LIPIDOMICS FORUM

12. - 14. November 2017

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

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

Coordination: Jutta Passarger

AbstractBook

Research Center Borstel
Leibniz-Center for Medicine and Biosciences

LIPIDOMICS
Informatics for Life Sciences

DGMS
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Program

Sunday | **November 12**

11:00 – 1:00 PM

LIFS – (Lipidomics Informatics for Life Sciences)
Hands-On Workshop on Lipidomics Softwares and Pipelines

1:00 PM

LUNCH

2:00 – 4:00 PM

LIFS – (Lipidomics Informatics for Life Sciences)
Hands-On Workshop on Lipidomics Softwares and Pipelines

4:00 PM

Registration

6:00 PM

OPENING KEYNOTE
Teymuras Kurzchalia

K 01 **In search for novel lipids with unknown functions**

Max-Planck-Institute for Molecular Cell Biology and Genetics, Dresden, Germany
kurzchalia@mpi-cbg.de

7:30 PM

DINNER AND COME TOGETHER
Music: Farmers and Firefighters Brass Band, Hartenholm

10:00 PM

Bus Transfer

Monday | **November 13**

9:00 AM — **SESSION 1 A** | Chair: Nicolas Gisch

Evelyn Rampler
T 01 **Simultaneous non-polar and polar lipid analysis by online 2D-HILIC-RP and high resolution MS**
Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Austria
evelyn.rampler@univie.ac.at

PRICE
Zhixu Ni
T 02 **High-throughput identification of unmodified and oxidized phospholipids from data-dependent LC-MS and shotgun lipidomics datasets using LipidHunter and LPPtiger**
Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy , Universität Leipzig, Germany
zhixu.ni@uni-leipzig.de

Simon Hammann
T 03 **Non-targeted lipid profiling of cereals and biomarker characterisation to identify cereal lipids in archaeological pottery**
University of Bristol, Department of Archaeology and Anthropology, United Kingdom
simon.hammann@bristol.ac.uk

10:00 AM — **COFFEE BREAK**

10:20 AM — **Session 1 B** | Chair: Nicolas Gisch

Hans Frieder Schoett
T 04 **Targeted high through-put quantification of human faecal sterols and 5-alpha_beta-isomeric stanols by high resolution liquid chromatography mass spectrometry Lessons learned from method development**
Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany
hans-f.schoett@gmx.de
Irene Pradas
T 05 **Lipidomic characterization of corticospinal motoneuron disease**
Department of Experimental Medicine, University of Lleida-Biomedical Research Institute of Lleida, Lleida, Spain.
irene.pradas@gmail.com

11:00 AM — **TUTORIAL**
Maria Fedorova
K 02 **Oxidized lipids and their quantitative analysis**
Center for Biotechnology and Biomedicine, Universität Leipzig, Germany
Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Germany
maria.fedorova@bbz.uni-leipzig.de

12:00 AM — **LUNCH**

1:00 PM — **KEYNOTE**
Todd Mitchell
K 03 **Nothing dries sooner than tears: unique tear film lipids and their role in the structure and function of tears?**
University of Wollongong, Australia
toddm@uow.edu.au

2:00 PM **SESSION 2 A** | Chair: Cristina Coman

Lukasz Marczak

T 06 **Lipidomics reveals molecular mechanisms leading to progression of cardiovascular disease related to chronic kidney disease**

Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland
lukasmar@ibch.poznan.pl

PRICE Christin Müller

T 07 **Oh What A Tangled Web We Weave – Coronavirus-Induced Replicative Organelles And Changes In The Lipid Profile Of Infected Host Cells**

Institute of Medical Virology, Justus Liebig University Gießen, Germany
Christin.mueller@viro.med.uni-giessen.de

2:40 PM **COFFEE BREAK**

3:00 PM **SESSION 2 B** | Chair: Cristina Coman

Carla Schmidt

T 08 **Protein lipid interactions studied by mass spectrometry**

HALOmem, Martin Luther University, Halle-Wittenberg, Germany
carla.schmidt@biochemtech.uni-halle.de

Fernando Martínez Montañés

T 09 **A time-resolved phospho-proteolipidomic platform demonstrates physiological regulation of global lipid metabolism**

VILLUM Center for Bioanalytical Sciences, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark

Jürgen Schiller

T 10 **Polyphosphoinositides: Interesting but difficult**

University of Leipzig, Medical Faculty, Institute of Medical Physics and Biophysics, Germany
juergen.schiller@medizin.uni-leipzig.de

4:00 PM **KEYNOTE**

Gennaro De Libero

K 04 **The immunology of glycolipid antigen recognition by T lymphocytes**

University of Basel and University Hospital Basel, Switzerland
Gennaro.delibero@unibas.ch

5:00 – 7:00 PM **POSTER SESSION**

Tommy Hofmann

P 01 **Identification and quantification of neuronal lipids.**

Interdisciplinary research center HALOmem, Martin Luther University Halle-Wittenberg, Germany
tommy.hofmann@biochemtech.uni-halle.de

David A. Peake

P 02 **Integrated Software For Lipid Data Analysis In Direct Infusion Ultra-High Resolution Accurate Mass Spectrometry Based Lipidomics Workflows**

Thermo Fisher Scientific, San Jose, California, USA
david.peake@thermofisher.com

Claire Dauly

P 03 **High Throughput Lipid Identification and Quantification Using a Directed HRAM LC-MS-MS approach on a Modified Quadrupole-Orbitrap Mass Spectrometer**

Thermo Fisher Scientific, Courtaboeuf, France
claire.dauly@thermofisher.com

PRICE Melissa Frick
P 04 **Mass Spectrometry of Liposomes**
Interdisciplinary research center HALOmem, Martin Luther University Halle-Wittenberg, Germany
melissa.frick@student.uni-halle.de

PRICE Angela Criscuolo
P 05 **Novel RPLC-MSMS method for the analysis of complex lipid mixtures**
Thermo Fisher Scientific, Bremen, Germany
angela.criscuolo@thermofisher.com

PRICE Regula Steiner
P 06 **Elucidation of metabolic pathway for 1-deoxymethylsphingolipids**
Univerisity Hospital Zürich, Switzerland
regula.steiner@usz.ch

Adam Wutkowski
P 07 **Software Platform to Improve Quality Control for Lipid Mediator Quantification**
Division of Bioanalytical Chemistry, Research Center Borstel, Borstel, Germany
awutkowski@fz-borstel.de

Norbert Reiling
P 08 **Acid sphingomyelinase deficient macrophages: Molecular characterization of magnetically isolated macropinosomes**
Research Center Borstel, Borstel, Germany
nreiling@fz-borstel.de

PRICE Patrick O. Helmer
P 09 **Structural characterization of cardiolipin oxidation products by means of LC-MS**
Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany
patrick.helmer@wwu.de

PRICE Viola Jeck
P 10 Elucidation of double bond positions in lipids by means of tandem MS
Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany
viola.jeck@wwu.de

PRICE Ansgar Korf
P 11 **Algorithm development for lipid identification in Chlamydomonas reinhardtii algae samples utilizing LC-HRMS**
Insitute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany
ansgar.korf@uni-muenster.de

PRICE Mike Lange
P 12 **Adipose Tissue Lipidomics Atlas**
Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Germany
mike.lange@uni-leipzig.de

PRICE Marcus Höring
P 13 **Development of a flow injection-high resolution MS method to analyze and quantify the lipid composition of neutral lipid rich samples**
Institute of Clinical Chemistry and Laboratory Medicine, University Hospital of Regensburg, Germany
marcus.hoering@ukr.de

PRICE Bing Peng
P 14 **High-throughput quantitative analysis of lipid mediators for platelet activation**
Leibniz-Institut für Analytische Wissenschaften – ISAS – e. V., Dortmund, Germany
bing.peng@isas.de

PRICE Cristina Coman
P 15 **SIMPLEX: from extraction to the biology of caveolin-3**
Leibniz-Institut für Analytische Wissenschaften – ISAS – e. V., Dortmund, Germany
cristina.coman@isas.de

PRICE Christiane Jung

P 16 **Development and application of a high resolution mass spectrometry method to identify and quantify faecal lipid species**

University Hospital of Regensburg, Germany
Christiane.Jung@ukr.de

Robert Ahrends

P 17 **Comprehensive analysis of the platelet lipidome reveals the central role of key lipids in activation and aggregation**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e. V., Dortmund, Germany
robert.ahrends@isas.de

Hande Karaköse

P 18 **Lipid Profile as Diagnostic Marker for Antimycobacterial Treatment Monitoring**

Research Center Borstel, 23845 Borstel, Germany
hkarakoese@fz-borstel.de

Dominik Kopczynski

P 19 **LipidCreator: a powerful Tool for Targeted Lipidomics**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e. V., Dortmund, Germany
dominik.kopczynski@isas.de

Nils Hoffmann

P 20 **LipidHome**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e. V., Dortmund, Germany
nils.hoffmann@isas.de

Kristaps Klavins

P 21 **Search for MDA modified phospholipids**

CeMM – Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria
kklavins@cemm.oeaw.ac.at

Canan Has

P 22 **SELFI: a meta platform for lipid identification**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany
canan.has@isas.de

Bettina Guertl

P 23 **Comprehensive lipidomics analysis using Orbitrap Fusion Lumos MS**

CeMM – Research Center for Molecular Medicine, Vienna, Austria
kklavins@cemm.oeaw.ac.at

PRICE Lars F. Eggers

P 24 **Lipidomes of human lung tissues and lung tumors change with histopathological phenotypes and the lifestyle of patients**

Research Center Borstel, Division of Bioanalytical Chemistry, Germany
leggers@fz-borstel.de

Fadi Al Machot

P 25 **A novel tool for the analysis of lipidomes homology based on LUX score**

Research Center Borstel, Division of Bioanalytical Chemistry, Germany
falmachot@fz-borstel.de

Andreas Wiesner

P 26 **LESA^{PLUS} enables an improved in-depth analysis of the lipidome in comparison to the traditional LESA (Liquid Extraction Surface Analysis) approach.**

Advion Ltd., Harlow, UK
awiesner@advion.com

Mariona Jové

P 27 **Lipidomic signature of extreme human longevity**

Department of Experimental Medicine, University of Lleida-Biomedical Research Institute of Lleida, Lleida, Spain.
mariona.jove@udl.cat

Julius Brandenburg

P 28 **Wnt6-induced signaling drives formation of triacylgylcerol-rich lipid bodies and promotes survival of Mycobacterium tuberculosis in macrophages.**

Division of Microbial Interface Biology, Research Center Borstel, Borstel, Germany
jbrandenburg@fz-borstel.de

Sandra F. Gallego

P 29 **Quantitative lipidomics reveals age-dependent perturbations of whole-body lipid metabolism in ACBP deficient mice VILLUM Center for Bioanalytical Sciences, Department of Biochemistry and Molecular Biology,**

University of Southern Denmark, Odense M, Denmark
sandrafg@bmb.sdu.dk

Kathrin M. Engel

P 30 **Separation of lipid oxidation products by TLC – the advantages of reversed-phase TLC**

University of Leipzig, Medical Faculty, Institute for Medical Physics and Biophysics, Germany
kathrin.engel@medizin.uni-leipzig.de

Ulf Sommer

P 31 **Targeted versus Untargeted Lipidomics**

Biocrates Life Sciences AG, Innsbruck, Austria
ulf.sommer@biocrates.com

Gerd Schmitz

P 32 **Evidence that plasmalogen species evolve as diagnostic targets in vascular metabolic and neurodegenerative disease.**

Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany
gerd.schmitz@ukr.de

7:00 PM **Dinner and Music**
Kurorchester Borstel

10:00 PM **Bus-Transfer**

Tuesday | November 14

9:00 AM **SESSION 3** | Chair: Robert Ahrends

Nestor González Roldán

T 11 **Filling the gap: lipids from pollen as orchestrators of the dynamics of allergic airway inflammation**

Junior Research Group of Allergobiochemistry, Research Center Borstel, Leibniz Center for Medicine and Biosciences, Airway Research Center North (ARCN), German Center for Lung Research (DZL)
ngonzalez@fz-borstel.de

David L. Marshall

T 12 **What Fat is That: Near-complete structure elucidation of OAHFAs by shotgun lipidomics**

Central Analytical Research Facility, Queensland University of Technology, Brisbane, Australia
d20.marshall@qut.edu.au

Kai Schuhmann

T 13 **Intensity-Independent Noise Filtering in FT MS and FT MSMS Spectra for Shotgun Lipidomics**

MPI of Molecular Cell Biology and Genetics, Brisbane, Australia
schuhman@mpi-cbg.de

Shane R. Ellis

T 14 **Automated and Parallel Acquisition of Both High Resolution Mass Spectrometry Imaging and Comprehensive Structural Identifications of Lipids in a Single Experiment**

M4I, The Maastricht Multimodal Molecular Imaging Institute, Maastricht University, The Netherlands
s.ellis@maastrichtuniversity.nl

10:20 AM **COFFEE BREAK**

10:45 AM **KEYNOTE**

Kim Ekroos

K 05 **High-definition FLUX Lipidomics through dedicated frameworks.**

Lipidomics Consulting Ltd., Finland

11:45 AM **POSTER AND TALK PRICES**

12:15 PM **LUNCH**

Talks Abstracts

In search for novel lipids with unknown functions

Teymuras Kurzchalia[1]

[1] Max-Planck-Institute for Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden

K 01

Investigations on nematodes in the recent two decades has shown that these organisms have very specific lipid composition. In addition to well-known lipid classes (e.g. PC, PE, PI etc.), worms appear to have some nematode-specific lipids. Moreover, some major components of mammalian cell membranes, like sterols, do not play any structural role in their membrane. In our laboratory, we have identified several novel lipids from nematodes *C.elegans* and *P. pacificus* (Maradolipids, Nematoil and Phosphorylated glycosphingolipids, PEGC) and started to investigate their functions. For instance, very recently we have shown that PEGCs are essential for cholesterol mobilization in worm and thus, for the switch of the developmental program via sterol derived hormone. Another very interesting lipid found in P. *pacificusis* Nematoil, an extremely long-chain polyunsaturated wax ester that covers the surface of the animal. The oily coat promotes congregation of up to one thousand individuals into stable "dauer towers" that can reach a beetle host more easily. We think that the investigation of lipids in different living organisms will provide information on novel, diverse molecular structures and consequently on their novel functions.

Simultaneous non-polar and polar lipid analysis by online 2D-HILIC-RP and high resolution MS

Evelyn Rampler[1],
Harald Schöny[1], Bernd Mitic[1], Yasin El Abiead[1], Michaela Schwaiger[1], Gunda Koellensperger[1]

[1] Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, evelyn.rampler@univie.ac.at

T 01

Comprehensive lipidomics demands efficient and robust separation methods to cover as many lipids as possible in a sample of interest. The combination of liquid chromatography and mass spectrometry has evolved as a state of the art coupling technique in order to analyse and quantify lipids. Although most lipids are amphiphilic, there are lipids with overall hydrophobic chemistry i.e. cholesterol, di- and triglycerides. Due to the distinct chemistry of polar and non-polar lipids, simultaneous chromatographic detection is challenging and time consuming. In this work, we present a novel online two-dimensional (2D) chromatographic method coupled with high resolution mass spectrometry (Orbitrap HF) for high coverage lipid analysis. The online combination of a hydrophilic interaction liquid chromatography (HILIC) specific for the head-groups (first dimension) and reversed phase chromatography (RP) using hydrophobic interactions (second dimension) enables fast and efficient separation for both polar and non-polar lipids within 15 min. The void volume of the first column is transferred via a heart-cutting process to the second chromatographic dimension and the hydrophobic lipids are eluted using high organic solvent concentrations. This setup enables the first reported online combination of HILIC with RP without any dilution (or use of a trapping column) in the second dimension, exploiting the strong chemical binding of hydrophobic lipids. The method proved to be robust with excellent retention time stability (RSDs < 1 %) even in the presence of complex biological matrix such as human plasma. The presented LC-MS/MS method enables fast analysis and quantification for a broad range of lipids belonging to the chemical classes of fatty acids, glycerolipids, glycerophospholipids, sphingolipids, and sterols. We strongly believe that lipidomics studies will benefit from the increased separation capabilities, enhanced sample throughput and broader lipid information of online 2D LC-MS methods.

High-throughput identification of unmodified and oxidized phospholipids from data-dependent LC-MS and shotgun lipidomics datasets using LipidHunter and LPPtiger

Zhixu Ni[1], Georgia Angelidou[1], Mike Lange[1], Ralf Hoffmann[1], Maria Fedorova[1]

[1] Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy , Universität Leipzig, Germany, zhixu.ni@uni-leipzig.de
[2] Center for Biotechnology and Bio-medicine, Universität Leipzig, Germany.

T 02 PRICE

Lipids are generally acknowledged as a dynamic constituent of biological systems capable to react and reflect any changes in physiological states. Thus, there is a large interest in lipid-derived markers for diagnostic and prognostic applications. However, screening efforts on larger cohorts are limited with automatic identification remaining a bottleneck of untargeted lipidomics. We have developed two open source software tools allowing high-throughput analyses of (modified) phospholipids in data-dependent LC-MS and shotgun datasets: LipidHunter [1] for phospholipids (PLs) and LPPtiger for oxidized PLs (oxPLs). LipidHunter provides an easy and efficient way to identify PL by resembling a workflow of manual spectra annotation. Lipid identification relies on MS/MS using defined fragmentation rules of each PL class. LipidHunter matches product and neutral loss signals obtained by collision-induced dissociation (CID) to a user-defined white list of fatty acid residues and PL class-specific fragments. The signals are searched first against elemental composition and bulk identification provided via LIPID MAPS online tools. The obtained bulk identifications is assigned to discrete forms by LipidHunter. The tabular and graphical reports provided by LipidHunter allow tracing back the main identification steps and to control data quality. This strategy identified 202 discrete lipid species in lipid extracts obtained from rat primary cardiomyocytes treated with a peroxynitrite donor. LPPtiger uses the PLs identified by LipidHunter to predict and identify oxPLs in the same dataset. The relative quantification of identified PLs and oxPLs allows monitoring dynamic reconfiguration of the cellular lipidome in response to mild nitroxidative stress. The source codes and Windows executable distributions of LipidHunter and LPPtiger are freely available at *https://bitbucket.org/SysMedOs*.

1 Zhixu Ni, Georgia Angelidou, Mike Lange, Ralf Hoffmann, and Maria Fedorova. LipidHunter Identifies Phospholipids by High-Throughput Processing of LC-MS and Shotgun Lipidomics Datasets. Analytical Chemistry, 2017

Non-targeted lipid profiling of cereals and biomarker characterisation to identify cereal lipids in archaeological pottery

Simon Hammann[1],
Lucy J. E. Cramp[1]

[1] University of Bristol, Department of Archaeology and Anthropology, simon.hammann@bristol.ac.uk

T 03

The analysis of lipid biomarkers from archaeological pottery offers unique insights into past foodways, technologies and cultural practices. These biomarkers and their isotopic signatures are usually analysed by gas chromatography (GC), coupled to mass spectrometry (GC-MS) or isotope ratio mass spectrometry (GC-IRMS). In this way, numerous research questions around animal-based food sources (e.g. dairy or aquatic sources) could be addressed. In contrast, despite their importance cereals and are virtually invisible with this approach due to a lack of robust and diagnostic biomarkers.

Goal of our work is to develop a set of specific and robust biomarkers for the detection and discrimination of cereal lipids using high resolution mass spectrometry data.

More than eighty cereal samples from seven families were collected from seed storage and research institutes. Total lipids were extracted with chloroform/methanol 2:1 (v/v) and the extracts were filtered through a short silica column. After trimethylsilylation the extracts were analysed by high temperature GC coupled to a QToF mass spectrometer. By using a short 15 m high temperature column we were able to analyse the original lipid composition without prior hydrolysis of high-boiling lipid species such as triacylglycerols. This approach allowed the detection and identification of several hundred individual compounds, including free fatty acids, sterols, tocopherols, alkylresorcinols and glycerol lipids. Processing of the data and statistical analysis (PCA) revealed the most discriminating compounds.

To investigate the applicability of GC-unamenable lipids as biomarkers, extracts will also be analysed by LC-MS using an orbitrap mass spectrometer. By comparing the LC-MS and GC-MS data mutually and exclusively detected features of both approaches and respective coverages will be assessed. This will give a set of compounds which could be used as diagnostic marker compounds to discriminate cereal families based on their lipid profiles and to identify cereal processing in archaeological pottery samples.

1 Evershed, R.P.. Organic residue analysis in archaeology: The archaeological biomarker revolution. Archaeometry, 50, 2008

2 Salque, M. Earliest evidence for cheese making in the sixth millennium BC in northern Europe. Nature, 493, 2013

Targeted high through-put quantification of human faecal sterols and 5-alpha_beta-isomeric stanols by high resolution liquid chromatography mass spectrometry Lessons learned from method development

Hans Frieder Schött[1],
Gerhard Liebisch[1], Silke Matysik[1]

[1] Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany, hans-f.schoett@gmx.de

T 04

A new high through-put method based on liquid chromatography - high-resolution mass spectrometry for the targeted determination of sterols and stanols in human faeces is developed and validated with respect to the FDA and EMEA guidance. Human faeces contain, depending on diet, different amounts of faecal sterols i.e. cholesterol and plants sterols and 5-α/β-isomeric stanols. Today it is evident that the microbiome in the intestine is mainly responsible for the formation of specific hydrogenated faecal stanols.

By means of this method development process we do present the major adjustment parameters that may, on one hand, improve the analyte determination or, on the other, rule out the reliable analyte quantification. The Orbitrap high resolution mass selective detector offers a plurality of different detection modes. The suitability and limitations of high resolution mass spectrometry in combination with high performance liquid chromatography for targeted sterol quantification by fast chromatography will be structured discussed. Further on does the chemical structure and physiological concentration of sterols and stanols challenge analysis.

Beside mass spectrometric challenges also the chromatographic separation of α/β-isomeric compounds is a critical analytical problem. Chromatographic solution and optimization steps for isomer separation will be presented and discussed. In detail the effects of additives in the mobile phase, column material, temperature, derivatisation, analyte structure and fragmentation, matrix effects, mass spectrometric detection modes and others will be compared and carefully evaluated. Finally, pitfalls and problem solutions will be presented as example for a diligent method development and reliable sterol determination.

Lipidomic characterization of corticospinal motoneuron disease

Irene Pradas[1],
Rosanna Cabré[1], Omar Ramírez[1], Joaquim Sol[1], Manuel Portero[1], Reinald Pamplona[1], Aurora Pujol[2] and Mariona Jové[1]

[1] Department of Experimental Medicine, University of Lleida-Biomedical Research Institute of Lleida, Lleida, Spain, irene.pradas@gmail.com
[2] Neurometabolic Diseases Laboratory, IDIBELL, L'Hospitalet de Llobregat, Spain; Centre for Biomedical Research on Rare Diseases (CIBERER), Institute Carlos III, Madrid, Spain; Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain.

T 05 PRICE

Background: X-linked adrenoleukodystrophy (X-ALD) is an inherited neurometabolic disorder caused by malfunction of the ABCD1 gene. X-ALD shows a wide range of phenotypes with different onset, progression, neurological afflictions and inflammatory patterns. However, all of them are characterized by a slowly progression of spastic paraplegia that affects corticospinal tracts, adrenal insufficiency and often they co-occur in the same family. Three of the main phenotypes are: i) adrenomyeloneuropathy (AMN); ii) cerebral form of AMN (cAMN), and iii) childhood cerebral adrenoleukodystrophy (cALD).

Purpose: To study the lipidomic profile of different X-ALD phenotypes in order to unravel the molecular bases of hereditary spastic paraplegia (HSP) and modelling corticospinal motoneuron disease.

Methods: Cerebrospinal fluid (CSF) samples from patients of the 3 main phenotypes; AMN (n = 13), cAMN (n = 5) and cALD (n = 13) with their corresponding age matched controls, were analyzed using a liquid chromatography-mass spectrometry platform. Univariate and multivariate statistics were used to modelling corticospinal motoneuron disease.

Results: AMN and cAMN phenotypes had more similar CSF lipidome profiles than cALD. Among the lipid species responsible for defining each X-ALD phenotype cholesterol esters, triacylglycerides, sphingolipids and phospholipids were identified. When the three phenotypes were considered as one we could define a specific lipidomic profile associated to HSP. The common features indicates higher levels of almost all differential lipids found, suggesting lipid metabolism as an important modulator of physiopathology in motoneuron diseases.

Conclusions: Using a lipidomic approach a better understanding of the physiopathology of neurologic disorders can be achieve as well as the role of lipid metabolism that could help to propose new therapeutic targets to enhance their treatment.

Correspondence to: Mariona Jové (email: mariona.jove@udl.cat) or Irene Pradas (email: ipradas@mex.udl.cat)

Oxidized lipids and their quantitative analysis

Maria Fedorova[1,2]

[1] Center for Biotechnology and Biomedicine, Universität Leipzig, Germany
[2] Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Germany

K 02

Current view on lipid peroxidation products (LPPs) underwent a significant paradigm shift – LPPs, previously seen as a toxic byproduct of free radical reactions, nowadays are recognized as an important mediator of various cellular responses and plays a significant role in organism redox balance. Significant success in deciphering the role of free fatty acid (FFA)-derived LPPs, especially iso- and neuroprostanes, established them as biomarkers of inflammatory pathways. More recently, potential biological activity studies were translated from FFA-derived LPPs to oxidized phospholipids (oxPLs). PL-bound LPPs have been demonstrated to play a significant role in platelet differentiation, induction of ferroptosis signaling, as well as an anti-inflammatory mediator in the context of atherosclerosis. Despite a relatively limited amount of available data, it becomes clear that the structure of LPPs is one of the main determinants of their diverse biological activities, including pro-inflammatory and death signaling as well as anti-inflammatory and pro-survival effects.

Systems wide profiling and identification of large number of oxidized lipids in biological samples are required to understand structure-functional relationships determining biological activities. Availability of state-of-the-art MS instruments characterized by improved sensitivity and high dynamic range of detection, combined with optimized analytical workflows, provides possibility for targeted and untargeted LPP profiling and quantification. The tutorial will cover analytical techniques used in LPPs research including lipid extraction, chromatography and mass spectrometry analysis, quantification and data integration.

Nothing dries sooner than tears: unique tear film lipids and their role in the structure and function of tears?

Todd W. Mitchell[1],
Sarah E. Hancock[1], David L. Marshall[2], Ramesh Ailuri[1], Michael J. Kelso[1], Simon H. J. Brown[1], Jennifer T. Saville[3], Venkateswara R. Narreddula[2], Nathan R. Boase[2], Burkhardt Schuett[4], Thomas Millar[4], Stephen J. Blanksby[2] and Mark D. P. Willcox[5].

[1] University of Wollongong, Wollongong, NSW, Australia 2522 toddm@uow.edu.au
[2] Queensland University of Technology, Brisbane, QLD, Australia 4000
[3] University of Adelaide, Adelaide, SA, Australia 5005
[4] Western Sydney University, Penrith, NSW, Australia 2751
[5] University of NSW, Sydney, NSW, Australia 2052

K 03

Dry-eye disease affects up to a third of the world's population and results in large medical and societal costs, estimated at up to 55 billion dollars in 2007 in the USA alone. It is a multifactorial disease defined as a disruption of the lacrimal functional unit, i.e., the eyelids, ocular surface, lacrimal glands and meibomian glands. A key role of this system is to deliver and maintain the thin film of primarily aqueous tears across the cornea, which is vital for the lubrication and protection of the ocular surface. The human tear film is composed of three layers; an inner glycocalyx layer rich in glycoproteins and mucins, an aqueous layer containing salts and proteins, and an outermost lipid layer secreted from meibomium glands. The physical properties of the tear film, including its stability and spreading, are intimately linked to the chemical composition of the lipid layer, and therefore its molecular lipid composition. Accordingly, we have developed and/or utilized a range of mass spectrometry-based techniques, e.g., differential ion mobility spectroscopy (DMS), ozone-induced dissociation, photo-dissociation and radical-directed dissociation to structurally identify tear film lipids. A particular focus has been on the novel (O-acyl)-omega-hydroxy fatty acids (OAHFAs) as a reduction in these lipidshas been linked to dry-eye disease. These polar lipids are secreted from the meibomian gland and are thought to play a significant role at the interface between the aqueous tear film and the neutral lipids, predominantly wax esters and cholesteryl esters, that make up the bulk of the lipid layer. We have also synthesised OAHFAs of vary chain lengths and double bond positions and tested their surface properties using a Langmuir trough. The complete structure of the most abundant OAHFAs present in the human tear film has been solved and trough experiments have defined their role in tear film stability.

Lipidomics reveals molecular mechanisms leading to progression of cardiovascular disease related to chronic kidney disease

Idkowiak J[1],
Tracz J[1], Marczak L[1], Luczak M[1], Perek B[2], Kostka-Jeziorny K[2], Podkowinska A[2], Stobiecki M[1]

[1] Institute of Bioorganic Chemistry Polish Academy of Sciences, lukasmar@ibch.poznan.pl
[2] Poznan University of Medical Science

T 06

Chronic kidney disease (CKD) is defined as progressive loss in kidney function over time. The diagnosis is based on glomerular filtration rate (GFR) which measures the standard kidney function.

Patients suffering from CKD since early stages are at strong risk of cardiovascular disease (CVD). It is known that major cause of death for all people with CKD are cardiac events nowadays. It was suggested at proteomic level that molecular mechanism of CVD related to CKD shows some kind of differences in reference to classical atherosclerosis. Nevertheless, disrupted metabolic pathways differentiating classical atherosclerosis between CVD related to CKD are not well recognized. In order to specify and to acquire knowledge about the dissimilarities in atherosclerosis development - lipid profiling of blood plasma samples was carried out.

The blood samples were taken from 24 healthy volunteers and 64 patients assigned in three groups:

1) CKD1-2 – patients at early stages of CKD and first symptoms of CVD;

2) CKD5 – patients at end-stage of CKD treated with renal replacement therapy with severe CVD symptoms;

3) CVD – patients suffering from advanced classical atherosclerosis and with normal renal function.

Extraction of lipids was performed according to protocol using MTBE extraction procedure proposed and optimized by Matyash and Shevchenko [1]. Q-Exactive Orbitrap (Thermo Fisher Scientific) coupled to TriVersa Nanomate (Advion) and UltrafleXtreme Maldi TOF/TOF (Bruker) were used as basic tools for non-targeted lipid profiling. In the next step the obtained profiles were compared in order to define the quantitative and qualitative differences. Differences in lipidome of the analyzed groups were found and developed dyslipidemia was confirmed. The abnormalities in metabolism of phospholipids and triacylglycerols were observed, that can be probably related to malnutrition or systemic inflammation leading to cardiovascular disease progression and in the effect, to cardiac events in CKD.

1 Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res. , 49(5): 1137-1146, 2008

Oh What A Tangled Web We Weave – Coronavirus-Induced Replicative Organelles And Changes In The Lipid Profile Of Infected Host Cells

Christin Müller[1],
Martin Hardt[4], Dominik Schwudke[3], Benjamin W. Neuman[2], Stephan Pleschka[1], John Ziebuhr[1]

[1] Institute of Medical Virology, Justus Liebig University Gießen, Christin.mueller@viro.med.uni-giessen.de
[2] Texas A&M University – Texarkana
[3] Division of Bioanalytical Chemistry, Research Center Borstel
[3] Imaging Unit, Biomedical Research Center, Justus Liebig University Gießen

T 07 PRICE

Similar to other +RNA viruses, coronaviruses induce membrane rearrangements in infected cells, resulting in organelle-like 'virus factories' that carry multi-subunit complexes that drive viral RNA synthesis. These replicative organelles (ROs) contain the viral and cellular proteins involved in viral replication/transcription and may also help to sequester viral factors from recognition by host defense mechanisms. There is increasing evidence that enzymes involved in cellular lipid metabolism have important roles in RO formation and, possibly, other steps of the coronavirus replication cycle. In this study, we analyzed the formation of human Coronavirus 229E (HCoV-229E) induced replicative organelles as well as the Virus-induced changes in the lipid profile of infected host cells (Huh-7 cells). We could demonstrate that Ceramides (Cer) and Lysophospholipids (LPL) are upregulated during the course of infection reflecting a possible role of those lipids in the formation of ROs. Additionally the inhibition of cytosolic phospholipase A2alpha (cPLA2a), which catalyzes the hydrolysis of membrane-associated glycerophospholipids at the sn-2 position, releasing a fatty acid and generating a lysophospholipid (LPL), blocks HCoV-229E replication and impairs the formation of ROs. Furthermore inhibition of cPLA2a activity resulted in reduced LPL levels, suggesting an involvement of LPLs in producing the membranous structures required for coronavirus replication. Finally, we were able to confirm that inhibition of cPLA2a activity affects the replication of several other +RNA viruses known to induce intracellular membrane rearrangements, such as MERS-CoV and Semliki forest virus, whereas poliovirus, human rhinovirus 1A, and vaccinia virus replication was not affected. Taken together, the data from this and a previous study provide strong evidence to suggest that cellular cPLA2a activity has important roles at different steps of the replication cycle of +RNA viruses from different families.

Protein lipid interactions studied by mass spectrometry

Carla Schmidt[1]

[1] HALOmem, Martin Luther University Halle-Wittenberg, carla.schmidt@biochemtech.uni-halle.de

T 08

Biological membranes separate the aqueous interior of cellular compartments from their mostly aqueous environment. This separation, however, makes the transport of information or material through the membrane necessary. This important task is carried out by the proteins that reside in the membrane. Membrane proteins therefore are embedded in the lipid bilayer and undergo interactions with the sur-rounding lipids. Hence, the main purpose of protein-lipid interactions is the stable fixation of the proteins in the membrane, although, a functional importance of protein-lipid interactions is gaining attention.

We studied various protein lipid complexes, including the ATP synthase from chloroplasts and bovine cytochrome c oxidase. We combined proteomics, lipidomics and native mass spectrometry to study the effects of lipids on the structure and function of these protein complexes. Using proteomics, we first confirmed the presence of protein subunits. We then identified associated lipids using LC-MS/MS. Notably, we identified new lipid species that specifically interact with both ATP synthase and cytochrome c oxidase. Native mass spectrometry revealed that both membrane complexes associate with a 'lipid plug' which is stabilizing the assemblies. Computational modelling further allowed generating a model of the protein-lipid assemblies giving insights into their structural arrangements. Currently, we are developing novel techniques to study protein-lipid interactions.

A time-resolved phospho-proteolipidomic platform demonstrates physiological regulation of global lipid metabolism

Fernando Martínez Montañés[1]
Albert Casanovas[2], Richard R. Sprenger[1], Magdalena Topolska[1], Martin Hermansson[1], Christer S. Ejsing[3]

[1] VILLUM Center for Bioanalytical Sciences, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark, fernandomm@bmb.sdu.dk
[2] Biomedical Research Institute of Barcelona, 08036 Barcelona, Spain
[2] Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany

T 09

Understanding how Saccharomyces cerevisiae cells modulate their proteome and lipidome during their growth adaptation has been facilitated by the advent of high-resolution mass spectrometry-based techniques. Hereof, we recently presented a time-resolved proteolipidomic platform that has revealed the extent of lipid metabolism regulation during physiological adaptations.

To gain further insight into the dynamic regulation of global lipid metabolism, we have now performed a phosphoproteomic analysis covering different yeast phases of proliferation from fermentative to respiratory metabolism.

Currently we are elucidating how reversible phosphorylation contribute to the observed metabolic changes. Hence, differentially phosphorylated lipid metabolic enzymes have been selected and the in vivo functionality of phosphorylation modifications is being addressed by growth phenotypic and metabolic flux analysis after phosphosite/s mutagenesis.

Taken together, our work provides new insights into lipid and protein (including phosphorylation) dynamics as quantitative resources for studying the regulation of lipid metabolism at the system level.

Polyphosphoinositides: Interesting but difficult

Jürgen Schiller[1],
Katharina Lemmnitzer[1], Rosmarie Süß[1]

[1] University of Leipzig, Medical Faculty, Institute of Medical Physics and Biophysics, juergen.schiller@medizin.uni-leipzig.de

T 10

(Poly-)phosphoinositides (PPI) represent an important group of phospholipids, although phosphatidylinositol (PI) and its phosphorylated derivatives phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4,5-bisphosphate (PIP2) constitute only about 5 % of total cellular lipids. PPI are crucial for cellular signaling: the hydrolysis of phosphoinositides by phospholipase C enables the transduction of extracellular signals through the plasma membrane by the formation of second messengers. Phosphoinositides that are phosphorylated at the third position of the inositol ring, like phosphatidylinositol-3,4,5-trisphosphate (PIP3), are of particular relevance.

Unfortunately, the analysis of PPI is rather challenging due to (a) their poor detectability combined with the small concentration in biological samples and (b) the lack of diagnostically relevant ions in MS/MS spectra. We have used a combination between MALDI-TOF MS, ESI-IT MS, thin-layer chromatography (TLC) and ^{31}P NMR to check the individual strengths and drawbacks of the different methods.

Using either MALDI or ESI all PPI can be detected. However, the detection limit increases considerably with the number of phosphate residues. This applies for positive and negative ion detection. According to our data it is not possible to differentiate the different isomers (for instance, PI-4P and PI-5P) by CID or PSD MS. Although the fatty acyl residues and the phosphorylated sugar ring can be easily identified, the position of the phosphate residue does not lead to differences in the fragment ion patterns. Therefore, previous separation is necessary. This can be achieved by TLC which enables the separation of the different isomers which can be subsequently identified by MS.

Finally, it will be shown that ^{31}P NMR is another convenient method to quantifiy different PPI even in complex lipid mixtures: since the chemical shifts of the different PPI isomers are different all isomers can be identified in a single spectrum and without the need of a major sample workup.

The immunology of glycolipid antigen recognition by T lymphocytes

Gennaro De Libero[1]

[1] Experimental Immunology, Department of Biomedicine, University of Basel and University Hospital Basel 1
Gennaro.delibero@unibas.ch

K 04

A central mechanism of immune response is antigen recognition. The dogma that only peptides stimulate T lymphocytes has become obsolete after the evidence that structurally different molecules are immunogenic for T cells.

The immunological relevance of T cells recognizing glycolipidantigens will be elucidated.

Glycolipids form stable complexes with CD1 antigen-presenting molecules and interact with T cells expressing specific T cell receptors. Complex glycolipids may be digested by glycosidases and lipases, thus generating small compounds suitable for CD1 binding. Glycolipid-specific T cells may recognize unique antigens preferentially enriched in tumor cells, this participating in tumor surveillance. Furthermore, the same T cells may provide efficient help to carbohydrate-specific B cells and facilitate the immune response against microbial carbohydrates.

The immunological implications of non-peptidic antigen recognition will be discussed in light of anti-microbial and anti-tumor immunity.

Filling the gap: lipids from pollen as orchestrators of the dynamics of allergic airway inflammation

Nestor González Roldán[1],
Regina Engel[1], Sylvia Düpow[1], Dominik Schwudke[2], **Katarzyna Anna Duda**[1]

[1] Junior Research Group of Allergobiochemistry, Research Center Borstel, Leibniz Center for Medicine and Biosciences, Airway Research Center North (ARCN), German Center for Lung Research (DZL), ngonzalez@fz-borstel.de
[2] Division of Bioanalytical Chemistry, Research Center Borstel, Leibniz Center for Medicine and Biosciences

T 11

Nearly 30 % cases of bronchial allergic asthma, are induced by grass pollen [1]. So far, research regarding allergy to pollen has focused on the study of proteins as allergens. However, the pollen coat contains not only proteins, but a large range of lipids (pollenkitt) that are essential for reproduction [2]. Previous reports showed that crude pollen-derived lipid extracts activated Natural Killer T (NKT) cells [3] and dendritic cells (DCs) towards an allergic phenotype [4]. Nevertheless, the detailed structure-activity relationship between lipid classes and reactive immune cells in the context of allergic inflammation remains poorly understood.

Therefore, we aimed to isolate and characterize the chemical structures of the lipid classes present in Timothy grass (Phleum pratense) pollen and to determine their role in allergic inflammation. We isolated different lipid species utilizing extraction with chloroform/methanol, separation on silica column followed by HPLC fractionation. The chemical structures of isolated compounds were determined by GC-MS, ESI-MSn and NMR analyses. The biological activity of the total pollen extract and subsequent fractions was tested on different murine and human cells-based systems using flow cytometry.

We found that fractions rich in phytoprostanes induced murine MC chemotaxis and IL-6 release; and enhanced IgE-dependent degranulation of human lung-derived and murine MCs, demonstrating a role in the immediate effector phase of allergic inflammation. On murine DCs, phytoprostanes selectively induced the upregulation of CD1d, likely preparing lipid-antigen presentation to NKT cells. Two additional fractions containing glycoglycero- and glycosphingolipids, induced proliferation of murine NKT cells ex vivo. Upon intranasal administration in mice, these fractions induced eosinophilic infiltration into the airways, a hallmark of allergic asthma. Finally, these fractions were recognized by human NKT and gammadeltaT cells from peripheral blood, evidenced by the expression of the activation marker CD69.

Taken together, our results provide a defined structure-activity relationship for lipid classes in the process of allergic inflammation.

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4 Claudia Traidl-Hoffmann, Valentina Mariani, Hubertus Hochrein, Kathrin Karg, Hermann Wagner, Johannes Ring, Martin J. Mueller, Thilo Jakob, Heidrun Behrendt. Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper 2 cell polarization. J Exp Med, 201(4): 627-635, 2005

What Fat is That: Near-complete structure elucidation of OAHFAs by shotgun lipidomics

David L. Marshall[1],
Sarah E. Hancock[2], Ramesh Ailuri[2], Michael J. Kelso[2], Todd W. Mitchell[2], Stephen J. Blanksby[1]

[1] Central Analytical Research Facility, Queensland University of Technology, d20.marshall@qut.edu.au
[2] Faculty of Science, Medicine and Health, University of Wollongong

T 12

(O-acyl)-hydroxy fatty acids (OAHFAs) are a recently discovered class of endogenous lipids, correlated with enhanced glucose tolerance in mice. Interestingly, the ω-OAHFA sub-class play a key role in stabilising the human tear film. Given the paucity of commercially available synthetic standards, developing high resolution tandem mass spectrometry tools for rapid and unambiguous structure elucidation of OAHFAs is essential to understanding their different physiological functions.

Nano-electrospray ionisation of OAHFAs produces abundant [M – H]– ions, which enables assignment of the total carbons and degree of unsaturation (sum composition) based on high resolution accurate mass measurements. Collisional activation of [M – H]– ions yield fatty acid (FA), hydroxy-fatty acid (HFA), and dehydrated HFA product ions, readily identifying the OAHFA constituents at the MS2 level. Further interrogation of the HFA in an MS3 experiment is required to produce diagnostic fragment ions that readily pinpoint hydroxylation position and thus, the original ester location of the OAHFA. [1] Gas-phase ozonolysis (OzID) is employed to identify double bond position within the fatty acyl chain – based on predictable neutral losses from the molecular ion. Moreover, cis/trans stereoisomerism can be resolved by their different rates of reaction with ozone.

Based on these analyses, OAHFAs – like phospholipids and triacylglycerols – can be categorised using a hierarchical description (e.g., lipid class, sum composition, structurally defined molecular lipid). Moreover, a general mechanistic understanding of the unimolecular dissociation of deprotonated OAHFAs is obtained, such that the structure of unknown OAHFAs can be assigned based solely on their MSn spectra, without reference to standards or chromatographic retention times.

1 DL Marshall, JT Saville, AT Maccarone, R Ailuri, MJ Kelso, TW Mitchell, SJ Blanksby. Determination of ester position in isomeric (O-acyl)-hydroxy fatty acids by ion trap mass spectrometry. Rapid Communications in Mass Spectrometry, 30, 2016

Intensity-Independent Noise Filtering in FT MS and FT MSMS Spectra for Shotgun Lipidomics

Kai Schuhmann[1],
Andrej Shevchenko[1]

[1] MPI of Molecular Cell Biology and Genetics, schuhman@mpi-cbg.de

T 13

Shotgun lipidomics provides a quantitative snapshot of lipid extracts composition and typically relies on the interpretation of high-resolution MS and MS/MS spectra. These spectra comprise >100.000 signals. But, only a few represent bona fide molecular ions of lipids.

We investigated the signal properties of PC and PC O- adduct ions in FT MS/MS- spectra from bovine heart PC whose abundance differed by 200-fold. The abundance of noise signals varied within the spectra in m/z-dependent manner and differed by > 100-fold concomitantly with the abundance of corresponding precursors. Therefore, arbitrary thresholds of signal-to-noise and peak intensity uniformly applied to all spectra in a dataset could bias the interpretation. At the same time, irrespectively of their intensity, > 90 % of noise signals were only detected in individual scans with low repetition rates, while signals related to bona fide PC/PC O- fragments were detected with high (>75 %) repetition rate. We, therefore, tested if repetition rate could be used as intensity independent threshold to eliminate noise. For the repetition rate filtering, the m/z range of each FT spectra was segmented into one mDa bins and signals were sorted by their accurate m/z. Signals are having the repetition rate < 65 % within a search window were eliminated. We found that this procedure reduced the dataset size by 95 %, while still being able to recognize 103 molecular species of PC/PC O- having the signal-to-noise ratio above the value of 10. When the same dataset was reduced to a similar size by applying a signal intensity cut-off threshold, 47 % of these species were lost.

In summary, repetition rate filtering enabled efficient reduction of shotgun datasets without compromising the confidence of identification and quantification of lipids, hence eliminating a major bottleneck in high throughput shotgun lipidomics.

Automated and Parallel Acquisition of Both High Resolution Mass Spectrometry Imaging and Comprehensive Structural Identifications of Lipids in a Single Experiment

Shane R. Ellis[1],
Martin. R. L. Paine[1], Berwyck L. J. Poad[2], Stephen J. Blanksby[2], Christer S. Ejsing[3], Ron M. A. Heeren[1]

[1] M4I, The Maastricht Multimodal Molecular Imaging Institute, Maastricht University, The Netherlands, s.ellis@maastrichtuniversity.nl
[2] Queensland University of Technology, Brisbane, Australia
[3] Department of Biochemistry and Molecular Biology, VILLUM Center for Bioanalytical Sciences University of Southern Denmark, Odense, Denmark and Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany

T 14

Mass spectrometry imaging (MSI) is a powerful tool for spatially profiling lipid distributions in tissues with resolution down to the low micrometre level. Translation of MSI to understanding localised lipid biochemistry has presented a major challenge, with one predominant reason being the limitations of MSI in structurally identifying the many hundreds of detected lipids.

To address this problem, we have developed parallelised data-dependant acquisition-MSI (DDA-Imaging) whereby both high resolution (240,000 FWHM @m/z 400) MSI and ion trap MS/MS data are acquired in parallel during an imaging experiment. Using this unique workflow, acquisition of MS/MS is "free" in that it does not prolong experiment time relative to a standalone MSI experiment. DDA-Imaging yields all of a high resolution MSI dataset, exact m/z of every detected peak and high quality fragment ion data of virtually every nominal mass. In this talk we demonstrate application of DDA-imaging to lipid imaging in the cerebellum. A prototype version of the ALEX123 software package was used to automatically identify lipids by both exact m/z and MS/MS. In a single DDA-Imaging experiment we could confidently identify over 100 molecular lipids species down to their individual fatty acyl composition while simultaneously providing their spatial distributions.

MS/MS and accurate mass cannot always differentiate lipid isomers, in particular those varying only in double bond position or the sn¬-position of fatty acyl moieties. Motivated by recent evidence suggesting isomer-specific roles of lipids, we also present results obtained from selective gas-phase ozonolysis during MSI experiments that reveal the distributions of lipid isomers. As an example, four distinct sn-positional isomers of PC(36:1) were observed: PC(18:0/18:1) and PC(18:1/18:0) were co-localized in the brain, however PC(16:0/20:1) was present much higher levels in the white matter, and depleted in the gray matter, relative to isomeric PC(20:1/16:0), suggesting the synthesis of individual lipid isomers is highly regulated.

Taking Lipidomics to the Sky and Catching the Details in Lipid Turnover

Kim Ekroos[1]

[1] Lipidomics Consulting Ltd., Esbo, Finland

K 05

During the last decades, we have developed novel mass spectrometry high-throughput technologies for the precise assessment of lipidomes enabled by advanced mass spectrometry, automation and software tools. We are now making another big leap forward by advancing the frameworks through the introduction of a first-ever cloud-based lipidomics software platform, offering high quality and throughput lipidomics data analysis directed towards big data analyses in standardized formats. In parallel, we continue to develop the precise determination of the turnover of molecular lipids in cells utilizing stable isotope labelled tracers. We have deployed this platform for monitoring the turnover rate of sphingolipids at unprecedented temporal resolution over 72 hours of culture, a time frame that covers several conserved metabolic programs and adaptations that takes place during cell growth. This work provides the first comprehensive time-resolved resource of the total sphingolipid turnover, permitting observations of lipid intermediates and metabolic flow of molecular sphingolipid species in mammalian cells. We are performing follow-up studies extending the molecular lipid species coverage and experimental conditions to ultimately outline the high-defined metabolic flow of the global lipid metabolism and turnover. We anticipate that the cloud-based computing and FLUX studies shape the future of lipidomics, providing better understanding of human health and the pathophysiology of lipid metabolic disorders, with foremost potential to discover new treatment strategies.

Posters

Identification and quantification of neuronal lipids.

Tommy Hofmann[1],
Carla Schmidt[1]

[1] Interdisciplinary research center HALOmem, Martin Luther University Halle-Wittenberg, Kurt-Moses-Straße-3, 06120 Halle (Saale) tommy.hofmann@biochemtech.uni-halle.de

P 01

Transfer of information through membranes can be accomplished by membrane fusion, e.g. in neuronal synapses during neurotransmitter release. Membrane proteins and their interplay with specific lipids trigger this process. However, the role of these lipids is poorly understood.

Therefore, we studied neuronal lipids by mass spectrometry to establish a reliable workflow for their identification and quantification in neuronal membranes. Standards representing various neuronal membrane lipid classes were subject to high-energy collisional dissociation (HCD) and collisional induced fragmentation (CID). Lipids were identified by diagnostic fragment ions generated by optimal collision energies determined for neuronal lipids. The performance of deuterated lipids for quantification purposes was examined by titration curves and isotopic cluster simulation for overlapping peak envelopes of unsaturated lipid subspecies.

Identification by diagnostic fragments proved reliable for mixtures and quantification using deuterated standard lipids was robust over a broad concentration range. Relative quantification by isotopic cluster simulation provided data on overlapping isomers, e.g. for Sphingomyelin (SM) a cluster was observed between m/z 729.6 and 735.6. From this we could determine the proportion of SM(34:0), SM(34:1) and SM(34:2) being 7 %, 83 % and 9 %, respectively. Molar titration of the deuterated and native lipids was used to benchmark their performance for absolute quantification. For this, we first successfully used Phosphatidylglycerols (PG) with different fatty acid chain length (PG(16:0/18:1) and PG(18:1/18:1)) to show that chain length has no effect on the linearity of the titration curve. With this, we were able to absolutely quantify the composition of complex lipid mixtures in direct infusion mass spectrometry.

Integrated Software For Lipid Data Analysis In Direct Infusion Ultra-High Resolution Accurate Mass Spectrometry Based Lipidomics Workflows

David A Peake[1],
Yasuto Yokoi[2], Reiko Kiyonami[1], Elena Sokol[1], Gavin Reid[3], Andrea Hühmer[1]

[1] Thermo Fisher Scientific, San Jose, California, USA, david.peake@thermofisher.com
[2] Mitsui Knowledge Industry Co., Tokyo, Japan
[3] University of Melbourne, Parkville, Victoria, Australia

P 02

Infusion MS-based lipidomics workflows using ultra-high resolution accurate mass spectrometry (UHRAMS) coupled with selective derivatization of lipid functional groups provide a convenient solution to address isobaric and isomeric mass lipid overlap1. One remaining bottleneck limiting wide-spread application of high-throughput untargeted lipidomics is the lack of integrated software tools. We describe here LipidSearch 5.0 software designed specifically for infusion lipidomics analysis.

Extraction of lipids followed by selective derivatization of amino phospholipids and plasmalogen ether-containing lipids was coupled with UHRAMS analysis using a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer. "Sum-composition level" lipid identification was performed by LipidSearch 5.0 software. Validation of the software performance was obtained using a mixture of labelled and unlabelled lipid standards with concentrations ranging over at least 3 orders of magnitude. MS analysis was performed at 120, 240 and 500K resolution (FWHM at m/z 200). MS/MS analysis was performed using both data dependent or independent approaches.

Key data processing features include mass re-calibration, Gaussian peak fitting and Poisson modelling to identify and correct isotopic overlaps. First the monoisotopic peak is assigned by searching against a user-defined database in SMILES format, enabling individual selection of lipid categories, class/subclass, total number of carbons and double bonds, positive or negative ion adducts and definition of "fixed" and "variable" modifications. Then, isotopic peaks from the identified lipid are removed and the process is repeated for all remaining peaks.

In a few seconds several hundred "sum-composition level" lipid species are confidently identified from crude lipid extracts. Normalized results are used for relative quantification and statistical comparison between groups. Positive and negative ion results are merged, providing higher confidence for lipid identification. MS/MS spectra are searched for product ions predicted from ions identified during MS data processing. Unique and non-unique product ions are assessed and MS/MS results are corrected for isotopic overlap.

1 Eileen Ryan, Gavin E. Reid. Chemical Derivatization and Ultra High Resolution / Accurate Mass Spectrometry Strategies for 'Top Down' Lipidome Analysis. Acc. Chem. Res., 49(9): 1596-1604, 2016

High Throughput Lipid Identification and Quantification Using a Directed HRAM LC-MS-MS approach on a Modified Quadrupole-Orbitrap Mass Spectrometer

Reiko Kiyonami[1],
David Peake[1], **Claire Dauly**[2], Andreas Huhmer[1]

[1] Thermo Fisher Scientific, San Jose, CA, USA
[2] Thermo Fisher Scientific, Courtaboeuf, France, claire.dauly@thermofisher.com

P 03

Lipids play a key role in cell, tissue and organ physiology. Diseases such as cancer and diabetes involve disruption of metabolic enzyme pathways. Lipidomics studies aim to identify and quantify thousands of cellular lipid species in order to provide a more detailed understanding of the biological function of lipids and subsequently to identify unique lipid biomarkers for early disease detection.

Our goal for this study is to develop a robust and reproducible HPLC MS-MS method which enables untargeted lipid identification and targeted screening of thousands major lipid molecule for getting estimated concentrations of identified lipid species in a single HPLC MS-MS run on a Thermo Scientific™ Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ mass spectrometer.

The method was developed with lipid extracts from bovine heart and human plasma. An isotopically labeled lipid standard mixture (SPLASH™ Lipidomix® Mass Spec Standard, Avanti Polar Lipids) was spiked into each sample prior to lipid extraction. A large inclusion list (positive and negative ions) covering 4074 lipid molecular species from 14 major lipid classes was generated in-silico using Thermo Scientific™ LipidSearch™ software and used to direct the LC MS-MS data acquisition. LipidSearch pre-release software was used for lipid identification and quantitation. The estimated concentrations of identified lipid species were calculated relative to the known concentration of internal standards included in the SPLASH mixture.

The results presented here demonstrate that thousands of individual lipid species can be identified and quantified from complex biological samples. The newly-developed workflow implements a very large pre-defined lipid precursor ion inclusion list for directed MS/MS data acquisition on a Q Exactive HF-X mass spectrometer. This comprehensive approach allows simultaneous unbiased novel lipid identification and determination of estimated concentrations for more than 1000 lipid species while targeting more than 4000 targeted lipid species across 14 lipid classes in a single HPLC MS-MS run.

Mass Spectrometry of Liposomes

Melissa Frick[1], **Carla Schmidt**[1]

[1] Interdisciplinary research center HALOmem, Martin Luther University Halle-Wittenberg, melissa.frick@student.uni-halle.de

P 04 PRICE

Liposomes are phospholipid bilayer vesicles which resemble cellular organelles and membranes. Due to their variability in size, composition and amphiphilic character, they are promising mimics of natural membranes. However, due to their heterogeneity and the ability of lipids to form large clusters in the gas phase, liposomes are not well-suited for mass spectrometric analysis.

The aim of this work was to optimize liposome composition, concentration and size to enable the analysis of intact proteins in their natural environment (liposomes) by mass spectrometry. Analysis of mixed liposomes containing DOPC: DOPS:DOPE:Cholesterol (50:20:20:10) revealed only small lipid clusters in the gas phase of the mass spectrometer. Most lipid clusters contained DOPC, the major component of these liposomes. Their fragmentation pattern showed no difference in size, composition and concentration suggesting that the analysis of liposomes is independent of these factors. However, at high collision energies larger lipid clusters dissociate and smaller clusters are obtained providing the basis for mass spectrometric analysis of proteo-liposomes. Currently, we are optimising the analysis of intact proteins in liposomes.

Novel RPLC-MSMS method for the analysis of complex lipid mixtures

Angela Criscuolo[1],
Maria Fedorova[2], Ken Cook[1]

[1] Thermo Fisher Scientific, Bremen, Germany, angela.criscuolo@thermofisher.com
[2] Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Leipzig, Germany

P 05 PRICE

Mass spectrometry based analysis of complex lipid mixtures represent a challenging task due to the extremely high complexity lipidomes in biological systems. Thus, LipidMAPS database (updated 30/01/2017) reports more than forty thousand unique lipid structures. Furthermore, many lipids are represented by isomeric (same elemental composition and therefore same molecular weight, but different structure) and isobaric (different elemental composition, but very similar molecular weight) compounds. To address high complexity of complex lipid samples, a novel RPLC-MS/MS workflow based on reverse phase chromatography and data-dependent acquisition was developed.
Lipids were extracted from human plasma according to the protocol reported by Matyashetal et al [1] to address a large variety of classes and molecular species. Lipids were separated using RPLC using an Ultimate 3000 HPLC system and five different columns:

- C18 Hypersil Gold (2.1 x 150 mm, 1.9 µm) column (column inner diameter x length, particle size)
- C18 Accucore (2.1 x 150 mm, 2.6 µm) column
- C30 prototype (2.1 x 150 mm, 1.9 µm) column
- C30 Accucore (2.1 x 150 mm, 2.6 µm) column
- C30 Acclaim (2.1 x 250 mm, 3 µm) column.

High mass accuracy MS analysis and data-dependent MS2 analysis were performed on a Q Exactive HF mass spectrometer to identify individual lipids molecular species. LipidSearch software version 4.1 SP1 was used for lipid identification. This workflow was applied to evaluate the separation performance of each column in terms of lipid species coverage. The optimized workflow successfully identified more than 700 lipid molecules.

1 Matyash V, Liebisch G, Kurzchalia T V, Shevchenko A, Schwudke D.. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics.. J Lipid Res. 49(5): 1137-1146, 2008

Elucidation of metabolic pathway for 1-deoxymethylsphingolipids

Regula Steiner[1],
Arnold von Eckardstein[1], Thorsten Hornemann[1]

[1] Univerisity Hospital Zürich, Switzerland, regula.steiner@usz.ch

P 06 PRICE

1-Deoxymethylsphinglipids (1-DeoxymethylSL) are atypical sphingolipids which are devoid of the C1 and the C1-OH-group present in canonical sphingolipids. They are formed by the condensation of palmitoyl-CoA and Glycine, instead of Serine as for the canonical sphingolipids. The biological role of 1-deoxymethylSL is so fare unclear but they have been found in mammalian cells and human plasma and they are elevated in the rare heredetary sensory neuropathy type 1. They were assumed to follow the same metabolic pathway as canonical sphingolipids, but as they are missing the C1 and hydroxyl-group 1-deoxymethylSL cannot be converted to complex sphingolipids nor phosphorylated and therefore not degraded via the lyase pathway. In this work, we tried to elucidate the unknown metabolic pathway of 1-deoxymethylSL and compared it to the canonical sphingolipid pathway.

HEK293 cells were fed with isotopic labeled or unlabeled 1-deoxymethylSL in several metabolic conversion assays. Five new downstream metabolites of 1-deoxymethylSL could be identified and the chemical structure was elucidated by the use of liquid chromatography and high resolution accurate mass spectrometry for the position of hydroxylation and unsaturation. The enzymatic pathway was demonstrated by feeding synthetic 1-deoxymethylSL to HEK293 or MEF cells with the inhibition or overexpression of suspected enzymes.

1-Deoxymethylsphinganine is converted to mono or di-unsaturated species or it gets mono- or di-hydroxylated. Several Enzymes of the canonical sphingolipid metabolism are involved in the formation of unsaturated or mono-hydroxylated species. Additionally, 1-deoxymethylSL can get di-hydroxylated over unknown pathway, which is not possible for canonical sphingolipids.

We could show a clear difference in the metabolic pathway of canonical sphingolipids and the atypical 1-deoxymethylsphingolipids.

Software Platform to Improve Quality Control for Lipid Mediator Quantification

Adam Wutkowski[1],
Matthias Krajewski[1], Navratan Bagwan[2], Sarah Ring[3], Bianca Schneider[3], Mathias Schäfer[4], Dominik Schwudke[1]

[1] Division of Bioanalytical Chemistry, Research Center Borstel, Parkallee 10, Borstel, Germany, awutkowski@fz-borstel.de
[2] Centro Nacional de investigaciones Cardiovasculares Carlos III (CNIC), Melchor Fernandez Almagro 3, 28029 Madrid, Spain
[3] Division of Colnfection, Research Center Borstel, Parkallee 30, Borstel, Germany
[4] Institute of Organic Chemistry, University of Cologne, Greinstraße 4, 50939 Köln, Germany

P 07

Lipid mediators (LM) are a class of bioactive compound which play an important role in many biological processes including inflammation, autoimmunity, allergy. The structure of LMs can be derived from three the precursor molecules Arachidonic Acid, Eiosanpentanoid Acid and Docosanhexanoid Acid. Generally, QqQ-MS with only unit resolution are applied to study LM metabolism focusing exclusively on selected fragments that are recorded using multiple reaction monitoring.

In this study, we applied parallel reaction monitoring (PRM) to identify and quantify LM in complex matrices. For a set of reference compounds MS2 spectra were recorded by direct infusion experiments on the Q Exactive and Q-TOF to generate two spectral libraries. For developing the spectral comparison score (SCS) both libraries containing 36 LMs were utilized. The score is based on the Spearman's rank correlation between fragment intensities in the query and library as well as the number of matched fragment. To automatize the scoring a software was developed to 1) process raw peak lists, 2) generate customized spectral libraries, 3) perform SCS calculations and 4) help to identify quantifier ions. A set of filters can be employed to delete background signals and/or select only fragments that fit the compositional constraint of $C_nH_{3/2n-y}O_z$ (n=4,5,6...22; y: 0,1,2; z=0,1,2..5). During evaluation of the spectra library, even numbered fragment ions (m/z) were detected for some LMs like 12-HETE, 11,12-EET, PD1 and Mar-1 that could be identified as radical ion species. These fragment ions could be associated to two structural features and can be employed to improve specificity of the SCS.

In summary, the developed SCS algorithm scored LMs in relation to their structural similarity enabling a well-defined quality control approach for PRM based quantitation. Finally, we applied the software for the evaluation of LMs quantitation during a co infection of mice with M. tuberculosis and influence.

Acid sphingomyelinase deficient macrophages: Molecular characterization of magnetically isolated macropinosomes

Victoria Sohst[1],
Maike Burmeister[1], Ute Distler[2], Christine Hess[1], Dieter Adam[3], Stefan Schütze[3], Ulrich E. Schaible[1], Stefan Tenzer[2], Norbert Reiling[1]

[1] Research Center Borstel, nreiling@fz-borstel.de
[2] Institute of Immunology, Johannes Gutenberg University Mainz
[3] Institute of Immunology, Christian-Albrechts-University Kiel

P 08

Intracellular membrane trafficking is regulated by the interaction of signaling molecules like membrane lipids and proteins. The acid sphingomyelinase (aSMase) is essential in membrane lipid metabolism. The enzyme hydrolyses sphingomyelins (SM) to ceramide in response to cellular stress. Ceramide is an important second messenger that mediates vesicle formation, cell signaling and the induction of cell death. The lack of aSMase results in lysosomal dysfunctions that impair pathogen clearance and dysregulate inflammatory processes. In humans aSMase deficiency causes the Niemann-Pick disease which is a severe lysosomal storage disorder characterized by the accumulation of SM in lysosomal compartments. The molecular and cellular consequences caused by changes in the membrane lipid composition are currently not known.

In this study we analyzed macrophages, being key cells in innate immunity, which continuously sample their environment for foreign antigens via macropinocytosis, a specialized way of endocytosis. Following internalization molecules are either recycled to the surface in early and recycling endosomes, or sorted for subsequent degradation in late endosomes and lysosomes. To isolate the compartments from primary mouse macrophages, we developed a new technique using colloidal and fluorescent beads. We have isolated fluid phase compartments from Smpdl+/+ and Smpdl-/- primary macrophages. This revealed in total 1803 proteins in five independent experiments, of which 1758 were present in at least three preparations (97.4%). We identified 58 differentially regulated factors of which 28 were significantly lower and 30 significantly higher expressed in compartments from Smpdl-/- macrophages. Taken together the study identified structural differences in the molecular composition of intracellular compartments of Smpdl+/+ and Smpdl-/- macrophages which might be involved in the lysosomal dysfunction in the context of an aSMase deficiency as observed in Niemann-Pick patients.

Structural characterization of cardiolipin oxidation products by means of LC-MS

Patrick O. Helmer[1], **Heiko Hayen**[1]

[1] Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany, patrick.helmer@wwu.de

P 09 PRICE

Cardiolipins (CL) are anionic phospholipids, which are exclusively located in mitochondrial membranes. Especially in the inner mitochondrial membrane, this lipid class plays a main role in energy metabolism. While it is essential for stability of protein complexes of the respiratory chain, it is also involved in various pathologies like neurodegenerative diseases. Oxidation of CL is known to destabilize these protein complexes and is associated with apoptotic events. To understand these processes it is important being able to analyze CL and its oxidation products. Due to four partly different acyl chains with various levels of saturation, CL themselves are very complex phospholipids. Furthermore, the oxidation increases this diversity by forming numerous oxidation species. Analyzing this divers lipid class is a very challenging task. Therefore, hyphenation of modern chromatographic techniques and mass spectrometric (MS) detection and software assisted data processing is a promising tool for CL characterization.

In order to simulate the occurrence of CL oxidation products, CL standards were oxidized by means of Fenton reaction conditions (H_2O_2/Fe^{2+}). Additionally, formed hydroperoxides were reduced by triphenylphosphine (TPP) to form hydroxides. After successful optimization of reaction conditions, the CL species were separated by reversed-phase high-performance liquid chromatography (RP-HPLC) using a phenyl hexyl stationary phase. Although chromatographic behavior of these oxidation products was quite similar, the retention order depends on the number of functional groups and not on the total number of oxygen atoms.

Using an Orbitrap MS after negative electrospray ionization, the CL species were identified by exact masses. The structural characterization was performed using a linear ion trap mass spectrometer by means of data dependent sequential (MS3) fragmentation experiments. Based on specific fragments the identity of various species and the oxidation state of unsaturated acyl chains of different species was successfully determined.

Elucidation of double bond positions in lipids by means of tandem MS

Viola Jeck[1],
Heiko Hayen[1]

[1] Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany, viola.jeck@wwu.de

P 10 PRICE

Lipids are a group of biomolecules with a broad variety of chemical structures, this makes them suitable for various kinds of tasks in a number of different organisms. Double bonds have an influence on lipids' chemical, biochemical and biophysical properties. Since localization of double bonds in lipids is still a difficult task regarding their structural diversity and possible complexities of available mixtures, the development of a new method addressing those challenges is required.

This work presents two approaches, taking advantage of chemical characteristics of respective double bonds. The first method is based on the photochemical reaction taking place between olefins and excited carbonyl compounds. The so-called Paternò Büchi reaction was carried out using acetone as reactant which binds to the double bond forming an oxetane ring.

The second method, basing on the double bonds' ability of being easily oxidized, was carried out by electrochemistry. Electrochemistry, a valuable and widely used tool for metabolism studies and drug discovery, is commonly used for oxidative and reductive experiments simulating metabolic pathways of relevant compounds. As for this application, the oxidative conditions were used to oxidize lipids' double bonds.

Respective products of each approach were further investigated by means of tandem mass spectrometry. By applying an appropriate energy, diagnostic fragments are formed which enable pinpointing lipids' double bond position.

Algorithm development for lipid identification in Chlamydomonas reinhardtii algae samples utilizing LC-HRMS

Ansgar Korf[1], **Christian Vosse**[1],
Heiko Hayen[1]

[1] Insitute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany, ansgar.korf@uni-muenster.de

P 11 PRICE

Recent technical advances regarding liquid chromatography and high resolution mass spectrometry enable the mapping of the lipidome of an organism with short data acquisition times and without resource and time consuming derivatization steps. However, the interpretation and evaluation of the resulting multidimensional datasets are challenging and are still the bottleneck regarding overall analysis times. Therefore, a set of tools for a computational analysis of the lipidome has been developed, using the Kendrick mass defect for graphical analysis and visualization of the results.

Most occurring lipid classes (digalactosyldiacylglycerol, diacylglyceroltrimethylhomoserine, monogalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol) and species in the green alga Chlamydomonas reinhardtii (C. reinhardtii) wild type have been identified by exact mass database matching and isotopic pattern comparison. The results have been mapped in a three-dimensional Kendrick mass defect plot. Mass spectrometry datasets have been pre-processed utilizing an optimized MZmine 2 workflow for feature list generation. Features have been annotated by database matching, using a self deployed exact mass database. Isotopic features have been grouped using in house developed Java code. The developed grouping algorithm calculates theoretical isotopic patterns for by exact mass annotated features of potential lipid species. In a next step, the algorithm searches for possible isotopic features and compares the retention time and the features intensities. If an isotopic features' retention time and intensity are in a set window, features' are grouped and annotated as an identified lipid species.

Using the developed analysis tools in combination with an optimized MZmine 2 workflow improved the identification of various lipid species of the four most occurring lipid classes in C. reinhardtii.

Adipose Tissue Lipidomics Atlas

Mike Lange[1], **Zhixu Ni**[1],
Georgia Angelidou[1], Maria Fedorova[1]

[1] Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, mike.lange@uni-leipzig.de
[2] Center for Biotechnology and Biomedicine, Universität Leipzig, Germany

P 12 PRICE

Obesity has reached epidemic proportions in modern societies with a prevalence of more than 20 % of the population and has been recognized as a risk factor for numerous metabolic disorders including type 2 diabetes, cardiometabolic, liver and renal diseases. Onset and disease progression is closely associated with metabolic reconfiguration of white adipose tissue (WAT), an important endocrine, paracrine and autocrine organ crucial for lipid storage and metabolism. Changes in WAT lipidome have been closely associated with pro-inflammatory signaling, adipocyte hypertrophy and membrane remodeling upon increased caloric uptake. WAT lipidomics is challenging due to the high abundance of triglycerides (TG) (up to 95 %) masking remaining lipids including sphingomyelins (SM), phosphatidylcholines (PC), cardiolipins (CL), lysophosphatidylcholines (LPC), free fatty acids (FFA) and very small amounts of acidic phospholipids. However, to understand the role of lipidome remodeling in obesity development and progression it is important to characterize the global WAT lipidome which can be used as a reference for further targeted and untargeted lipidomics studies.

In order to create a human WAT lipidomics atlas a combination of several fractionation, separation and MS analysis techniques were used. Differential solid phase extraction (dSPE) protocol was optimized for initial fractionation of lipid classes which were further separated using two dimensional chromatography (HILIC/RP) and detected using data-dependent acquisition on QTOF-MS instrument. WAT lipidome was identified using in-house developed open-source software LipidHunter (https://bitbucket.org/SysMedOs/lipidhunter) [1].

1 Z. Ni, G. Angelidou, M. Lange, R. Hoffmann, M. Fedorova. LipidHunter identifies Phospholipids by High-Throughput Processing of LC-MS and Shotgun Lipidomics Datasets. Analytical Chemistry, ahead of Print, 2017

Development of a flow injection-high resolution MS method to analyze and quantify the lipid composition of neutral lipid rich samples

Marcus Höring[1],
Sabine Schweizer[2], Josef Ecker[2], Gerhard Liebisch[1]

[1] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital of Regensburg, Germany, marcus.hoering@ukr.de
[2] Institute of Nutritional Science, Technical University of Munich, Germany

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Excess lipid storage is associated with diseases such as metabolic syndrome, which results from complex interactions between genetic and environmental factors. Detailed lipid profiles of samples derived from patient studies and animal models may provide further insight into physiology and pathophysiology of fat storage, mobilization and metabolism. However, high quality data of polar lipids may not be generated by direct lipidomic methods for sample types like adipose tissue and fecal samples due to their high amount of neutral lipids (mainly triglycerides (TG)).

Therefore we developed a three-step lipid extraction method to improve the detection and quantification of polar lipid classes in neutral lipid rich samples. First, total lipid extracts were prepared according to the protocol by Bligh and Dyer (B/D). This extract is used for analysis of neutral lipid classes. To remove the excess of neutral lipids the B/D-lipid extracts were washed in a second step with an apolar organic solvent. In a third step, the washed lipid extracts were cleaned by a second B/D-extraction. Both lipid extracts were analyzed by electrospray flow injection analysis high resolution mass spectrometry (ESI-FIA-HR-MS). Subsequently, data evaluation needs to be done, which includes species assignment, several isotopic correction steps (13C and sodium), quantification and visualization. In a pilot experiment we could compare the polar lipid profiles of different mouse adipose tissue samples: inguinal white adipose tissue (iWAT), epididymal white adipose tissue (eWAT) and interscapular brown adipose tissue (iBAT). Thus, we could show that the triglyceride and polar lipid species profiles of iBAT are significantly different in comparison to white adipose tissue samples.

In summary, ESI-FIA-HR-MS offers a high throughput method to analyze and quantify lipid species profiles of different biological samples. The three-step extraction of TG-rich samples can improve the detection of polar lipid classes.

High-throughput quantitative analysis of lipid mediators for platelet activation

Bing Peng[1],
Sascha Geue[2], Patrick Münzer[2], Oliver Borst[2], Robert Ahrends[1]

[1] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany, bing.peng@isas.de
[2] Medizinische Klinik III, Kardiologie und Kreislauferkrankungen, Universitätsklinikum Tübingen, Otfried-Müller-Straße 10, 72076, Tübingen, Germany

P 14 PRICE

Platelets are the central building block of coagulation and homeostasis and their aggregation process is regulated by a network of signal transduction pathways. Pathway markers, which are part of lipid mediators, are generated from the oxidation of polyunsaturated fatty acids (arachidonic acid and eicosapentaenoic acid). Previous studies presented that prostaglandins, one class of mediators, are potent inhibitors of platelet aggregation, while 12-HETE showed both platelet pro- and anti-aggregate activity. This study is aiming to provide a rapid quantification for the main lipid mediators and to investigate the roles and contribution of these bioactive lipid mediators in platelet activation and function. Platelets of mice were treated with multiple activation approaches by thrombin, or collagen related peptide, or the combination of both. After platelet activation, both the pellets and supernatants of each condition were analyzed for a comprehensive profile of mediator distribution. In conclusion, we established a reliable and rapid approach to analyze a wide range of mediators. The workflow includes liquid-liquid extraction, high performance liquid chromatography-tandem mass spectrometry, and high-throughput data analysis.

SIMPLEX: from extraction to the biology of caveolin-3

Cristina Coman[1],
Andreas Roos[1], Evelyn Rampler[2], Gunda Koellensperger[2], Robert Ahrends[1]

[1] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany, cristina.coman@isas.de
[2] Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna

P 15 PRICE

"Caveolinopathies" are caused by mutations in the sarcolemmal protein Caveolin-3 and cover a group of autosomal inherited muscular disorders with a broad range of clinical severity ranging from limb-girdle-muscular to hyperCKemia dystrophy and cardiomyopathy. 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 apply SIMPLEX (SImultaneous Metabolite, Protein, Lipid EXtraction procedure) which offers a fundamental turn in study design, since multiple molecular classes can be accessed in parallel from one sample with equal efficiency and reproducibility.

The cardiac muscles from a transgenic TgCav3P104L mouse model presenting the limb-girdle pattern and cardiomyopathy as well as the wild-type littermates were subjected to the SIMPLEX workflow. In brief the tissue was incubated with cold MeOH, MTBE was added and water was utilized to induce phase separation. The individual fractions containing lipids (top phase), metabolites (lower phase) and proteins (pellet) were then subjected to the individual omics workflows. Here we demonstrate how (i) to investigate the interlinked proteome, lipidome and metabolome at the systems scale, and (ii) to study how a Cav3 missense mutation influences the heart metabolism of mice.

Application of this method in mass spectrometry based workflows allowed the simultaneous quantification of lipids, metabolites and proteins from cardiac muscle samples. The versatility of this method is shown in the transgenic Cav3 mutants, where strong metabolic shift towards a Duchenne like phenotype at the protein, lipid and metabolite level was revealed. Thereby we proved the robustness of the SIMPLEX approach to investigate interconnected systems in tissues and pointed out that SIMPLEX provides a superior strategy compared to conventional workflows and thereby allows profound insights into pathophysiological processes such as the molecular basis of cardiomyopathy.

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

Christiane Jung[1],
Marcus Höring[1], Hans-Frieder Schött[1], Gerhard Liebisch[1]

[1] University Hospital of Regensburg, Christiane.Jung@ukr.de

P 16 PRICE

The effect of the gastrointestinal system in health and disease is a factor that should not be underestimated. Obviously the intestinal microbiome seems to play a key role in human metabolism. Faecal materials reflect the microbial activity and the analysis of remaining unabsorbed metabolites including lipid species provides an estimate of metabolic interaction between gut microbiota and host. Up to now only few reports of the faecal lipidome exist due to the lack of suitable methodology.

Therefore, new mass spectrometric methods for identification and quantification of faecal lipid species should be established. Homogenized faecal samples were extracted according to the protocol of Bligh and Dyer. Crude lipid extracts were analysed by electrospray flow injection analysis high resolution mass spectrometry (FIA-HRMS). Although, the amount of faecal sample corresponds to the same dry weight we observed a high diversity in the mass spectra between different faecal samples. Using both accurate mass and MS/HRMS we could identify a number of glycerophospholipids, sphingolipids and neutral lipids including triglycerides, diglycerides and other classes typically observed in microbiota. Interestingly, in majority of samples high amounts of triglycerides (TG 52, TG 54) and diglycerides (DG 34, DG 36) were detected including highly unsaturated species.To get more insight into polar lipid species, TG content was reduced by washing the lipid extracts with isooctane. This way we could significantly increase the intensity of polar lipid species.

In the next step we will establish quantification of faecal lipid species by FIA-HRMS. Due to the complexity of the samples, additional separation techniques like hydrophilic interaction liquid chromatography will be coupled to MS to increase the specificity and sensitivity of identification. The application of these methods in various samples should provide a comprehensive picture of the faecal lipidome and contribute to our understanding of the role of the microbiome in human health.

Comprehensive analysis of the platelet lipidome reveals the central role of key lipids in activation and aggregation

Robert Ahrends[1], **Oliver Borst**[2],
Bing Peng[1], Sascha Geue[2], Cristina Coman[1], Patrick Münzer[2], Dominik Kopczynski[1], Florian Lang[3], Albert Sickmann[1], Meinrad Gawaz[2]

[1] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany, robert.ahrends@isas.de
[2] Department of Cardiology and Cardiovascular Medicine, University of Tuebingen, Germany
[3] Department of Physiology, University of Tuebingen, Germany

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Platelet integrity and function critically depend on lipid composition. We therefore examined the lipidome of murine platelets, using lipid-category tailored protocols. The development of a quantitative lipidomics platform allowed the establishment of the first quantitative description of the platelet lipidome which comprises almost 400 lipid species and covers a concentration range of seven orders of magnitude. The quantitative lipidomics platform presented here permits, for the first time, a systematic assessment of the lipidomics network in resting and activated murine platelets, indicating the feasibility of comprehensive absolute and differential quantitative lipidome analyses from isolated platelets. Lipids with low abundance in resting platelets, such as 12-HEPE with only five hundred molecules per cell, were quantified simultaneously with lipids of high abundance, such as cholesterol, with 0.1 billion molecules per cell. Using our established protocol, it was further shown that sphingomyelin phosphodiesterase 1 (Smpd1) deficiency (smdp1-/-) resulted in a very specific modulation of the platelet lipidome with an order of magnitude up-regulation of lyso-sphingomyelin, and subsequent modification of dense-granule release and platelet aggregation. In conclusion, this first comprehensive quantitative lipidomic analysis of blood platelets sheds light on novel mechanisms important for platelet function, and has the potential to open up novel diagnostic and therapeutic opportunities.

Lipid Profile as Diagnostic Marker for Antimycobacterial Treatment Monitoring

Hande Karaköse[1], **Nicole Zehethofer**[1],
Julius Brandenburg[1], Michael Weinkauf[1], Verena Scholz[1], Stefan Niemann[1], Norbert Reiling[1], Christoph Hölscher[1], Kerstin Walter[1], Dominik Schwudke[1]

[1] Research Center Borstel, 23845 Borstel, Germany, hkarakoese@fz-borstel.de

P 18

A fast and reliable diagnostics of tuberculosis (TB) remains as an urgent need in pre-clinical and clinical trials of TB. Current methodologies for TB detection lacks sensitivity as in sputum smear test or requires weeks for diagnostic results as in culture methodology test. There are currently no markers available for reflecting antibiotic efficacy and completion of the therapy by clearance of MTB at site of infection. Therefore, by using lipidomics approach, we investigate lipid molecules that can serve as diagnostic markers during TB therapy.

In our shotgun lipidomics analysis of MTB genotypes of Haarlem, Beijing, Uganda, EAI, WA2 and Canetti, Phosphatidylinositol (PI) (35:0) was found to be the most abundant lipid specie within all genotypes. In negative ion mode, PI (35:0) fragments to fatty acids of FA (16:0) with m/z 255.2, FA (19:0) with m/z 297.3 referred as tuberculostearic acid (TSA) and to PI head group with m/z 241.0. We screened several biomaterials such as plasma, plasma peripheral blood mononuclear cell, cell cultures from mouse and human origin and targeted PI (35:0) as a novel marker molecule to count MTB in cell culture system avoiding the time consuming CFU determination.

We can show that 10,000 MTB are detectable with this approach. The currently applied MS method is sensitive enough to detect PI and TSA in presence of $3.0 \cdot 10^5$ macrophages infected with 10^5 bacteria with no false positive results from 36 cultures.

Furthermore, in our lipid analysis with MTB infected IL-13TG and Wildtype mice (IL-13WT), we can show linear correlation between PI (35:0) amounts and CFU level in lung homogenates.

The analysis of PI (35:0) by ESI/MS is a promising approach to empower TB research in cell and animal models. We will further investigate how antibiotic quantitation and quantification of PI (35:0) can be integrated into one workflow.

LipidCreator: a powerful Tool for Targeted Lipidomics

Dominik Kopczynski[1],
Bing Peng[1], Nils Hoffmann[1], Robert Ahrends[1]

[1] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany, dominik.kopczynski@isas.de

P 19

During the past decade, the analysis of lipids gained more and more importance due to their involvement in cellular functions such as membrane building blocks, energy storage, or signaling. However, this research field still lacks sophisticated tools covering multiple steps of computational lipidomics. Here, we present LipidCreator, a novel and powerful kick-off tool for targeted lipidomics which is the first tool for easy providing of transition lists and in-silico created spectral libraries for lipid annotation and analysis. In total, five lipid categories (namely glycerolipids, glycerophospholipids, sphingolipids, mediators, and cholesterols) are provided containing about 70 of the most important lipid classes in eukaryotic organisms. A large set of lipid fragment ions derived from authentic standards or literature were integrated. LipidCreator is the first tool computing spectral libraries to validate targeted lipidomics results. These libraries are not only used for SRM / PRM data inspection but also will be the base for further data independent acquisition (DIA) analysis. On top, a user-friendly and intuitive graphical user interface is provided, where the user can easily select and assemble favored lipids. In conjunction with Skyline, LipidCreator improves all steps in targeted lipidomics such as transition calculation, parameter optimization, data integration as well as validation [1]. The recently introduced nomenclature of lipid structures [2] was utilized and is consecutively enhancing for MS2 annotation for LipidCreator. Taking advantages of the powerful analysis tool Skyline [3], LipidCreator can be launched either standalone or directly connected via Skyline as an external tool with the advantage to transfer all data directly back into Skyline. Therefore, it fills the gap of initial data acquisition in targeted lipidomics and is designed to be compatible with the established state-of-the-art tools such as Bibliospec and Panorama.

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LipidHome

Nils Hoffmann[1],
Fadi Al Machot[2], Dominik Schwudke[2], Kenneth Haug[3], Claire O'Donovan[3], Robert Ahrends[1]

[1] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany, nils.hoffmann@isas.de
[2] Bioanalytical Chemistry, Research Center Borstel - Leibniz-Center for Medicine and Biosciences, Parkallee 1-40, 23845 Borstel
[3] EMBL-EBI Hinxton, Metabolomics, Wellcome Genome Campus, CB10 1SD, United Kingdom

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Lipidomics as a discipline has seen a steady increase in research output throughout the last decade. With the advent of high-throughput lipidomics platforms based on high resolution mass spectrometry, the need for a central, well connected and comprehensive resource for both experimental and computational scientists has increased.

With Lipid Compass, we want to simplify the exploration of the lipidomic structural space from different angles, following the structural hierarchy, as established by LipidMaps and the proposed extension to further levels based on high-resolution MS technologies, as proposed by Liebisch et al. [1], which has been implemented in the LipidHome database for theoretical lipids [2]. Based on the hierarchy of in-silico lipid structures for two main lipid categories in LipidHome, currently Glycerolipids and Glycerophospholipids, we plan to extend the available categories with further lipid categories in the near future.

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

To simplify the submission of experimental data and thus evidence for theoretical lipids in the database, we will integrate with the data deposition workflow established by MetaboLights [3] using mzTab as an additional format for summary and detail information on lipids.

Lipid Compass will furthermore be an integration point for multiple lipid-related web services, such as LUX Score and LipidXplorer as part of the Lipidomics informatics for life-science (LIFS) project [4].

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Search for MDA modified phospholipids

Juliane Weisser[1], **Kristaps Klavins**[1],
Bettina Guertl[1], Elena Sokol[2]

[1] CeMM – Research Center for Molecular Medicine of the Austrian Academy of Sciences, kklavins@cemm.oeaw.ac.at
[2] Thermo Fisher Scientific

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Malondialdehyde is one of the most abundant lipid peroxidation product. This highly reactive compound is generated when a cell experiences oxidative stress, and can in turn cause additional damage by covalent attachment to biomolecules. The two aldehyde groups of MDA enable reactions with amino groups of proteins and other biomolecules. Protein adducts derived from MDA are highly immunogenic and are recognized and neutralized by several different effectors of the innate immune system. While proteins are the more prominently described target of MDA-derived modifications, in theory, MDA modifications could occur on head groups of phospholipids containing primary amino groups e.g. phosphatidylethanolamines (PE) and phosphatidylserines (PS). To date, however, no extensive studies have been performed on assessing these type of lipid modifications.

In this work, we searched for MDA modified PE species in apoptotic cell samples using lipidomics workflow based on reversed phase and HILIC separations combined with Orbitrap Fusion Lumos MS detection and employing LipidSearch and Skyline for the data processing. First, we generated MDA lipid adduct in vitro using PE 18:1/18:1 as a model lipid. The CID and HCD fragmentation pattern as well as the chromatographic retention behaviour of MDA adducts were characterized with our lipidomics workflow. Afterwards, we used UV-irradiated Jurkat cells as a model system for the generation of MDA-derived adducts in vivo. After UV irradiation with 100 mJ/cm^2 cells underwent apoptosis for 16 h. The theoretical masses of the MDA adducts for the most abundant PE species identified in control samples were calculated and screened in apoptotic cell samples. Despite the fact that we could successfully generate MDA-derived modification in vitro, this type of modification was not detected in apoptotic cell samples.

SELFI: a meta platform for lipid identification

Canan Has[1], **Robert Ahrends**[1]

[1] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany, canan.has@isas.de

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Untargeted mass spectrometry based lipidomics enables identification and quantification of lipid molecules in a given cell without any specific monitoring of certain lipid classes. Two main strategies have been performed in untargeted MS pipelines; shotgun lipidomics and LC-MS/MS based lipidomics. While shotgun lipidomics aims at identifying lipid species by direct injection of lipid mixtures to mass spectrometer, liquid chromatography coupled tandem mass spectrometry is considered as a powerful technique to analyze low abundant lipids. The fast and accurate identification and quantification of high-throughput spectra remains challenging in untargeted MS-based lipidomics. Several open-source algorithms like LipidXplorer[1], LipidHunter[2], Greazy[3] and commercial algorithms such as LipidSearch have been released [4] to perform lipid identification based on untargeted measurement strategy.

Here, in this work, we aim to generate a meta search platform which will enable (1) spectra conversion from raw input files to mzXML, mzML standard input files (2) pre-processing of spectra by PeakStrainer algorithm to remove spurious peaks in order to decrease false-positive identifications as well as runtime (3) usage of multiple open source search engines such as LipidHunter, LipidXplorer and Greazy. As shown previously for MS-based proteomics, consensus identification that combines results of multiple identification algorithms yields higher sensitivity and specificity. With the aid of this platform, users will run multiple algorithms with the same shared settings such as precursor and fragment ion tolerance on the input spectra simultaneously. The results by these engines will be combined by the platform as taking the common majority vote.

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3 Kochen, M.A., et al.. Greazy: Open-Source Software for Automated Phospholipid Tandem Mass Spectrometry Identification. Anal Chem, 88(11): 5733-41, 2016

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Comprehensive lipidomics analysis using Orbitrap Fusion Lumos MS

Bettina Guertl[1],
Elena Sokol[2], Hatoon Baazim[1], Kristaps Klavins[1]

[1] CeMM – Research Center for Molecular Medicine, kklavins@cemm.oeaw.ac.at
[2] Thermo Fisher Scientific

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Recent advances in high-resolution, accurate-mass spectrometry have increased sensitivity and speed for analysis of a variety of lipid species in complex biological samples. Especially the state of the art Orbitrap Fusion Lumos mass spectrometer offers unique possibilities that significantly improves confidence for lipid identification. Besides the extensive parallelization of mass analysis, Orbitrap Fusion Lumos provides multiple dissociation techniques including HCD and CID to enable MSn capabilities. However, in order to ensure reproducible and reliable analysis it is important to understand how different mass analysis and dissociation techniques influence obtained results.

In this work, lipids in the bovine liver lipid extract (purchased from Avanti Polar Lipids, Inc.) were analysed employing reversed phase separation combined with Orbitrap Fusion Lumos MS detection. Several MS method parameters were tested including polarity switching, Orbitrap detection, ion trap detection, ddMS2 with CID and HCD, ddMS3 with CID and HCD, and MS analysis with inclusion list. The obtained data were processed with LipidSearch and several parameters including mass accuracy, signal intensity, points per peak, and number of identified lipid species were compared. Based on the results the measurement method comprising inclusion list, polarity switching, CID ddMS2 for phospholipids, and HCD ddMS3 for glycerolipids was generated. This method provided optimal performance in terms of throughput and number of identified lipid species. As a matter of fact, using this method more than 800 lipid species were identified in mouse serum samples and significant changes in lipid levels between different biological conditions were observed.

Lipidomes of human lung tissues and lung tumors change with histopathological phenotypes and the lifestyle of patients

Lars F. Eggers[1],
Julia Müller[2], Verena Scholz[1], Henrik Watz[3], Christian Kugler[4], Klaus F. Rabe[4], Torsten Goldmann[2], Dominik Schwudke[1]

[1] Research Center Borstel, Division of Bioanalytical Chemistry, Parkallee 1-40, 23845 Borstel, Germany, leggers@fz-borstel.de
[2] Pathology of the University Hospital of Lübeck and the Research Center Borstel, Clinical and Experimental Pathology, 23538 Lübeck and 23845 Borstel, Germany
[3] Pulmonary Research Institute at LungenClinic Großhansdorf, Wöhrendamm 80, 22927 Großhansdorf, Germany
[4] LungenClinic Großhansdorf, Wöhrendamm 80, 22927 Großhansdorf, Germany
[5] Airway Research Center North, German Center for Lung Research, Wöhrendamm 80, 22927 Großhansdorf, Germany

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Lipid metabolic processes were linked to respiratory diseases including chronic obstructive pulmonary disease and lung cancer. A pilot study [1] comprising lipidomes of 43 tissue samples showed distinct alterations between lung tumor tissues and matched normal alveolar lung tissues. Particularly phosphatidylglycerol characterized alveolar lung tissues most likely due to its presence in the pulmonary surfactant, a lipid/protein complex lining the alveoli. The heterogeneity of tissue samples was accounted by a modelling approach based on partial-least-squares regression (PLSR) and showed distinct lipidome alterations with regard to the histopathological phenotypes such as vital tumor cells, necrosis and stroma. The presented study aims to further investigate potential parameters influencing the lipidome composition of human lung tissues by an advanced PLSR modelling strategy.

Lipidomes comprising 350 species from 14 classes of human lung tumor tissues and normal alveolar tissues of 92 patients were analyzed together with characterizations of the histopathological phenotypes and clinical parameters. PLSR models were used to evaluate different tissue lipidome influencing parameters.

Specific changes between non-small-cell-lung-cancer (NSCLC) tissues and carcinoid tumors were found in their ceramide metabolism. We were able to identify lipids differentiating adenocarcinomas (ADC) and squamous-cell carcinomas (SCC), the main fractions of NSCLC. The tissue composition, in particular the content of necrosis and metabolically active tumor cells, showed direct correlations to the lipidome composition. Necrosis was associated with triacylglycerols whereas vital tumor content correlated to phosphatidylcholines and phosphatidylethanolamines. Alveolar lipidomes from patients with ADC and SCC diagnosis were distinct, indicating fundamental lipid metabolic changes in lung cancer. We found indications for age dependence of alveolar lipidomes. Further, correlations with gender were indicated.

In summary, our study evaluates numerous parameters that affect the lung lipidome. Investigation of specific lipids responsible for the development of pathologies opens up great potential for personalized therapeutic strategies in precision medicine.

1 Lars F. Eggers, Julia Müller, Chakravarthy Marella, Verena Scholz, Henrik Watz, Christian Kugler, Klaus F. Rabe, Torsten Goldman .
Lipidomes of lung cancer and tumour-free lung tissues reveal distinct molecular signatures for cancer differentiation, age, inflammation, and pulmonary emphysema. Scientific Reports, 7, 2017

A novel tool for the analysis of lipidomes homology based on LUX score

Fadi Al Machot[1],
Dominik Schwudke[1]

[1] Research Center Borstel, falmachot@fz-borstel.de

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The importance of lipids in health and disease, initiated a number of technological advances to improve lipidome analyses of cells, tissues and organisms. Recently, we developed the LUX score approach to determine the homology between lipidomes solely based on lipid structures [1].

Until now the computation of the LUX score suffered from the following problems, (a) it supports only Linux using command line approach which is not a user friendly environment, especially for non-experts, (b) it is slow, which makes processing of complex lipidomes not a feasible task, and (c) it does not support the error modeling functionality.

Consequently, we developed a user friendly version including a guided installation routine and provide a graphical user interface. Moreover, we implemented the error modeling functionality into the software workflow to integrate robustness evaluations into the general output.

The new version of the LUX score is able to process lipidomes of different sizes and complexity much faster. We tested LUX score on already published datasets using lung lipids [2], Drosophila lipids and Yeast lipids. The benchmark results showed improved processing times by factor of 2,8 for Yeast, factor of 20 times faster for Drosophila and a factor of 102 for the human lung lipidome.

The software runs under Linux and Windows operation systems using state-of-the-art methods in software engineering. The LUX score approach can now be utilized to study large lipidome datasets to advance our understanding of lipidome composition in functional context of cell differentiation and disease development.

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2 Lars F. Eggers, Julia Müller , Chakravarthy Marella, Verena Scholz, Henrik Watz, Christian Kugler, Klaus F. Rabe, Torsten Goldmann , Dominik Schwudke. Lipidomes of lung cancer and tumour-free lung tissues reveal distinct molecular signatures for cancer differentiation, age, inflammation, and pulmonary emphysema. Scientific Reports, 2017

LESA^{PLUS} enables an improved in-depth analysis of the lipidome in comparison to the traditional LESA (Liquid Extraction Surface Analysis) approach.

Andreas Wiesner[1],
Daniel Eikel[2], Reinaldo Almeida[1]

[1] Advion Ltd., Harlow, UK, awiesner@advion.com, awiesner@advion.com
[2] Advion Inc., Ithaca, NY, USA

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Spatial lipid composition, distribution and regulation are very important factors for mediating lipid functionality and, when disrupted, can cause pathophysiological processes leading to cancer, obesity, atherosclerosis, and neurodegeneration.

The LESA^{PLUS} (LESA = Liquid Extraction Surface Analysis) approach combines the standard liquid extraction surface analysis with an additional step of a nanoliquid chromatography separation. This combination is ideally suited to investigate lipids, small molecule drugs or metabolites from thin tissue sections and here, we make a comparison between LESA^{PLUS} and LESA in the analysis of lipids from mouse brain.

Standard liquid extraction surface analysis allows for a rapid sample analysis from a multitude of locations across tissue sections. For a shotgun lipidomics analysis approach, a 5 minutes infusion experiment provides sufficient time to investigate lipid composition in detail. However, due to the sample complexity and matrix involved, some minor components may show a signal intensity insufficient for detailed MS analysis. In those cases, LESA^{PLUS} adds an additional dimension of analyte separation and allows an improvement in both spatial resolution as well as analyte sensitivity. Furthermore, LESA^{PLUS} can separate isobaric lipid species and therefore, allows a more in-depth analysis of the lipidome.

The TriVersa NanoMate offers both modes of operation within the same automated nanoelectrospray source and enables rapid analyte screening for surface profiling as well as in- depth lipid analysis.

Lipidomic signature of extreme human longevity

Mariona Jové[1],
Kevin Huynh[2], Alba Naudí[1], Natalia Mota[1], Juan Gambini[3], Consuelo Borras[3], Jose Viña[3], Peter Meikle[2], Reinald Pamplona[1] and Irene Pradas[1]

[1] Department of Experimental Medicine, University of Lleida-Biomedical Research Institute of Lleida, Lleida, Spain, mariona.jove@udl.cat
[2] Baker Heart and Diabetes Institute, Melbourne, Vic., Australia.
[3] Department of Physiology, University of Valencia, Spain.

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Background: Human longevity is partially explained by genetic factors and metabolome, as the final step of cellular biochemical activity, reflexes the changes in gene expression and its interaction with the environment. Particularly, some lipid species and their characteristics have been previously described to be associated with animal longevity. Besides, human aging and longevity phenotypes are very heterogeneous and centenarians can be considered the best example of successful aging.

Purpose: Describe the plasma lipid profile of centenarians and define which lipid species are a signature of healthy aging.

Methods: Plasma samples from centenarian (n=29), octogenarian (n=30) and adult (n=30) subjects were analysed using a liquid chromatography-mass spectrometer platform. Univariate and multivariate statistics were used to describe which lipid species are altered in each experimental group.

Results: Principal component analysis and hierarchical clustering algorithm revealed the existence of a specific lipidomic signature in centenarian subjects conferring them a more oxidative stress resistance context, which could contribute to their healthy aging. This resistant signature involves lipids such as ceramides, gangliosides and glycerophospholipids species. Interestingly, among glycerophospholipids, all plasmalogen species detected in these samples had different values in centenarian and adult subjects respect to aged ones.

Conclusions: Extreme longevity phenotypes can be defined by their plasmatic lipid profile and some ceramides or lipid unsaturation can be used as biomarkers of longevity. Changes in centenarian plasma lipid species are focused on achieve an oxidative stress resistant phenotype.

Correspondence to: Mariona Jové (email: mariona.jove@udl.cat) or Irene Pradas (email: ipradas@mex.udl.cat)

Wnt6-induced signaling drives formation of triacylgylcerol-rich lipid bodies and promotes survival of Mycobacterium tuberculosis in macrophages.

Julius Brandenburg[1],
Dominik Schwudke[2], Sebastian Marwitz[3], Jochen Behrends[4], Martina Hein[4], Thomas Scholzen[4], Torsten Goldmann[3], Andreas Kispert[5], Michael Leitges[6], and Norbert Reiling[1]

[1] Division of Microbial Interface Biology, jbrandenburg@fz-borstel.de
[2] Division of Bioanalytical Chemistry,
[3] Division of Clinical and Experimental Pathology,
[4] Core Facility Fluorescence Cytometry, Research Center Borstel, Leibniz Center for Medicine and Biosciences, 23845 Borstel, Germany
[5] Institut für Molekularbiologie, Medizinische Hochschule Hannover, 30625 Hannover; Germany,
[6] The Biotechnology Centre of Oslo, University of Oslo, Oslo, Norway

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB),has developed multiple strategiesto survive within its main host cell, the macrophage. During pathogenesis of TB, lipid-laden foamy macrophages appear within the granuloma, a cell type which is thought to provide a favorable environment for the pathogen. Since we have observed that the Wnt ligand Wnt6 is mainly expressed by neutral lipid-rich cells within the lung of Mtb-infected mice, we hypothesized that Wnt6 is a novel, foamy macrophage-promoting factor during Mtb infection *in vivo*. In the current study, we demonstrate that Wnt6 over-expression in a cell line modulates expression of key regulatory metabolic enzymes, promotes triacylglycerols (TAG) accumulation, as well as formation of neutral lipid-rich cytoplasmatic organelles termed lipid bodies. Experiments in primary murine macrophages substantiate these findings by showing that *Wnt6*-deficiency affects fatty acid-induced formation of TAG-rich lipid bodies and expression of key metabolic enzymes during Mtb infection. Although bacterial uptake, phagosome acidification and nitric oxide production are not affected by the absence of Wnt6, we observe that Mtb´s ability to replicate within *Wnt6*-deficient macrophages is impaired. Notably, this growth defect in Wnt6-deficient macrophages could be rescued by addition of fatty acids or could be mimicked by inhibition of key metabolic enzymes, suggesting that these factors contribute to susceptibility of *Wnt6*-sufficient primary murine macrophages to infection. Indeed, we show that inhibition of distinct metabolic factors severely impairs Mtb growth also in human primary macrophages. Taken together, our data indicate that Wnt6 and downstream metabolic processes enhance the survival of Mtbin macrophages by promoting accumulation of TAG-rich lipid bodies and foam-cell formation.

Quantitative lipidomics reveals age-dependent perturbations of whole-body lipid metabolism in ACBP deficient mice

Sandra F. Gallego[1],
Richard R. Sprenger[1], Ditte Neess[1], Josch K. Pauling[1], Nils J. Færgeman[1], Christer S. Ejsing[1,2]

[1] VILLUM Center for Bioanalytical Sciences, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark, sandrafg@bmb.sdu.dk
[2] Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany

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The acyl-CoA binding protein (ACBP) plays a key role in chaperoning long-chain acyl-CoAs into lipid metabolic processes and acts as an important regulatory hub in mammalian physiology. This is highlighted by the recent finding that mice devoid of ACBP suffer from a compromised epidermal barrier and delayed weaning, the physiological process where newborns transit from a fat-based milk diet to a carbohydrate-rich diet. Although these hallmarks of ablating ACBP function are readily observed on the macroscopic level, we still know very little about the molecular underpinning that evokes these metabolic and physiological dysfunctions on the whole-body level.

To determine how loss of ACBP function affects lipid metabolism at the whole-body level we performed a comprehensive and comparative global lipidomics analysis of liver, skeletal muscle and plasma from Acbp knockout and wild type mice. The lipidomics analysis was executed on a high resolution Orbitrap Fusion mass spectrometer equipped with an automated nanoelectrospray ion source and afforded monitoring the absolute abundance of 613 distinct lipid species across the three different tissues. Our results reveal that ACBP deficiency affects primarily lipid metabolism of liver and plasma during weaning. Specifically, we find that ACBP deficient mice have elevated levels of hepatic cholesteryl esters, and that lipids featuring an 18:1 fatty acid moiety are increased in Acbp depleted mice across all tissues investigated. Our results also show that the perturbation of systemic lipid metabolism in Acbp knockout mice is transient and becomes normalized and similar to that of wild type as mice grow older.

These findings demonstrate that ACBP function is important for systemic lipid homeostasis in mice during weaning and that ablation of ACBP prompts lipid metabolic effects in liver, plasma and skeletal muscle, three highly important and centrally positioned tissues that govern whole-body lipid homeostasis.

Separation of lipid oxidation products by TLC – the advantages of reversed-phase TLC

Kathrin M. Engel[1],
Jürgen Schiller[1]

[1] University of Leipzig, Medical Faculty, Institute for Medical Physics and Biophysics, Leipzig, Germany,
kathrin.engel@medizin.uni-leipzig.de

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Oxidized lipids are of great interest because they play important roles in human health and disease. Their impact on cells is often studied using in vitro systems. Even though the demand on oxidized lipids is high, their commercial availability is limited. Therefore, they need to be synthesized in the lab by suitable approaches. These artificial oxidations (for instance by oxygen exposure or Fenton reaction) result in mixtures of different lipid oxidation products that can be divided into primary (long chain) oxidation products, such as peroxy, epoxy and hydroxy compounds, and secondary (short chain) oxidation products, such as lyso lipids, aldehydes and carboxylic acids. Aldehydes and carboxylic acids are generated by the scission of a double bond position. Identification and separation of these products are challenging even if defined lipids are oxidized and require a suitable chromatographic approach coupled to mass spectrometry (MS). There are many reports on the coupling of liquid chromatography (LC) to electrospray ionization (ESI). Nevertheless, thin-layer chromatography (TLC) coupled to MS is a promising alternative method that possesses several advantages compared to LC that will be explained in this presentation. Furthermore, the reversed-phase approach of TLC (RP-TLC), which has only been sparsely used so far, seems to be superior to normal phase TLC in the separation of lipid oxidation products according to their functional groups. It will be shown that dioxo-, dihydroxy- and mixed lipid oxidation products of unsaturated phosphatidylcholines such as PC 16:0/18:1 or PC 16:0/18:2 can be separated in a single step.

Targeted versus Untargeted Lipidomics

Ulf Sommer[1],
Therese Koal[1]

[1] Biocrates Life Sciences AG, ulf.sommer@biocrates.com

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In mass spectrometry based lipidomics research untargeted experiments are carried out using high-resolution, high-mass accuracy instruments measuring as many signals as possible but allow only relative quantitation, while targeted analyses are (semi)quantitative, usually performed at low resolution and on a limited number of annotated metabolites [1, 2]. We are discussing here two very different studies applying both approaches [3, 4], and the respective reasons to use a targeted and/or an untargeted approach.

Wolbachia bacteria have a protective effect on mosquitos against viral infections. Case 1 is a small FT-ICR MS based study on mosquito cells with and without Wolbachia infection. An extraction protocol had to be worked out and a number of unknown metabolites had to be identified. Significant changes were observed for whole lipid classes (including insect-specific ones), different chain lengths, and degree of saturation.

Case 2 is a study by the Alzheimer's Disease Neuroimaging Initiative; a total of 730 participants in three different cohorts were screened using fasting serum samples and the Biocrates AbsoluteIDQ-p180 kit (targeting 186 metabolites), and changes in sphingomyelins, ether-containing phosphatidylcholines and acylcarnitines could be observed as either disease-related or disease-progression-related changes.

Case 1 is a small-scale, explorative study on changes in a non-human system in which even large-scale targeted lipidomics systems would have missed important information. Based on the significant changes observed additional research is warranted. Case 2 is a large-scale study in several parts, based on a plethora of prior knowledge which required a validated, standardized (ring trial) and quantitative method to enable data comparison over several cohorts. The relatively high number of metabolites targeted by the kit makes an additional prior untargeted experiment less desirable.

While not every planned study might be that easily categorized, we hope that this discussion can guide the decision.

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Evidence that plasmalogen species evolve as diagnostic targets in vascular metabolic and neurodegenerative disease.

Gerd Schmitz[1],
Stefan Wallner[1]

[1] Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany, gerd.schmitz@ukr.de

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Plasmalogens contain vinylether bound triplets of 16:0; 18:0; 18:1 fatty acids (FAs) in sn-1 for each FA-species esterified in sn-2. Plasmalogen species decrease upon aging, vascular- and metabolic diseases, and AD.

Each cell type involved in vascular- and metabolic disease shows a cell type (Leidl,K. et al. BBA (2008) and differentiation dependent plasmalogen profile (Wallner, S. et al.Chem. Phys.of Lipids (2011). We found, that PUFA-PE P-species at sn-2 (20:4; 22:4; 22:5; 22:6) are high in monocytes, while shorter and less desaturated PE P-species at sn-2 (18:1; 18:2; 18:3) predominate in neutrophils. Differentiation of monocytes to macrophages increases PE P-species with 1; 2; or 3 double bonds in sn-2 and decreases 4-5 times desaturated PE P-species.(Wallner, S. et. al. PLOS ONE 2014) leading to a PE P-profile of macrophages similar to granulocytes, indicating terminal phagocytic differentiation and that PE plasmalogens discriminate the stage of monocyte-derived macrophage differentiation. Lipid loading of macrophages with eLDL and oxLDL showed a n-2-saturation dependent pattern of changes in treated cells. Especially eLDL strongly lowered saturated, mono-unsaturated and three-times unsaturated PE P-FAs in sn-2. oxLDL slightly lowered saturated species and increased mono- and double-unsaturates. Unloading with HDL3 only had minor effects on the plasmalogen pattern. Cells that were previously loaded with eLDL showed increases in sn-2 16:1 and 20:5 PE P species. HDL3 unloading after oxLDL loading led to decreases in sn-2 18:0, 16:1, 18:1, 18:2 PE P, as well as increases in sn-2 16:0 and 20:5 PE P (Wallner, S. et al. PLOS ONE 2017, submitted). Comparing PE P-species responses during differentiation of adipocytes, macrophages, granulocytes and lung maturation it is obvious that the SAFA-, MUFA- and PUFA-species show a unique cell specific increase in 1-2 double bond FAs and a decrease of SAFA- and PUFA-species in glycerophospholipids and plasmalogens which become dysfuctional in diabesity.

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