples of genetically altered mice will be investigated. It will be shown that the newly developed approach provides data which are comparable to standard methods of lipid analysis.

Oxylipin and Fatty Acid Pattern following Feeding of Mice with n3-Polyunsaturated Fatty Acids

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Dietary intake of long chain n3-polyunsaturated fatty acids (n3-PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is associated with beneficial health effects, e.g. a reduced risk for inflammatory or cardiovascular diseases. It is believed that a significant portion of these effects is mediated by their oxidative metabolites – oxylipins – formed in the arachidonic acid (AA) cascade. Conversion by cyclooxygenases (COX), lipoxygenases (LOX), cytochrome P450 (CYP) monooxygenases, soluble epoxide hydrolase (sEH) or other enzymes leads to a large number of oxylipins with different physiological properties. Prostaglandins from AA e.g. are involved in the regulation of pain and fever or epoxides from AA, EPA and DHA have anti-inflammatory, analgesic and vasodilatory effects.

In the present study we investigated the nutriokinetics of a high n3-PUFA diet (1 % EPA and DHA ethyl ester). Over a supplementation period of 45 days, the fatty acid composition of plasma and tissues was monitored by gas chromatography with flame ionization (GC-FID). The oxylipin pattern was analyzed by liquid chromatography-mass spectrometry (LC-MS) based targeted metabolomics allowing the parallel quantification of 120 oxylipins.

On the poster presentation, absorption and distribution of n3-PUFAs is characterized and correlated with changes in the n3- and n6-PUFA oxylipin metabolome. Furthermore, changes in the oxylipin pattern in plasma and tissues are compared. Tissue specific n3-PUFA metabolites are identified and discussed with regard to enzymatic routes of formation.

Ostermann, A. I.; Muller, M.; Willenberg, I.; Schebb, N. H., Determining the fatty acid composition in plasma and tissues as fatty acid methyl esters using gas chromatography - a comparison of different derivatization and extraction procedures. Prostaglandins, leukotrienes, and essential fatty acids 2014, 91, (6), 235-41.

Schebb, N. H.; Ostermann, A. I.; Yang, J.; Hammock, B. D.; Hahn, A.; Schuchardt, J. P., Comparison of the effects of long-chain omega-3 fatty acid supplementation on plasma levels of free and esterified oxylipins. Prostaglandins & other lipid mediators 2014, 113-115, 21-9.

Willenberg, I.; Ostermann, A. I.; Schebb, N. H., Targeted metabolomics of the arachidonic acid cascade: current state and challenges of LC-MS analysis of oxylipins. Anal Bioanal Chem 2015, 407, (10), 2675-83.

Unexpected Products of the HOCl-Induced Oxidation of Oleic Acid: a Study using TLC-ESI MS, MALDI MS and NMR Spectroscopy

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Reactive oxygen species (ROS) play important physiological roles and are of particular relevance in the pathogenesis of inflammatory diseases such as hepatitis or arthritis. At inflammatory conditions hypochlorous acid (HOCl) is generated (beside many other ROS such as hydroxyl radicals) via the enzyme myeloperoxidase (MPO) which is abundant in neutrophilic granulocytes. Although HOCl induces primarily the oxidative modification of thiol and amino residues, HOCl reacts also with the double bonds in the fatty acyl residues of (phospho)lipids under formation of chlorohydrins as the main products. Chlorohydrins are comparably stable products and can be easily identified by mass spectrometric methods.

Using oleic acid (OA), the simplest unsaturated fatty acid which contains just a single double bond, as a model system, we carefully investigated all products after reaction with HOCl, including the chlorohydrin, by a combination of thin-layer chromatography (TLC), electrospray ionization (ESI) mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. In contrast to phospholipids, the reaction between oleic acid and HOCl leads not exclusively to the formation of chlorohydrin (isomers) but is much more complex: there are also significant amounts of dimeric and (to a minor extent) trimeric products which indicate the involvement of free radicals during the reaction. Although we were so far not able to elucidate the structures of all generated isomers in detail, the formation of oligomeric products could be verified by all used methods. The obtained products after OA chlorination were also compared with the reaction products of 1-palmitoyl-2-oleoyl-sn-phosphatidylcholine (POPC) and HOCl. The reasons why different products are obtained at both conditions will be discussed and potential physiological consequences regarding the metabolism of oxidized fatty acids highlighted.

Matching Thin-Layer Chromatography, Overlay Immunodetection, and Desorption Electrospray Ionisation (DESI) – Mass Spectrometry for Glycosphingolipid Analysis

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Introduction:

The analysis of complex and heterogeneous biological samples often poses a serious challenge which can be addressed by use of mass analysers providing a very high resolving power such as FT-ICR mass spectrometers. Alternatively, sample complexity can be reduced by chromatographic separation, e.g. by thin-layer chromatography (TLC) preceding MS analysis. Coupling a DESI ion source to a mass spectrometer provides a convenient technique to probe a TLC plate under ambient conditions [1]. Here, we show that the hyphenation of DESI with an FT-ICR mass spectrometer enables accurate and precise analysis of immunodetected glycosphingolipids (GSLs) which act as receptors of Shiga toxins (Stxs) released by enterohemorrhagic *Escherichia coli* (EHEC) [2].

Materials and Methods:

Neutral GSLs from human erythrocytes were applied bandwise onto high-performance normal phase silica TLC plates, separated, and detected by antibody-overlay assays [2]. Subsequently, plates were submitted to DESI FT-ICR-MS analysis. Tandem MS experiments were performed by use of IRMPD.

Results and Discussion:

TLC-separated Stx GSL receptors globotriaosylceramide (Gb3Cer), globotetraosylceramide (Gb4Cer) and Forssman GSL were detected by overlay assays with GSL-specific antibodies as well as Stx1a, Stx2a and Stx2e subtypes, combined with secondary antibodies and subsequent staining with a chromogenic substrate. Stained bands were probed by DESI FT-ICR MS and immunopositive GSLs could be desorbed and ionised directly from the TLC plate without interferences from the antibodies, Stxs or the staining reagent. The observed fragmentation patterns in IRMPD experiments are similar to those obtained by low energy collisional activation. Evaluation of the MS/MS spectra allowed for structural elucidation of Stx receptors both with respect to glycan sequence as well as ceramide moiety. The results presented in this study demonstrate the potential of the hyphenation of planar chromatographic separation and formation of gaseous ions directly from the surface of the TLC plate by DESI for the analysis of immunostained GSLs.

1   Z. Takáts, J. M. Wiseman, B. Gologan, R. G. Cooks, Science, 2004, 603, 471.

2   I. Meisen, M. Mormann, J. Müthing, Biochim. Biophys. Acta, 2011, 1811, 875.

Lipid-Profiling by LC-ESI-MS/MS

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Important representatives of lipids are the phospholipids and glycolipids, which inherit multiple functions in biological systems. The lipids are divided into classes by reference to the polar head group, which is bonded to the glycerol backbone or to the glycerolphosphate backbone. Important representatives of phospholipids are phosphatidylcholines (PC), phosphatidyl-ethanolamines (PE), phosphatidylserines (PS), phosphatidylglycerols (PG), phosphatidyl-inositols (PI), and bis(monoacylglycero)phosphates (BMP), whereas mono- (MGDG) and digalactosyldiacylglycerols (DGDG), sulfoquinovo-syldiacylglycerols (SQDG) and diacylglyceroltrimethylhomoserines (DGTS) are interesting glycolipids.

Lipid-profiling of various organisms is mandatory to gather information on the response to environmental stress. For example, the adaptation of algae to excessive light can be investigated on the glycolipid level. Furthermore, lipids are often involved in the pathogenesis of various diseases. Several studies indicate that BMP can be used as biological marker for breast cancer. Diseased tissue is characterized by higher contents of BMP.

Important structural information can be obtained by use of MS^n. However, the constitutional isomers PC and BMP show highly similar MS/MS spectra. Therefore differentiation of these lipids solely based on MS is not possible, and hence chromatographic separation is mandatory. Hydrophilic interaction liquid chromatography (HILIC) provides a good opportunity to separate the polar lipid classes.

The applicability of the developed lipid-profiling method based on HILIC-ESI-MS/MS is demonstrated by analysis of polar lipids from baker's yeast (*Saccharomyces cerevisiae*) and from the green algae *Chlamydomonas reinhardtii*.

Probing the Effect of ROS on Properties of Mitochondria and Liposomes made of Synthetic and Natural Cardiolipins

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Cardiolipins are a class of phospholipids that is predominantly present in the inner mitochondrial membrane. Since there are various oxidative processes taking place in mitochondria it is most likely that these lipids are exposed to high concentration of reactive oxygen species. Therefore we are investigating the effect of ROS on properties of biologically relevant membranes. Recent results have proven that mitochondria [1], thrombocyte vesicles [2] and liposomes [3, 4] can adhere and spread on the hydrophobic surface of a static mercury electrode leading to capacitive current peaks due to the formation of lipid monolayer islands. Analysis of adhesion-spreading kinetics provides information on the phase transition temperature as well as on the flexibility of the membranes. The vesicles are treated with hydroxyl radicals generated by the catalytic decomposition of hydrogen peroxide on platinum, thus avoiding any other chemical or physical effect on the liposomes. Mass spectrometry of the products of this radical attack has shown that one alkyl chain is abstracted from synthetic cardiolipin forming a lysocardiolipin that can result in changes of the membrane fluidity.

1   Hermes, M. et al. Electrochemical signals of mitochondria: A new probe of their membrane properties. Angew. Chemie - Int. Ed. 50, 6872 - 6875 (2011).

2   Agmo Hernández, V. et al. The adhesion and spreading of thrombocyte vesicles on electrode surfaces. Bioelectrochemistry 74, 210 - 216 (2008).

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4   Zander, S. et al. Membrane fluidity of tetramyristoyl cardiolipin (TMCL) liposomes studied by chronoamperometric monitoring of their adhesion and spreading at the surface of a mercury electrode. J. Solid State Electrochem. 16, 2391 - 2397 (2012).

Site Specific Release of Liposomal Nanotheranostic Agents through Enzymatic Sensitizing of Liposomes by Phopholipases in Combination by Remote Activation by Alternating Magnetic Field

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Several small molecules have to short circulation time in the blood. Liposomal delivery systems have been used to compensate this phenomenon in drug delivery and in imaging. At the moment liposomal delivery systems have a problem that the encapsulated substances remain entrapped inside the liposomes. Our purpose was to develop magnetoenzymatic sensitive liposome (MESL) carrier systems for imaging and targeted release of multifunctional nanotheranostic agents. The method utilizes alternating magnetic fields (AMF) and liposomal nanoparticles enzymatically sensitized to apoptosis, tumor and inflammation processes. We have used this phenomenon to develop more advanced image guided tumor and inflammation targeting and therapy methods utilizing aSMase secretion in the target tissues. We have also studied different aSMase levels in different tumor cells and tumor xenografts and inflamed tissues. Our theranostic delivery system offers a method to avoid this problem by adding site specific release of the content.

The liposomal construct was assembled as ICG-iron nanoparticle containing MESL nanocarrier. The construct was evaluated by using DLS, SAX, and electron microscopy. Long term stability and in vitro activation assays were done by using leakage assays. In vitro cells studies were performed by using SCC and pancreas tumor cell lines. Activated SMase levels were analyzed and the SMase level changes impacts of the leakage rates was assessed. Preliminary in vivo imaging studies were performed at the Molecular Imaging North Competence Center (MOIN CC). Liposome payload accumulation was tested by using optical imaging (NightOwl and FMT 2500) and MRI. ROI analysis was used to assess tumor to background and tumor to organ ratios. In preliminary in vivo experiments 10-30 orthotopic xenograft mice were used per cell line for imaging, targeting and PK parameter assessment. Arthtritis and Colitis models were used as models for inflammation targeting). All administrations were IV injections. The liposome accumulation was studied by using ICG fluorophores encapsulated in to the liposomes. The dye content in a target organ was assessed by using ICG fluorescence or absorbance.

There was clear synergy in using AMF and enzymatic activation in tandem. MRI and optical methods can be used to assess and develop this platform for further refinement. This method offers total improved way to do imaging and drug delivery and has possible perspectives to be clinically translated.

Chemically Cleavable Linkers for Target Identification

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Covalent chemical probes are important tools in chemical biology for the study of enzymes, mechanisms of covalent drugs and post-translational modifications. In these studies, mass spectrometry plays a crucial role in the identification of the probe targets and modification sites. However, the enrichment of probe targets is often accompanied by background, complicating unambiguous target identification.

We have embarked upon the development of chemically cleavable linkers in order to reduce background protein identifications. We will here present our latest results on the design, synthesis and evaluation of cleavable trifunctional tags, incorporating an azide for click chemistry, a fluorophore for sensitve detection and a biotin for enrichment purposes.

Rapid Phospholipid Characterization Using a Novel Intelligent Workflow on a Tribrid Orbitrap Mass Spectrometer

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High energy HCD/MS/MS is widely used for the detailed characterization of complex lipid extracts. Both positive and negative MS/MS data are often required for characterization of individual molecular lipid species, in particular PC species. However, it takes extra instrument time to collect MS/MS data in both polarities and the ionization efficiency in the negative mode is generally lower. Additionally, a choice of the dissociation method (HCD or CID) is important for analysis since each of the techniques produces a different fragmentation pattern. In order to address all these issues, we developed a novel workflow on a Tribrid Orbitrap MS instrument, which uses HCD MS/MS for characterization of major lipid classes and combined HCD/CID MS/MS for characterization of PC molecular lipid species. To benchmark the workflow egg PC extract was analyzed by reverse-phase chromatography and data were acquired using three different MS approaches: 1) HCD/CID MS/MS in positive ion mode, 2) DDA (top 15) acquisition in both positive and negative mode, and 3) DDA (top 10) acquisition using polarity switching. The HR/AM MS and MS/MS data obtained using these three approaches were processed by LipidSearch. The software automatically combines HCD and CID MS/MS data collected from the same precursor ion as a single MS/MS spectrum and aligns positive and negative ion data from multiple raw files for automatic lipid identification and characterization. The automated data analysis enables confident identification of lipid species for all three approaches. In order to evaluate the improvement in productivity and sensitivity of the presented workflow the total number of PCs identified by their sum and molecular composition, and the percentage of confidently characterized PCs were compared for each MS-approach. More than one hundred PCs were identified and characterized using fragment ion information from the combined HCD and CID MS/MS spectra or alignment of positive/negative MS/MS data. Importantly, we were able to identify more low abundant PC species using the novel HCD/CID MS/MS. In summary we report a novel approach for detailed characterization of PC molecular lipid species within a single alternating positive and negative ion LC-MS analysis using complementary HCD and CID MS/MS data.

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