LIPIDOMICS FORUM 2024

1. - 3. September

Research Center Borstel, Leibniz Lung Center

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K1 *MYCOBACTERIUM TUBERCULOSIS* (MTB): THE PATHOGENIC GREASEBALL.

Author: David G. Russell

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Lipids and fatty acids feature extensively in the life cycle of Mtb. Mtb has evolved from saprophytic actinomycetes, and its free living relatives have genomes that are 50% larger (*M. smegmatis*; 6.3K genes, Mtb; 4.3K genes). Clearly genome downsizing has been significant, likely due to Mtb's "simpler" life style involving one major host species and no free-living stage. Therefore, logically, what it keeps is important to its success and upon sequencing the Mtb genome it was noted that lipid metabolism, synthesis and catabolism, was the most common single theme[1]. I'd like to discuss 3 areas where lipids play a critical role in the biology of this pathogen.

Cell wall lipids: Mtb cell wall is rich in lipidoglycans that have marked immunomodulatory activities. The activation of the innate immune sensing pathways of the host has major impact on both infection at the cell level and disease progression at the tissue level.

Bacterial nutrition: Atypically for a pathogen, Mtb exploits host fatty acids and cholesterol as its primary carbon sources *in vivo*. Limiting access to these nutrients by either genetic or pharmacological intervention suppresses bacterial growth within its host cell in culture and *in vivo*.

Host macrophage metabolism: While it was originally thought that all macrophages originated from blood monocytes this is now known not to be true. Tissues, such as the lung, are populated by self-renewing resident lineages, including alveolar macrophages, during embryogenesis. These macrophages are differentially-programmed epigenetically, and this is reflected in their metabolic bias towards OXPHOS and FAO. The metabolic states of these macrophage lineages is central to disease outcome *in vivo*.

This presentation will explore the biology of these host and bacterial lipid pathways and discuss their potential significance to therapeutic approaches to combat this disease, which, following COVID, is back as the most frequent cause of death by a single infectious agent.

1. Cole, S. T. *et al.* Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**, 537-544, doi:10.1038/31159 (1998).

K2 DETECTING BACTERIA IN COMPLEX BIOLOGICAL MATRICES USING METABOLIC BIOMARKERS

Author: Nicole Strittmatter

Affiliation: Technische Universität München, Garching, Germany

Fast and reliable identification of bacteria directly in clinical samples is the holy grail of microbiological diagnostics. Current approaches predominantly require time-consuming bacterial isolation and enrichment procedures, delaying stratified treatment. Here, we describe a biomarker-based, hierarchical strategy that utilises conserved bacterial small molecular metabolites and lipids for direct mass spectrometric detection of bacteria in clinical samples. Marker discovery was carried out by creating a metabolic library of 232 bacterial species and mining this library for markers showing specificity at various taxonomic levels. We have found 359 bacterial taxon-specific markers (TSMs) via a combination of univariate and multivariate statistical analysis methods. We applied the concept of TSMs to the *in situ* detection of bacteria directly from complex clinical specimens, using healthy and cancerous colorectal tissues as well as faecal samples. To show the method independent nature of these TSMs, samples were analysed using chemical histology as well as traditional metabolomics approaches. TSMs were found in >90% of samples tested, suggesting the general applicability of this novel workflow to detect bacterial presence with standard MS-based analytical methods.

K3 LIPIDS GO VIRAL: THE ROLE OF LIPIDS IN VIRUS REPLICATION

Author: Eva Herker

Affiliation: University of Marburg, Marburg, Germany

All intracellular pathogens rely on metabolic pathways of the host for infection and replication. In the case of positive-strand RNA ((+)ssRNA) viruses, host cell lipids are directly needed for formation of membranous replication organelles (ROs) and in the case of enveloped viruses for formation of the envelope. Additionally, viruses can induce mobilization and metabolization of lipids as building blocks and for energy that is needed for multiplication.

We performed shotgun lipidomics of cells infected with different flaviviruses including hepatitis C virus (HCV) that causes chronic infection and arthropod-borne orthoflaviviruses that cause acute infections. All viruses investigated induce profound lipid remodeling in infected cells. Altered lipid metabolic pathways were probed for mechanistic relevance using RNAi and specific inhibitors. These studies revealed that lipid remodeling by flavivirus infections includes distinct changes but also common patterns shared by several viruses that are needed for efficient infection and replication.

K4 - Sponsor Talk: COMPREHENSIVE DISCOVERY LIPIDOMIC WORKFLOW WHICH UTILIZES A PROTOTYPE, MULTI-REFLECTING TOF WITH INTEGRATED INFORMATICS, PROVIDING HIGHLY CONFIDENT LIPID CHARACTERIZATION AND QUANTIFICATION

Authors: Nyasha Munjoma (1), Lee Gethings (1), David Heywood (1), Paolo Tiberi (2), Laura Goracci (3), Jayne Kirk (1), Richard Lock (1)

Affiliations: 1 Waters Corporation, Wilmslow, UK; 2 Mass Analytica, Barcelona, Spain; 3 University of Perugia, Perugia, Italy

Lipidomics allows researchers to probe changes in the lipidome as a result of disease, treatment, lifestyle, etc. Analysis of these lipids in a discovery mode is normally performed by a combination of liquid chromatography (LC) and accurate mass spectrometry (MS). Despite developments in analytical technology the detection and identification of lipids remains a significant challenge. Here we show the key benefits of a novel, benchtop MS and the features it provides to help overcome some of the drawbacks outlined for lipid analysis. Combining this novel MS data with third-party informatic solutions, demonstrates a powerful lipidomic workflow. The benefits of this approach are demonstrated using plasma samples from colon and rectum cancer and healthy control plasma.

Lipid extracts originating from the standard mixes was first assessed to establish instrument performance. Data were acquired using either data dependent (DDA) or data independent analysis (DIA). Resolution and mass accuracy were initially evaluated, providing 100,000 (FWHM) and 500ppb respectively for each lipid component. Based on previous literature, the most commonly identified lipids from human plasma (based on the NIST standard) highlighted the reduction in false positive identifications following database searching due to the high mass accuracy provided. A dynamic range of 5-orders was routinely demonstrated, whilst data acquisition rates of 100Hz were utilized for the MS methods, providing the ability to run with faster gradient profiles. Cancer-based study samples were prepared using the same protocol described for the lipid standards. Data processing via third-party informatics was used for peak picking, data normalization and lipid identification. Statistical analysis involving a range of MVA tools showed clear differentiation between the cancer types and healthy controls. Identification of the differential markers responsible for the group separation, was conducted using a lipid-specific database, highlighting phosphocholines, sphingomyelins and ceramides as the main lipid classes.

K5 What can archaeal lipids tell us about the past, present and future?

Author: Nicole Bale

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A distinct characteristic of the Archaea is their unique membrane lipids that set them apart from the other two domains of life. Archaeal lipids contain ether linkages between glycerol and isoprenoid chains and certain archaea however produce membrane spanning lipids, comprising 40 carbon isoprenoid chains, commonly referred to as GDGTs. Archaeal GDGTs are ubiquitous lipids in both marine and terrestrial environments and are used extensively in geological and environmental studies.

One GDGT-forming phylum of archaea of particular interest to geochemists is Thaumarchaeota (recently renamed Nitrososphaerota). This globally distributed phylum makes GDGTs with 0 to 4 cyclopentane rings and a very specific GDGT called crenarchaeol which contains 4 cyclopentane rings and one cyclohexane ring. To date, crenarchaeol has been detected only in Thaumarchaeota and hence is considered to represent a specific biomarker for members of this phylum. In Thaumarchaeota the distribution of GDGT lipids depends on growth temperature, the basis of the widely applied TEX86 paleotemperature proxy. This paleotemperature proxy has been applied to estimate sea surface temperatures as far back as the middle of the Jurassic era (~160 million years ago). Paleotemperature reconstructions are essential for future climate modeling. Indeed, to better predict and understand our future with a warmer and rapidly changing climate we can reconstruct different environmental parameters over past climate fluctuations and sudden warming events.

It is not just the lipids of Thaumarchaeota that fascinate geochemists. We are also interested in a wide range of archaea including extremophiles, such as halophiles and thermophiles. Many of these produce distinct lipids that can serve as biomarkers in geological studies. Our ongoing research into archaeal lipidomes both in culture and in samples from the environment is providing insights into the ecology of archaea as well as related topics such as membrane adaptation and lipid biosynthesis.

Here I will present one such study into the archaeal symbiont *Ca.* Nanohaloarchaeum antarcticus, which is dependent on its archaeal host *Halorubrum lacusprofundi* for lipids. We explored the lipidome dynamics of the *Ca.* Nha. antarcticus – *Hrr. lacusprofundi* symbiotic relationship during co-cultivation. We did this using a comprehensive untargeted lipidomic methodology, which processes Orbitrap mass spectrometry using MZmine software and the GNPS platform. Our study revealed that *Ca.* Nha. antarcticus selectively recruits 110 lipid species from its host, i.e., nearly two-thirds of the total number of host lipids. Lipid profiles of co-cultures displayed shifts probably associated with changes in membrane fluidity and improved resistance to membrane disruptions, consistent with compensation for higher metabolic load and mechanical stress on host membranes when in contact with symbionts. Our work emphasizes the strength of employing untargeted lipidomics approaches to provide details into the dynamics underlying an archaeal symbiont-host system.

T1 Lipidomics Minimal Reporting Checklist

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The number of lipidomics experiments is rapidly growing in the previous 10 years and so does the frequency of published articles in scientific journals describing these experiments. However, many publications do not provide complete or transparent set of information to either comprehend, reproduce, or even reuse the data or results from these experiments. These reports may lack a controlled vocabulary, a clear structure, or even no access to the raw data. Another issue emerges, when non-lipidomics specialized journals send articles for peer review to non-domain experts for a sophisticated assessment of the described methods.

In a community-wide effort, we implemented an interactive lipidomics reporting checklist [1, 2] to overcome these limitations. The Lipidomics Standards Initiative (LSI) compiled guidelines for minimum reporting standards with the assistance of the lipidomics community of the International Lipidomics Society (ILS). The interactive checklist is a web-based questionnaire. Users can select several lipidomics methods such as *direct infusion* or *separation* to customize their report. The checklist guides the user through eight sections, i.e., *Overall study design*, *Preanalytics*, *Lipid extraction, Analytical platform, Lipid Identification & Quantification, Quality control, Method qualification and validation*, and *Reporting.* Each section demands a minimum set of information in order to continue. The resulting pdf report can further be, e.g., attached as supplementary to an article, and/or published separately via the Zenodo platform. This system intends to support analytical chemists to ensure that their provided data are of highest level as well as educate young scientists in this field to learn the crucial requirements within this discipline. The questions in the checklist provide profound explanations and link to literature for further details. The checklist is free of charge and can be used without any limitations.

[1] MacDonald, J.G., Ejsing, C.S., Kopczynski, D. et al. (2022). *Nature Metabolism*. **4**, 1086-1088.

[2] https://lipidomicstandards.org/

T2 Lipids in Eukaryotes – Definition of Core Lipidomes with Help of Lipid Compass

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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 metabolomics-platforms based on chromatography and high-resolution mass spectrometry, the need has increased for a central, well connected, and comprehensive resource for both experimental and computational scientists.

With LipidCompass, we want to offer a FAIR resource to simplify the exploration of quantitative and semi-quantitative lipidomics data from different angles and help establish a collection of core lipidomes for different organisms, tissues, and cell types, starting with eukaryotic model organisms. This is achieved using semantically enriched data, using controlled vocabularies and ontologies like NCIT, PSI-MS, NCBITaxon and lipid names following the structural hierarchy induced by the shorthand nomenclature for lipids. Lipidomics and Metabolomics tools that support mzTab-M as an output format can submit their data to LipidCompass. With automated mapping of lipid shorthand names to LIPID MAPS, SwissLipids and ChEBI, we open the possibility to integrate lipidomics data in a larger, systems biology context. LipidCompass provides comprehensive data exploration, comparison and interactive visualization features that simplify the detection of differences between samples within the same study, but, for the first time, also allow analysis and visualization of similarities and differences on a large scale between studies and to compare datasets against established qualitative and quantitative baselines.

LipidCompass will be the central integration hub for multiple lipid-related web services and can serve as a comprehensive resource for the establishment of core lipidomes, capturing the expected composition and structural variability of eukaryotic lipidomes in a first step. Further collaboration with the International Lipidomics Society interest groups on standardization and clinical lipidomics will integrate support with the upcoming lipidomics checklist.

A1 Utilizing High-Throughput Techniques and Computational Approaches for Lipid A Annotation

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Lipopolysaccharides are unique bacterial lipids and components of the outer membrane of Gram-negative bacteria. Lipopolysaccharides in the outer membrane provide membrane integrity, a mechanism for interaction between bacteria and other surfaces, and resistance to different antibiotics. They are also potential factors in the pathogenicity of Gram-negative bacteria. The three main parts that compose lipopolysaccharides are the membrane anchor, the core oligosaccharide, and the O-polysaccharide. Specifically, the membrane anchor, called Lipid A, can vary in structure, saccharide composition, and size among bacterial species and even individual strains. Its unique structure contributes to the outer membrane's properties, resistance to antibiotics, and activation of a pro-inflammatory response.

The central core of Lipid A is a glucosamine disaccharide with three to seven acyl chains (ester or amide bonds) attached to the N-acetylglucosamine sugars. Most Gram-negative bacterial strains exhibit some variation in Lipid A structure, including differences in the length and number of aliphatic chains and the location of acyl groups. Furthermore, Lipid A can vary in degrees of phosphorylation and the presence of substituents, such as phosphoethanolamine and 4-amino-4-deoxy-L-arabinose. Additionally, saturated, unsaturated, hydroxylated, and branched fatty acids have been described as constituents of the molecule, and these fatty acids can vary in number, acyl chain length, and degree of acylation.

Due to this structural complexity, analyzing Lipid A presents numerous challenges. Currently, the main methods for elucidating Lipid A structure are NMR and mass spectrometry. While mass spectrometry cannot provide absolute structural information about the target, the general components of the molecule can be detected and studied, allowing for comparative profiles. However, even this limited interpretation of the data requires manual annotation of full scan and fragmentation spectra, which strongly limits throughput.

To the best of our knowledge, no automatic tool currently exists to annotate Lipid A in mass spectrometry data. Only a few individual Lipid A species can be found in online databases and there is no consistent nomenclature that describes the variations in the structure of this complex group of molecules. Currently, individual Lipid A species are named after the bacteria in which they are found.

Here, we present an innovative approach to address the challenge of identifying and annotating Lipid A features from Liquid Chromatography-Mass Spectrometry and data-dependent tandem mass spectrometry measurements. We created a library containing the structures of 100 published Lipid A from a variety of biological backgrounds and used this library to identify unique structural features, such as the natural isotopic pattern, to identify additional and previously undescribed Lipid A structures in untargeted datasets. In addition, we established a comprehensive system to categorize Lipid A structures based on their structural characteristics, such as the number of hydroxyl fatty acids and fatty acid esters of hydroxy fatty acids, which will facilitate future annotation, interpretation, and comparison. Lastly, we developed a Python-based script that predicts putative Lipid A structures based on their accurate mass and natural isotope distribution.

The combination of our literature-based in-house library, comprehensive sorting system, and unique script for structural prediction will significantly advance Lipid A research.

A2 FAT IS LIFE: LIPID ACQUISITION BY MYCOBACTERIA DURING THEIR INTRACELLULAR LIFE

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Mycobacteria predominantly rely on host-derived lipids as carbon and energy sources during infection, but the dynamics of lipid acquisition during intracellular growth remains to be explored. While residing inside cells, mycobacteria can access various lipid sources depending on their location inside the MCV (mycobacteria-containing vacuole) or in the cytosol. In this study, we aim to elucidate those dynamics using *Dictyostelium discoideum*/*M. marinum* as an infection model system, offering a well-established platform to investigate the interplay between intracellular pathogens and cell-autonomous defense mechanisms. We first showed that the genetic knock-out of mycobacterial systems involved in the import (Mce transporters) and utilization (Facl6 and LipY for incorporation, Icl1 for detoxification) of lipids led to significant intracellular growth defects in *D. discoideum*, while their initial capacity to infect cells was not altered, indicating a limited ability to replicate. Lipid profiling by thin-layer chromatography highlighted an unbalanced metabolism for some mutants, notably the ones affected in lipid incorporation, with a defect in the production of neutral lipids and a slight increase in cell-wall associated lipids such as PDIM/PGL. Investigations by immunofluorescence microscopy revealed an altered intracellular location, with some being affected in their ability to properly establish their MCV. We then investigated lipid transfer from the host to the bacterium by following lipid droplet (LDs) dynamics. LDs accumulate in the vicinity of MCV early during the infection and are eventually translocated inside the MCV where they provide a source of nutrients for *M. marinum*. By high-content microscopy, we showed that LD clustering close to the bacteria was significantly reduced when *M. marinum* failed to damage their MCV (using a strain mutated in its region of difference 1 (ΔRD1), encoding the ESX-1 secretion system), while the total cellular LD content remains similar. On the contrary, when bacteria were able to escape earlier to the cytosol (in absence of autophagy repair and restriction), a larger LD accumulation was observed. By using a fluorescent fatty acid analog, we confirmed the reduced lipid transfer from the host to *M. marinum* ΔRD1, underlining that the ability of *M. marinum* to induce damage to its compartment is somehow correlated to lipid acquisition. Overall, these results suggest that the use of host-derived lipids by mycobacteria might represent a crucial step early in manipulating the phagocytic process leading to MCV genesis. Further investigations are ongoing to understand the precise mechanism of LD translocation inside the MCV.

A3 LAMB MUSCLE LIPIDOMIC PROFILES AND RUMEN MICROBIOME CAN BE BENEFICIALLY MODIFIED BY DIET

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Omega-3 fatty acids are polyunsaturated fatty acids found in foods and when consumed at optimal levels are reported to have health benefitting properties including promoting cardiovascular health and playing an essential role in cell structures and the inflammatory response. Rich sources of n-3 PUFA include oily fish, seeds, nuts, and brassica vegetables. There are two main avenues, which are responsible for altering the lipid profiles and fat content of meat products 1) genetics, and 2) diet of the animal. Grass is known to be rich in alpha-linolenic acid (C18:3 n-3), which is a known precursor for longer chain fatty acids, however, undergoes significant hydrogenation in the rumen, limiting its deposition in muscle. Increasing n-3 PUFA using feed supplements has limitations due to LC-PUFAs such as C20:5, C22:5 and C22:6 n-3 PUFA being toxic to rumen microbiomes. Microalgae is reported to have protective properties which allows the LC n-3 PUFA to bypass without being exposed and damages by rumen processes.

This study investigate how animal diet impact fatty acid composition, specifically omega-3 PUFA, and the concentration and distribution of lipid species across classes. The aim of this study was to investigate the effect of lamb diet on production performance, muscle lipid profiles and effect on rumen microbiome. At the start of the study, all the ruminal volatile fatty acids (VFA) were similar between the control and the microalgae treatment. At time of the slaughter propionic acid was the only VFA with significant difference between both treatments and the microalgae treatment lowered the propionic acid concentrations from 24 to 15 mmol/L. A methane reduction of 23% was estimated by microalgae treatment compared to control. The number of bacteria and archaea genera present in gastrointestinal samples during the study decreased similarly across the different treatments.

Dried lipid extracts of *Longissimus* and *Leg* muscle samples (n=26) from the grass, concentrate and high microalgae as well as grass and concentrate treatments, respectively were reconstituted in 900 µL 20% of mobile phase B (mobile phase B was 90% isopropanol, 10 mM ammonium formate and 0.1% HCOOH in ultrapure water) and 100 µL chloroform and centrifuged. 3 µL was injected into the UHPLC/MS system. Lipids were then directly analyzed using a Vanquish UPLC-System (Thermo Scientific, Waltham, MA, USA) with a heated electrospray ionization (HESI) QExactive plus Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) in positive ion mode. Chromatographic separation took place on a reversed-phase column (Accucore Polar Premium 100×2.1 mm (2.6 µ) with guard column: Accucore Polar Premium 10×2.1 mm (2.6 µ)) from Thermo Scientific (Waltham, MA, USA). Identification and quantification of individual lipid species were performed by LipidSearch Software 5.1 from Thermo Scientific (Waltham, MA, USA) on product level (MS/MS fragmentation).

Results of LC-MS analysis have shown that several C18:3 n-3 lipid species increases two to tenfold only in *Longissimus* and *Leg* muscle of grass treatment and not in algae treatment in comparison to concentrate treatments. Main lipid class of C18:3 n-3 in which the increase occur is alkenyl-PE. An increase of C18:3 n-3 in the TG class could be detected only in *Leg* muscle samples of grass treatment compared to concentrate treatment and not in *Longissimus* muscle. The main lipid classes with C22:5 and C22:6 n-3 increase are alkyl and alkenyl-PE and alkyl-PC. The highest increase (by 3 to 10) is seen for algae treatment. Furthermore a tenfold increase of PI 18:0_22:6 and AcCa 22:6 is detected in *Longissimus* muscle after microalgae treatment in comparison to concentrate treatment. In microalgae treatment of *Longissimus* muscle further incorporation of C22:6 n-3 occurs in specific C22:6 n-3 TG species with medium-chain fatty acids with less or equal than 10 carbons.

This detailed lipidomics study of lamb muscle diet treatments shows that C18:3 n-3 lipid species are mainly incorporated into phospholipids of *Longissimus* and *Leg* muscle after grass diet treatment but also into TGs of exclusively *Leg* muscle. The algae diet treatment lead to a considerable increase of C22:5 and C22:6 n-3 in specific phospholipids and TGs that is not seen in the grass diet treatment.

A4 Organism-Wide Lipid Flux by ^{15}N Labeling and Shotgun Ultra-High Resolution Mass Spectrometry

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Assessment of lipid turnover (i.e. rates of synthesis, breakdown, and remodeling) is crucial in understanding lipid metabolism. The flux is typically monitored by relating the amount of newly synthesized and endogenous lipids by pulse-chase isotopic labeling experiment. In contrast to common ^{13}C labeling of lipids by feeding ^{13}C-glucose or ^{13}C-glycerol, ^{15}N labeling offers simple isotopic profile and distinguishes the lipid turnover from fatty acid synthesis. However, the molecular peaks of ^{15}N labeled lipids are not distinguishable from ^{13}C peaks of endogenous lipids by conventional means of mass spectrometry.

We report a workflow that combines *in vivo* organism-wide ^{15}N metabolic labeling and shotgun ultra-high resolution mass spectrometry (sUHRMS) to measure absolute abundance and estimate the turnover rates of membrane lipids and major lipid precursors in body fluids and tissues of mice. Metabolic labeling was carried out by feeding young- and old-aged mice with a ^{15}N-enriched SILAM diet over five time points. To achieve ultra-high resolution (~1.5M @ *m/z* 200), we coupled Q Exactive Orbitrap MS with an external data acquisition system (Booster X2) and accessed time-domain signals (transients). The transients were then processed using Peak-by-Peak software and lipids were identified and quantified by LipidXplorer software.

With sUHRMS workflow, we resolved ^{13}C isotopes of unlabeled and monoisotopic peaks of ^{15}N labeled lipid species (Δm = 0.00633 Da). We determined the molar abundance and turnover rates of over 120 nitrogen-containing species covering major classes of membrane lipids in mouse plasma, whole blood, four distinct regions of brain and liver. Furthermore, a broad-spectrum of specimen-specific, lipid class and molecular species-characteristic turnover kinetics were observed across ages. Notably, ethanolamine- and serine-containing lipids showed rapid turnover and relatively higher (ca. 2-fold) rate in comparison to the choline-containing lipids. The kinetics of turnover strongly differed among diacyl glycerophospholipid species in contrast to lysolipids. Moreover, brain regions (cortex, cerebellum, striatum, and hippocampus) exhibited much delayed and lower (ca. 4-5 fold) lipid turnover rates and discrete kinetic profiles than body fluids and liver. In the end, to check the reliability of our measures, we computed the abundance of ^{15}N in the intracellular precursor (e.g. choline, serine) and intermediate (e.g. P-Chol, P-Etn, P-Ser, CDP-Chol, CDP-Etn) molecules that are available in the organism for lipid synthesis using shotgun metabolomics.

Taken together, for the first time we monitored lipid flux rates at the full organism level across ages, which will serve as a useful resource for a better understanding of the dynamics (metabolism and transport) of lipid species in mammals.

A5 Investigation of branched-chain fatty acid containing phospholipids of plant-pathogenic bacteria by LC-MS/MS

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Plant-pathogenic bacteria are one of the major constraints on agricultural yield. In order to selectively treat these bacteria, it is essential to understand the molecular structure of their cell membrane. Previous studies have focused on analyzing hydrolyzed fatty acids due to the complexity of bacterial membrane lipids. These studies have highlighted the occurrence of branched-chain fatty acids (BCFA) alongside normal-chain fatty acids (NCFA) in many bacteria. The presence of isomeric FA complicates lipid analysis, because several fatty acids are bound in the intact phospholipids. Furthermore, commercially available reference standards do not fully cover potential lipid isomers.

Therefore, we have developed a reversed-phase high-performance liquid chromatography (RP-HPLC) method with tandem mass spectrometry (MS/MS) to analyze the phospholipids of various plant-pathogenic bacteria with a focus on BCFA containing phospholipids. The study revealed the separation of three isomeric phosphatidylethanolamines (PE) depending on the number of bound BCFA to NCFA. The validation of the retention order was based on available reference standards in combination with the analysis of hydrolyzed fatty acids through gas chromatography with mass spectrometry (GC/MS) after fractionation.

In addition, the transferability of the retention order to other major lipid classes, such as phosphatidylglycerols and cardiolipins, was thoroughly examined. Using the information regarding the retention behavior, the phospholipid profile of six plant-pathogenic bacteria was elucidated. Furthermore, the developed LC-MS/MS method was used to classify the plant-pathogenic bacteria based on the number of bound BCFA in the phospholipidome.

Several bacteria could be differentiated by the developed in-depth phospholipid profiling. The deduced retention order could now pave the way for comprehensive analysis of bacterial phospholipids containing BCFA in further studies, and thus, may provide broader insights into the biological background of bacterial membrane stability and susceptibility.

A6 POLYOL LIPID PROFILING IN THE YEAST-LIKE FUNGUS *AUREOBASIDIUM PULLULANS* BY HPLC WITH PARALLEL CHARGED-AEROSOL AND HRMS DETECTION

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Polyol lipids, also known as liamocins, are a class of surface active glycolipids [1]. These microbiologically produced lipids (alongside others) are also referred to as biosurfactants. Compared to chemically synthesized surfactants derived from fossil fuels, biosurfactants provide a more environmentally friendly and less toxic alternative. These compounds typically consist of a hydrophilic head group made up of a sugar component and a hydrophobic tail composed of fatty acids. In case of polyol lipids, the chemical structure includes a hydrophilic head region comprised of diverse sugar alcohols, such as mannitol or arabitol, connected to a hydrophobic tail formed by three or four 3,5-dihydroxydecanoic acids (DHDA). Additionally, acetylation at the 3-OH position of the 3,5-dihydroxydecanoic acid immediately attached to the sugar alcohol is possible. Other potentially relevant biosurfactant structures have also been described in *Aureobasidium pullulans*, including exophilins, also known as tri- and tetradihydroxydecanoic acids (3DDA and 4DDA) [1]. Due to its rich portfolio of potential biosurfactants, research has increasingly focused on the dark, yeast-like fungus *Aureobasidium pullulans*.

Because of the high structural diversity of polyol lipids, chromatographic separation and high resolution mass spectrometry (HRMS) are necessary for identification. Reversed-phase liquid chromatography (RP-LC) effectively separates various classes of polyol lipids. High resolution mass spectrometry using electrospray ionization (in positive mode) and data-dependent tandem mass spectrometry (MS/MS) allow for a comprehensive detection and characterization of polyol lipids and similar substances. Post-column splitting via a t-piece enables a simultaneous detection and relative interclass quantification via a charged aerosol detector (CAD). By examining several mutant strains and different conditions during cultivation of *Aureobasidium pullulans*, its versatility as a producer of biosurfactants is showcased.

In this study, an efficient method for separating and identifying polyol lipids present in *Aureobasidium pullulans* strains was developed. Furthermore, this study provides a relative quantitative analysis of various polyol lipid classes and their distribution in different mutant strains and cultivation conditions. The results presented in this work highlight potential different parameters for optimizing the biotechnological production of biosurfactants in *Aureobasidium pullulans.*

[1] Tiso, T.; Welsing, G.; Lipphardt, A.; Sauer, D. F.; Chi, Z.; Blank, L. M.; Hayen, H. J Surfact Deterg. **2024**, *27*, 459-461.

A8 LIPID A IN OUTER MEMBRANE VESICLES SHIELDS BACTERIA FROM POLYMYXINS

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The continuous emergence of multidrug-resistant bacterial pathogens poses a major global healthcare challenge, with *Klebsiella pneumoniae* (*Kp*) being a prominent threat. We conducted a comprehensive study on *K. pneumoniae*'s antibiotic resistance mechanisms, focusing on outer membrane vesicles (OMVs) and polymyxin, a last-resort antibiotic.

Our research demonstrates that OMVs protect bacteria from polymyxins. OMVs derived from polymyxin B (PB)-stressed *K. pneumoniae* exhibited heightened protective efficacy due to increased vesiculation, compared to OMVs from unstressed *Klebsiella*. OMVs also shield bacteria from different bacterial families. This was validated *ex vivo* and *in vivo* using precision cut lung slices (PCLS) and *Galleria mellonella*. In all models, OMVs protected *K. pneumoniae* from PB and reduced the associated stress response on protein level. We observed significant changes in the lipid composition of OMVs upon PB treatment, affecting their binding capacity to PB. The altered binding capacity of single OMVs from PB stressed *K. pneumoniae* could be linked to a reduction in the lipid A amount of their released vesicles. Although the amount of lipid A per vesicle is reduced, the overall increase in the number of vesicles results in an increased protection because the sum of lipid A and therefore PB binding sites have increased. This unravels the mechanism of the altered PB protective efficacy of OMVs from PB stressed *K. pneumoniae* compared to control OMVs. The lipid A-dependent protective effect against PB was confirmed *in vitro* using artificial vesicles. Moreover, artificial vesicles successfully protected *Klebsiella* from PB *ex vivo* and *in vivo*.

The findings indicate that OMVs act as protective shields for bacteria by binding to polymyxins, effectively serving as decoys and preventing antibiotic interaction with the cell surface. Our findings provide valuable insights into the mechanisms underlying antibiotic cross-protection and offer potential avenues for the development of novel therapeutic interventions to address the escalating threat of multidrug-resistant bacterial infections.

A9 ELEVATING LIPID IDENTIFICATION CONFIDENCE IN MS IMAGING WITH MZMINE

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Mass spectrometry (MS) imaging enables the spatial mapping of compound distributions within tissue sections. When applied to biomedical imaging, lateral resolutions in the lower µm range can provide insight into tissues affected by disease states or other conditions. Therefore, MS imaging is becoming more prominent in the life sciences. However, the technique mostly relies on annotations by *m/z* only, which is unsuitable for reliably identifying lipids due to isomeric and isobaric overlap of lipid species. One reason is that most instruments lack data-dependent acquisition of MS/MS spectra. State-of-the-art ion mobility spectrometry (IMS)-enabled mass spectrometers provide collisional cross sections (CCS), improving annotation confidence in IMS-MS imaging. While the provided CCS values allow for more robust annotations, the community still lacks MS/MS acquisition strategies. Therefore, we present two different strategies to increase confidence for MALDI imaging based lipid identification.

The first strategy aims to integrate results from LC-IMS-MS/MS and MALDI-IMS-MS imaging experiments.[1] The LC-IMS-MS/MS data is used for confident lipid annotation, using a rule-based approach and subsequent quality control by including equivalent carbon number models, as well as Kendrick mass defect plots.[2]

The second strategy does not rely on additional LC-MS/MS data, because MS/MS spectra are acquired as part of the MALDI imaging experiment. For this, we present the spatial ion mobility-scheduled exhaustive fragmentation (SIMSEF) workflow to plan dataset-dependent MS/MS acquisition and subsequently acquire MS/MS spectra on a TIMS-QTOF-MS.[3]

Both strategies were integrated into the open-source software mzmine. Full data analysis workflows, covering raw data processing, annotation, integration of datasets from LC-IMS-MS/MS and MALDI-IMS-MS, as well as scheduling MS/MS experiments across the tissue by applying SIMSEF, will be compared.

[1] Schmid, R., Heuckeroth, S., Korf, A. *et al.* Integrative analysis of multimodal mass spectrometry data in MZmine 3. *Nat Biotechnol* **41**, 447–449 (2023).

[2] Korf, A., Vosse, C., Schmid, R. *et al.* Three-dimensional Kendrick mass plots as a tool for graphical lipid identification. *Rapid Commun Mass Spectrom*, ***32***, 981-991 (2018).

[3] Heuckeroth, S., Behrens, A., Wolf, C. *et al.* On-tissue dataset-dependent MALDI-TIMS-MS2 bioimaging. *Nat Commun* **14**, 7495 (2023).

A10 LIPIDOMICS AND GENOMICS APPROACHES FOR DISCOVERY OF LIPID BIOACTIVITY AND ECOLOGICAL FUNCTION IN MARINE MICROPHYTOBENTHIC BIOFILMS

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Microphytobenthic biofilms are complex communities of microeukaryotes, cyanobacteria, other bacteria and archaea that occur on intertidal mudflats and sandflats. These biofilms are a significant contributor to the total primary production of coastal ecosystems and persist and function despite extreme variation in environmental conditions over short timescales and high biological competition between taxa. Given the extent of biological competition and co-evolution, highly variable environmental conditions, and their accessibility, intertidal microphytobenthic biofilms are also suitable targets for marine natural product discovery. Despite their important natural role and potential for human application, comprehensive studies of community composition, function and chemical diversity such as metabolites are lacking. In this study, microphytobenthic biofilms were sampled from intertidal settings in temperature waters across Ireland. A multi-omic workflow to characterise biological diversity using culture-independent sequencing technologies and lipid diversity using mass spectrometry-based approaches is being conducted. The qualitative and quantitative distribution of lipids were analysed by targeted and untargeted GC-MS and LC-MS with data dereplication and compounds identification being assisted using the Global Natural Product Social molecular networking platform. Preliminary data highlight significant differences in community composition and abundance between sites. Community composition, biogeography and lipid diversity differences are matched by significant variation in anticancer, antimicrobial and antioxidant potential based on bioactivity screening of lipid extracts.

A12 HILIC BASED FRACTIONATION OF POLAR LIPID CLASSES FOR THE QUANTIFICATION OF ESTERIFIED OXYLIPINS IN HUMAN CELLS

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Several oxylipins are potent lipid mediators. They can be formed by enzymatic and non-enzymatic oxidation of polyunsaturated fatty acids (PUFA) such as arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid. The enzymatic oxidation of PUFAs is catalyzed by lipoxygenases, cyclooxygenases and CYP450 monooxygenases leading to a wide range of different biologically active oxylipins such as prostaglandins and leukotrienes. A dysregulation of the endogenous oxylipin formation has been implicated in the pathogenesis of several inflammatory diseases, including asthma and atherosclerosis. These effects were attributed to free i.e., non-esterified oxylipins. However, the majority of oxylipins in biological samples is present in esterified form, being bound to phospholipids or other lipids. Not much is known about esterified oxylipins in polar lipids and it's unclear in which phospholipid class they are bound.

The best option for quantification of esterified oxylipins in biological samples is currently the targeted liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) based analysis of free, i.e. non-esterified oxylipins and total oxylipins after saponification. Our established method allows the quantification of more than 230 oxylipins in a single run using LC-MS/MS in multiple reaction monitoring mode[1,2]. However, the information in which lipid class the esterified oxylipins are bound is lost in the saponification step. In this project solid phase extraction (SPE) in hydrophilic liquid interaction chromatography (HILIC) mode is used to fractionate phospholipid classes before quantifying oxylipins to pinpoint in which lipid class the oxylipins are bound.

In the presentation, the development and optimization of a HILIC SPE procedure is summarized. It allows the separation of phospholipid classes i.e. phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in individual fractions. The separation efficacy was evaluated using spiking experiments with one representative phospholipid for each class. In combination with non-targeted lipidomics[3] and feature detection using MS-DIAL, the fractionation for all fractionated lipids was evaluated.

This method was applied on the analysis of human cells. Using different cell models, the pattern of oxylipins in the phospholipid classes was analyzed to investigate and compare the incorporation of endogenously formed and exogenously supplemented oxylipins.

1. Hartung, N. M., et al., *Bioanal. Chem.*, **2023**, 415, 913.

2. Ostermann A. I., et al., *Prostaglandins Other Lipid Mediat* . **2020**, 146, 106384.

3. Rund, K.M., et al., Anal Bioanal Chem **2024**, 416, 925–944.

A14 Targeting Fatty Acid Activation to Increase Mycobacteria Eradication: Effects on Vacuole Escape and Cell Viability

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Pathogenic mycobacteria use fatty acids (FAs) released from host lipid droplets to fuel their central metabolism during infection. To import FAs, the bacteria are equipped with intricate transport machineries. However, the process of esterifying FAs with coenzyme A, an essential step for their further turnover, remains elusive.

Employing bioanalytical analysis such as thin layer chromatography and lipidomics, alongside advanced microscopy techniques like spinning disc imaging as well as structured illumination microscopy and serial-block face EM, we revealed that *Mycobacterium marinum* (*Mmar*) FA-CoA ligase (FACL) 6 is essential for FA esterification particular favoring C16 FAs. Deletion of FACL6 impedes FA uptake, reduces triacylglycerol levels and increases the sensitivity towards free FAs.

We postulate that unutilized free FAs interact with the membrane of the *Mycobacterium*-containing vacuole (MCV) rendering it susceptible to membrane damage. Indeed, when FACL6 is absent, markers for membrane repair (ALIX-GFP and GFP-VPS32) are recruited to the MCV more efficiently leading to an increase in phagosome escape. Consequently, the *Mmar* Δ*facl6* mutant forms microcolonies in the host cytosol but is compromised by xenophagy and attenuated in *Dictyostelium* and in BV-2 cells. In immortalized BMDMs, FACL6 deletion triggers both plasma and phagosome membrane damage, resulting in increased pyroptosis.

Understanding the interplay between lipotoxicity, membrane damage and cytosolic translocation is vital to develop novel host-directed therapies aimed at disrupting the sequential stages of the infection cycle (such as phagosome escape and cell-to-cell spreading by pyroptosis) in favor of the host cell.

A17 Lipidomic Phenotyping of Cancer Cells, Organoids and Tissues by Laser Ablation Rapid Evaporative Mass Spectrometry – Biomarkers for Disease Subtypes and Therapy Resistance

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Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is an ambient ionisation method developed for the analysis of aerosols formed on cold or thermal ablation of biological tissues. REIMS data features primarily lipid species from fatty acids through phospholipids to triglycerides among others. While REIMS has been proven to be an excellent tool for the analysis of surgical aerosols and developing mass spectrometry-guided surgical approaches, these tools were proven to be impractical for laboratory testing of cells or tissues. More recently we have developed picosecond mid-infrared resonant laser systems using four wave mixing (FWM) and continuous wave-seeded optical parametric amplifier technologies to obtain lasers operating close to 3 µm wavelength in the picosecond pulse width range with beam profiles allowing to focus them below spot sizes comparable to the wavelength. These lasers were shown to be able to produce meaningful mass spectra by evaporating 30-100 pg of biological tissue corresponding to subcellular amounts (for comparison a single HeLa cell is estimated to weigh 500 pg).

We demonstrated the applicability of LA-REIMS integrated in an automated platform for the high-throughput lipidomic and metabolomic profiling of living and frozen cells, with minimal or no pre-treatment. We performed validation experiments with 5 breast and 5 colorectal cancer cell lines, to establish the classification performance and the molecular coverage of the method. Classification accuracy using an independent dataset was found to be 99.5%, while the method was able to detect more than 400 lipid species, including fatty acids, glycerophospholipids, diacyl and triacylglycerides, and cardiolipins. Additionally, the method was tested against an already proven biological hypothesis, shown enhanced cPLA2 activity and arachidonic metabolism in PIK3CA mutant breast cancer cell lines. In a separate experiment we have tested 70 cancer cell lines and 8 matched patient-derived organoids. The mass spectrometric data revealed 0.97 area under curve results in ROC analysis for the detection of PIK3CA mutations in this case, where the organoids were all correctly classified using a support vector machine model generated from the cell line data. The therapy resistance to various cytostatic agents was predicted with >75% accuracy, with very long chain fatty acids and lysophospholipids driving the classification.

A18 MALDI-2 Mass Spectrometry Imaging of Peptide Toxins and Lipopeptides in *Bacillus subtilis* Biofilms

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Introduction

Bacillus subtilis is a Gram-positive, spore-forming soil bacterium known for its ability to form robust, yet adaptive biofilms on various surfaces. It serves as an important model organism in microbiological research. To improve our understanding about the complex communication and cell differentiation processes occurring during biofilm formation, MALDI-2 mass spectrometry imaging (MSI) can be used. Here we advanced this method towards the analysis of thin sagittal sections across bacterial biofilms at 5-10 µm pixel size and for the top-view analysis of single and multiple *B. subtilis* knock-outs that are lacking cannibalism-associated peptide toxins and defense strategies.

Experimental

B. subtilis biofilms of wild type NCIB 3610 and NCIB 3610-derived mutant strains were grown at 28 °C for up to 10 d using a mixed cellulose ester filter membrane (mean pore size, 0.22 µm) as substrate, placed on minimal medium (MSgg) agar. For cryo-sectioning at 10 µm thickness, samples were embedded in carboxymethyl cellulose gel; whole biofilms were analyzed without embedding but following chemical fixation in 10% paraformaldehyde for 30 min. For MALDI analysis, all samples were spray- coated with 2,5-dihydroxyacetophenone MALDI matrix. A timsTOF fleX MALDI-2 mass spectrometer (Bruker Daltonics) was used as mass analyzer. Bright-field and phase contrast images were recorded with a VS200 microscopy scanner (Evident).

Results

In the course of evolution, *B. subtilis* has developed sophisticated strategies of cell-to-cell communication and division of labor to orchestrate colony growth in response to the environment, as for example availability of nutrients and presence of competing microorganisms. Cannibalism, a scenario in which a sub-population of cells is sacrificed to ensure colony survival, is for instance controlled via the expression of a set of peptide toxins. In particular, the roles of the epipeptide EPE, the sporulation killing factor SKF and the sporulation delaying peptide SDP, comprising 17, 26, and 42 AA, respectively, are currently studied to better understand these processes. Our MALDI-(2-)MSI analysis of cross-sections of *B. subtilis* wt strains enabled visualization of these peptides, exhibiting molecular masses between 2.1 and 4.3 kDa, at a spatial resolution in the low 10 µm-range. In addition, *B. subtilis* lipopeptides surfactins and plipastatins, as well as numerous further analytes in the *m/z* range below 1000 were detected (e.g. structural phospholipids) following adjustments in the matrix coating protocol. Confirmed by high-resolving microscopy, the intricate structures constituting the biofilms, including crypt and microchannel formation, became observable on a molecular level. Our MALDI-MSI analysis of single and multiple knockouts of the *epe*, *sdp* and *skf* operons, in this case obtained solely from whole matrix-coated biofilms, added yet another biological and biochemical dimension to these findings. For example, our experiments visualized distinct " wave-like" expression of the toxins across the biofilm.

Together, our mass spectrometry imaging data reflect an exceedingly high and – in case of the studied *B. subtilis* model system – partly unknown level of cellular differentiation during bacterial biofilm development.

A19 Elucidating Cellular Metabolism with a Full Suite of Next-Generation Omics Tools

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With cellular research models you often need a comprehensive view of metabolism at not only the cellular but also the metabolite level to generate accurate biological insights and identify potential therapeutic targets. This presentation highlights the ability to use Seahorse XF technology with LC/MS synergistically to deliver these critical holistic biological insights with enhanced confidence.

In this study we identified two candidate tyrosine kinase inhibitors that had desirable impacts on aerobic metabolism in cancer cells with a Seahorse XF screening strategy. Additionally, by leveraging Seahorse Mitochondrial Stress Test we assessed candidate therapeutics for spare respiratory capacity to assess potential for metabolic switching. To follow up on this mechanistically we conducted both targeted and untargeted metabolomics and lipidomics approaches with Agilent LC/MS systems. From these omics assays we confirmed that one of the drug candidates permitted the cells the ability to switch substrates to beta-oxidation-based metabolism and relieve inhibition.

Methods

The THP-1 cancer cell line was utilized in this study. Cells were treated for 2 or 18 hours with two tyrosine kinase inhibitors or a vehicle control. Flow cytometry was used for cell count normalization for both Seahorse XF and LC/MS analyses to ensure sampling of the same number of cells from each treatment group. Treated cells were plated into Seahorse XF plates using recommended guidelines and oxygen consumption and extracellular acidification rates were monitored. Separately, treated cells were aliquoted for LC/MS analysis and prepared using a room temperature cell lysis and quenching method. Metabolites and lipids were fractionated with a Captiva EMR-Lipid plate using a Bravo Metabolomics Sample Prep Platform. Metabolite extracts were separated with HILIC-LC, and lipid extracts were separated with RP-LC, and eluents were analyzed with an Agilent Revident LC/Q-TOF and MassHunter Explorer software for chemometrics in a discovery workflow. To identify endogenous metabolites, a database search was performed against a 471-compound subset of the Agilent METLIN Personal Compound Database and Library curated with HILIC-Z retention times from authentic chemical standards, resulting in 101 annotated compounds. To enable lipid annotation, MassHunter Lipid Annotator software was first used to build a lipid database based on in silico MS/MS spectral library matching from MS/MS spectra that were acquired from representative pooled cellular extracts. Specifically, a set of six positive-ion and six negative-ion mode iterative MS/MS data files were analyzed. This resulted in 562 lipids, representing 16 classes, annotated for positive-ion mode, and 500 lipids, representing 22 classes, annotated for negative-ion mode.

P1 ORGAN AND PLASMA SPECIFIC LIPIDOME ALTERATIONS IN RESPONSE TO ACUTE MYOCARDIAL INFARCTION

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The composition of the plasma lipidome in a tightly regulated environment can reflect underlying phenotype in health and disease states. Unfortunately, this precludes conclusions about lipid composition and dynamics within the heart e.g. after myocardial infarction (MI) or other subsequently affected organs.

Hence, we developed a workflow which allows for the investigation of both low abundant lipid species (like eicosanoids) and major membrane components (e.g. cholesterol) from the infarct or remote area of the same heart. The tissue was washed, homogenized and subjected to either MTBE or MeOH/MTBE/CHCl3 extraction. The analysis incorporated internal lipid standards at both the species and lipid-class levels in order to cover 4 analysis platforms: HR-MS/MS shotgun lipidomics and LC-MS/MS for sphingolipids, sterols, signaling lipids and metabolites[1,2,3]. Following structural analysis, identification, and quantification, the absolute amounts corresponding to more than 700 molecules were analyzed using Pearson correlation and cosine similarity clustering, accounting for both positive and negative correlations. The generated data was compared to the matched condition upon reperfusion. To investigate how the body responds to MI, we applied the same analysis setup on the plasma and liver of the corresponding mice. By integrating our findings, we were able to observe changes in the liver and plasma of the mice as early as 0.5 or 1h after MI which relate to our observations in heart. This indicates an extensive overall effect and connection within the organs and provide a first look on early lipidome alterations following both MI and reperfusion.

1. Coman, C., Solari, F. A., Hentschel, A., Sickmann, A., Zahedi, R. P., Ahrends, R. (2016) Simultaneous Metabolite, Protein, Lipid Extraction (SIMPLEX): A Combinatorial Multimolecular Omics Approach for Systems Biology. Molecular & cellular proteomics: MCP 15, 1453-1466.

2. Rubenzucker, S., Manke, M-C., Lehmann, R., Assinger, A., Borst, O., Ahrends, R. A Targeted, Bioinert LC–MS/MS Method for Sensitive, Comprehensive Analysis of Signaling Lipids, Anal. Chem. 2024, 96, 23, 9643–9652.

3. Troppmair, N., Kopczynski, D., Assinger, A., Lehmann, R., Coman, C., Ahrends, R. (2023) Accurate Sphingolipid Quantification Reducing Fragmentation Bias by Nonlinear Models. Analytical Chemistry 95, 41, 15227-15235.

P2 Lipidome Projector: Visualization, comparison, and analysis in a vector space

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We have developed a software package for the interactive visualization and analysis of lipidome datasets. A shallow neural network was used to embed lipid structures in a 2- or 3-dimensional space with the goal that structurally similar species are assigned similar vectors. Tests on complete lipid databanks show that the method automatically produces distributions which follow conventional lipid classifications. The embedding is accompanied by the software “Lipidome Projector”, which parses and projects user lipidomes onto the vector space. The data is displayed as 2D or 3D interactive scatterplots for quick exploratory analysis, quantitative comparison and interpretation at a structural level. The software is intended to be used as a web-application accessed on a remote server but is also available for local installation.

P3 Towards targeted and quantitative 4D Lipidomics - From PASEF to prm-PASEF

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Parallel accumulation–serial fragmentation (PASEF) combined with trapped ion mobility spectrometry (TIMS) allows mobility-resolved fragmentation and increased targeted precursor throughput compared to conventional MS/MS. High-confidence rule-based lipid annotations from 4D-Lipidomics data in MetaboScape serve as a basis for targeted screening and quantitation. For targeted lipidomics, parallel reaction monitoring (prm)PASEF showed optimal data quality. We present a workflow from untargeted to targeted and quantitative 4D-Lipidomics.

By phospholipid profiling of NIST SRM 1950 plasma lipids, a deep lipidome coverage of 93 high confidence annotations based on retention time, isotope pattern, collisional cross section and fragmentation behavior was obtained in MetaboScape. The transfer of the annotated lipids including lipid species-dependent fragment ions to the targeted/quantitative software solution TASQ facilitates the seamless generation of a prmPASEF MS/MS transition list for a quantification method. Where applicable, MS/MS transitions were used to monitor indicative qualifier ions as an additional tool for identification to support reliability in lipid quantitation on species or molecular species level.

Using HILIC-MS/MS, a lipid class separation was achieved which favors quantitation by using one isotope-labeled internal standard per lipid class. However, this coelution also favors isobaric type-II overlaps that result from the natural isotopic pattern of lipid species with an additional double bond. Through the additional TIMS dimension, separation of the isobaric overlap could be achieved. For accurate quantification of non-baseline separated overlaps in TIMS, we recommend the use of the M+1 signal for quantitation, which is also implemented in the TASQ software. With this method, quantitation accuracies of for example SM 34:0;02 and PC 36:1 could be increased by 74% and 42%, respectively. All in all, the combination of MetaboScape and TASQ software solutions for targeted Lipidomics by HILIC-TIMS-MS/MS and prmPASEF served for a precise and user-friendly lipid quantitation workflow with high confidence, offering potential for applications in clinical lipidomics.

P4 ACCURATE CERAMIDE QUANTIFICATION WITHOUT FRAGMENTATION BIAS BY NON-LINEAR MODELS

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Quantitative sphingolipid analysis is crucial for comprehending the roles of these bioactive molecules in various physiological and pathological contexts [1]. Molecular sphingolipid species are typically quantified using sphingoid base-derived fragments relative to a class-specific internal standard. However, the commonly employed 'one standard per class' strategy fails to account for fragmentation differences presented by the structural diversity of sphingolipids. To address this limitation, we have developed a novel approach for quantitative sphingolipid analysis. Building on a previously developed LC-ESI-MS/MS-based approach for the comprehensive analysis of sphingolipids in biological samples, we focused on optimizing the quantification process through post-acquisition data correction. To prevent overlaps of species that differ by only one double bond, we employed a fragment of the long-chain base for quantification, in addition to chromatographic separation.

Using only one internal standard for the entire lipid class, we developed a fragmentation model for ceramides. This approach uses information on the double bonds and hydroxy groups of the long-chain base, as well as the fatty acid, for a rough calculation of the correction factor and the chain length for a more precise one. The determined response factors are based on experimental data and are independent of the employed instrumentation, collision energies, or matrix, and may be extended to another internal standard or different sphingolipid classes.

To further enhance the usability and efficiency of our method, we have integrated it into a user-friendly workflow using the software tool “Konstanz Information Miner” (KNIME) [2]. This workflow automates data processing after acquisition, such as the calculation of species-specific correction factors, significantly reducing the time and effort required. Overall, the workflow has proven suitable for processing data from ceramide analyses of complex biological samples, such as fat cells [3], delivering the same quantitation results as manually obtained from the same data set, in a markedly shorter timeframe.

[1] B. Peng et al., A comprehensive high-resolution targeted workflow for the deep profiling of sphingolipids. Anal. Chem. 2017, 89, 22.

[2] M. R. Berthold et al., KNIME: The Konstanz Information Miner. Springer 2008.

[3] R. Ahrends et al., Controlling low rates of cell differentiation through noise and ultrahigh feedback. Science 2014, 344, 6190.

P5 Analysis of bioactive lipids in biological matrices using LC-MS/MS: method development and practical implications

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Introduction

Bioactive lipids like oxylipins, lysophosphatidic acids, ceramide-1-phosphates, and sphingosine-1-phosphates govern many cellular processes, including cell growth, death, and migration, and are key players in inflammatory processes [1-2]. Comprehensive methods for quantifying bioactive lipids are therefore essential to advance the understanding of their role in biological processes. While state-of-the-art methods cover a few of these lipid classes [2], there is yet no workflow that enables the quantification of all relevant lipid classes in one analytical run. Challenges include a wide dynamic range, isomeric and isobaric lipid species, and, most of all, these analytes' very different structures and chemical properties [2]. We herein present the first LC-MS/MS method capable of analyzing close to 400 signaling lipid species in a single run.

Methods

Lipid standards from more than 15 different lipid classes were used to establish a LC-MS/MS method on a QTrap 6500+ coupled to a Vanquish Flex. MS source and sMRM parameters were optimized for each lipid class. Different column hardware, gradients, and salt and acid concentrations were tested for optimum chromatographic separation. The final method consisted of a 20 min chromatographic run employing a linear gradient on a YMC-Accura Triart C18 column (150×2.1 mm, 1.9 µm). Optimized sample preparation consisted of a one-step extraction procedure adapted from literature [3]. The method was validated in plasma and plateletmatrix. Finally, the method was used to investigate the human plasma and murine platelet signaling lipidome.

Results

Free phosphate group-containing lipids like lysophosphatidic acids, sphingosine-1-phosphates and ceramide-1-phosphates showed extensive carryover when conventional column hardware was used for RP-LC. Switching to a bioinert column greatly reduced these carryover effects and further optimization of the solvent composition in regards to acid and salt concentration lead to negligible carryover, enabling the robust analysis of all lipid classes of interest. Different sample extraction protocols were adapted from literature and tested to ensure optimal recovery of all lipid classes: BuMe [4,5], BuEt [2] and MMC [3]. The average extraction recovery over all analytes was 80%, 75%, and 62% for MMC, BuMe, and BuEt, respectively. Furthermore, 88% of the analytes had an extraction recovery of ≥80% for the MMC protocol, whereas for BuMe and BuEt, this threshold was only reached for 47% and 6% of the analytes. MMC was chosen as the most suitable extraction method due to its high recoveries and ease of use. The established workflow was validated in plasma and platelet matrix. The method showed excellent retention time stability over multiple batches (RSD<0.71%), inter- and intraday repeatability (RSD<15%) and almost no carryover (<0.28%), even for difficult analytes like ceramide-1-phosphate. LLOQs were in the low nM range for all lipid classes in all tested matrices.

Applying our workflow to human plasma, we identified 307 lipid species, spanning a dynamic range of 6 orders of magnitude. The estimated concentrations are in good agreement with values previously published for plasma and NIST reference material.Investigating the signaling lipidome during platelet activation we identified 267 lipids, of which 85% were regulated upon stimulation. The high coverage of our established method furthermore enabled the detailed investigation of distinct lipid signaling pathways for glycerophospholipid-derived and sphingoid-based signaling lipids on a molecular lipid species level, providing the first comprehensive report of signaling lipid regulation during platelet activation.

[1] Peng, B. et al., Identification of key lipids critical for platelet activation by comprehensive analysis of the platelet lipidome, Blood **2018**, 132 (5), e1-e12. **[2]** Schoeman, J. C. et al, Development and application of a UHPLC-MS/MS metabolomics based comprehensive systemic and tissue-specific screening method for inflammatory, oxidative and nitrosative stress, Analytical and Bioanalytical Chemistry **2018**, 410 (10), 2551-2568. **[3]** Pellegrino, R. M. et al., LC/MS lipid profiling from human serum: a new method for global lipid extraction, Anal Bioanal Chem **2014**, 406 (30), 7937-48. **[4]** Lofgren, L. et al., The BUME method: a novel automated chloroform-free 96-well total lipid extraction method for blood plasma, J Lipid Res **2012**, 53 (8), 1690-700. **[5]** Hoering, M. et al., Benchmarking One-Phase Lipid Extractions for Plasma Lipidomics, Anal Chem **2022**, 94 (36), 12292-12296.

P7 EMPLOYING VACUUM JACKETED COLUMNS AND PROTOTYPE BENCHTOP MULTI REFLECTING TIME-OF-FLIGHT (MRT) TO INCREASE LIPIDOMIC THROUGHPUT WHILST MAINTAINING HIGHLY CONFIDENT IDENTIFICATIONS

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Introduction
One common theme between all omics applications is the ever-increasing size of patient cohorts, driven by the need for identification of novel disease biomarkers and increasing the power of the studies. However, as these studies scale to thousands of patient samples throughput becomes the limiting factor. It may be possible to decrease separation time, however this comes at the cost of peak capacity and feature detection. Vacuum jacketed columns (VJC) can significantly increase peak capacity and narrow peak widths, thereby allowing faster chromatography when combined with fast scanning acquisitions without information loss, or maintaining chromatography duration while increasing peak capacity. Vacuum jacketed columns were therefore applied to the analysis of the lipidome of healthy controls and patients with different cancers.

Methods
Lipids from serum samples of healthy controls and patients with bladder, colon or kidney cancer were extracted using IPA and spiked with EquiSPLASH™ to act as an internal control. Lipids were separated on a 1mm diameter Phenyl-Hexyl column, consisting of either 100 or 50 mm length. Corresponding columns in VJC format were also applied for the separation. A standard 10-minute gradient was used with all columns before scaling down to sub-1 minute gradients. The eluate was directed towards a prototype benchtop multi reflecting time-of-flight (MRT) mass spectrometer operating at 100Hz scan speed in a data-independent mode of acquisition. Generated data were analysed using a combination of in-house and third-party informatic tools.

Preliminary Data
Generated datasets were aligned, peak picked and normalised using Lipostar informatics. Lipid identifications were returned from searching the LipidMAPS™ structure database. Initially data acquired by employing a conventional 2.1mm column using a 10-minute gradient were analysed for benchmarking purposes. Several highly abundant biomarkers could be identified using this method, which related to cell proliferation and signalling pathways (including lysophosphatidylcholines and phosphatidylcholines) and relative abundance differences observed between healthy controls and cancer groups. Chromatographic methods were then scaled down to 1mm ID and column length reduced to maintain the gradient time which resulted in a similar number of features being identified. Increased flow rate and lowering of gradient time(s), reduced the peak capacity as expected and as such fewer features were detected. However, major biomarkers were still observed and samples could be separated based on abundance. Finally, methods were transferred to a 1mm vacuum jacketed column and when a 10-minute gradient was employed, peak capacity was shown to have increased when compared to conventional column formats, which facilitated a larger number of identified features. Statistical analysis comprising of multi-variate statistics, resulted in similar profiles being generated via unsupervised principal component analysis (PCA), regardless of the chromatographic methodology employed. This ultimately resulted in the most statistically significant features between the patient groups being identified for 1mm and VJC-based data. The high scan speed afforded by the prototype benchtop MRT instrument allowed adequate profiling of sub-1 second peaks observed during VJC separations without compromising on mass accuracy at the ppb level and resolution. Application of VJC using a reduced gradient time compared favourably to conventional columns and separation time, allowing for an 30% increase in throughput whilst maintaining equivalent chromatographic performance.EquiSPLASH is a trademark of Avanti Polar Lipids, LLC. LIPID MAPS is a trademark of The Regents of the University of California.

Novel Aspect
High-throughput, highly confident identification and relative quantification of lipid cancer biomarkers from human sera

Conflict of Interest Disclosure
All authors are employees of Waters Corporation, a vendor of mass spectrometers.

P8 Exploration of Single Cell Lipidomics with a Novel Multi-reflecting Q-Tof Platform

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Introduction
The development of cutting-edge mass spectrometers with unbeknownst sensitivity and resolution has led to single-cell OMICs gaining significant momentum in recent years. Now, previously unanswerable questions can be probed through the distinct measurements of single cells in lieu of a bulk cell average. Here we present a single-cell lipidomic workflow, established with cultured human cell lines using liquid chromatography (LC) coupled to a novel multi-reflecting Q-Tof mass spectrometer (MS).

Methods
A dilution series of EquiSPLASH® (Avanti Polar Lipids) at a concentration of 0.5-1000 ng/mL (equivalent to single cell level) was used to benchmark instrument sensitivity. Following optimisation of liquid chromatography and mass spectrometry parameters, bulk cells (THP-1, PC-3) were extracted using methanol and IPA, and a dilution series down to the equivalent of a single cell was undertaken and analysed. Bulk cells were also used for column conditioning prior to single cell analyses. Single cells were isolated from trypsinised cell cultures using FACS and lysed directly with methanol and IPA. Data were collected and processed with in-house software, in addition to being processed with third party informatics such as Skyline and Lipostar (Molecular Discovery).

Preliminary Data
Initial experiments consisted of lipid extracts analysed with an LC-MS workflow, which was primarily configured with a 2.1mm column. The LC was directly connected to a novel multi-reflecting Q-Tof, demonstrating high mass resolution (100,000 FWHM), sensitivity down to typical single cell levels of <5 ng/mL and a dynamic range of 5-orders. Further method optimisation was conducted with the LC configuration, which consisted of benchmarking 2.1mm (12-minute gradient) and 1 mm (5-minute gradient) columns to establish potential gains in sensitivity and lipid species detection. Results from the EquiSPLASH® based experiments, highlighted that lipid features could be readily detected to 5 ng/mL based on 2.1mm chromatography. Levels of 0.5 ng/mL was repeatedly achieved for 1mm. instrument optimisation and benchmarking with lipid standards, data relating to single cell lipid extracts originating from PC-3 and THP-1 cells were collected using the 1 mm LC configuration. The use of 1 mm columns provided enhanced sensitivity and the option to acquire with shorter analysis times and hence provide a high throughput strategy for single cell analysis, whilst also ensuring robustness of the methodology was maintained. The novel MS platform provided high quality MS and MS/MS data for confident lipid identification. The adaptability of the platform was also confirmed for rapid chromatographic methods using narrow bore columns, whereby acquisition rates of up to 100 Hz were implemented, providing more than 20 points across the chromatographic peak for accurate quantification but also maintaining resolution over the mass range of 50-1200 Da. Greater than 200 features were detected from single cells of PC-3 and THP-1, with the main lipid classes relating to phospholipids, ceramides and triglycerides. A high degree of confidence could be applied to the lipid identifications following database searching, due to the high mass accuracy afforded by the instrument, providing identifications within 200 ppb.

Novel Aspect
Establishing single cell lipidomic LC-MS workflow using a novel, multi-reflecting Q-Tof.

Conflict of Interest Disclosure
The authors declare no competing financial interest

P9 Analysis of novel *Caenorhabditis elegans*-specific phosphorylated sphingolipids using LC-TIMS-MS/MS and MassQL

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Introduction

High-resolution mass spectrometry (HR-MS) is an important tool for lipid identifiaction in different biological samples, with enhancements from orthogonal information such as retention times (RT) and collisional cross sections (CCS). The nematode *Caenorhabditis elegans* represents an important model organism in biomedical research, with many recent studies investigating intact lipids. As the entire *C. elegans* lipidome is not known yet, correct lipid annotation and identification are crucial. While for known lipid classes this can be achieved in reproducible manner e.g. by rule-based annoation, the systematic search for novel lipid classes and species needs dedicated data analysis strategies. Here we present the analysis of phosphorylated glycosphingolipids from *C. elegans* using Mass Spec Query Language (MassQL) to query for lipid characteristic MS/MS data.

Methods

C. elegans reference samples were obtained from the University of Athens and extracted with different lipid extraction protocols: Bligh and Dyer, MTBE, alkaline MTBE, BUME and MeOH. Analysis was performed using an Elute UHPLC coupled to a timsTOF Pro 2 (Bruker Daltonics). Lipid separation was achieved on a Waters Cortecs C18 column (150 mm x 2.1 mm ID; 1.6 µm particle size) with a gradient from 40% H2O / 60% ACN to 10% ACN / 90% iPrOH, both with 10 mM ammonium format and 0.1% formic acid. Data was acquired in positive and negative mode using DDA-PASEF and data analysis was performed in MetaboScape 2023b, which includes a beta-version of MassQL.

Preliminary Data

Boland *et al.* [1] recently described novel phosphorylated sphingolipids in *C. elegans*. Although the sphingolipidome of *C. elegans* has been thoroughly analyzed in previous studies [2, 3], this particular class of lipids was only detected during a cholesterol deprivation study. These sphingolipids are derivatives of glucosylceramides, characterized by an unusual phosphoethanolamine or monomethyl phosphoethanolamine residue linked to the sugar moiety. Currently, there are no commercial reference standards for these lipids, and no reference MS/MS spectra have been deposited in public spectral repositories.

To investigate the prevalence of this lipid class in *C. elegans*, we extracted lipids using various methods and analyzed them using LC-TIMS-MS/MS. We then employed MassQL to generate queries in the acquired data, based on previously described fragmentation patterns for these novel phosphorylated sphingolipids. For instance, we looked for the neutral loss of mass 285.06134 ($C_8H_{16}O_8NP$) in phosphoethanolamine glucosyl ceramides (PEGCs) and mass 299.0770 ($C_9H_{18}O_8NP$) in monomethyl phosphoethanolamine glucosyl ceramides (mmPEGC).

Our approach led to the identification of six novel species with varying N-acyl chain lengths, in addition to five known species. To validate these MS/MS-based annotations, we examined trends along RT and CCS to study chromatographic and ion mobility behavior. This study marks the first time this lipid class has been reported with ion mobility. The obtained CCS values and fragmentation patterns can aid in the identification of these lipids in future studies.

[1] doi:10.1038/nchembio.2347
[2] https://doi.org/10.1016/j.chemphyslip.2019.04.009
[3] https://doi.org/10.1016/j.chroma.2021.462481.

P10 IPRM-PASEF: AN INTEGRATED WORKFLOW FOR THE ANALYSIS AND INTERPRETATION OF SPATIAL ON-TISSUE TANDEM MASS SPECTROMETRY OF LIPIDS

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Spatial tandem-MS-based molecular identification remains a challenge in mass spectrometry imaging. While several automated data acquisition and annotation approaches exist, they mostly rely on “profiling” where the spatial relationship between precursors and fragments is severely reduced. Here we present iprm-PASEF, a novel workflow that integrates CCS-enabled MALDI Imaging of lipids with a targeted tandem-MS imaging approach that allows the fragmentation and analysis of up to 25 precursors in a single acquisition, and which comes with a fully integrated computational workflow to generate accurate annotations with spatial fidelity.

CCS-enabled MALDI Imaging of lipids was performed on rat kidney cryosections using a timsTOF fleX. Data was imported into SCiLS Lab and T-Rex^3 feature finding was performed. A selection of CCS features was exported as precursor list for the iprm-PASEF acquisition. Tandem-MS data were imported into SCiLS Lab. Using MetaboScape-powered rule-based Lipid Species Annotation, fully integrated into SCiLS Lab, fragments and precursors were annotated and identified respectively. Several chimeric tandem-MS spectra were observed, since evidence for multiple explanations at the molecular species level were found in a single tandem-MS spectrum. For example, m/z 766.54 was annotated as PE 38:4 at the species level based on the headgroup fragment. However, additional side chain fragments confirm the presence of PE 18:0_20:4, PE 16:0_22:4, PE 18:1_20:3 and PE 18:2_20:2. Based on fragment intensities, distributions and MS/MS score, PE 18:0_20:4 was found to be predominant.

This workflow allows for a complete and comprehensive analysis of spatial tandem-MS data in an intuitive and integrated manner and enables confident tandem-MS-based molecular annotation directly from tissue.

P11 Effect of long-term exercise intervention on plasma lipids in young sedentary adults in the ACTIBATE randomized controlled trial

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Background

Regular physical activity is important not only for physical health but also for mental health. Systemic changes in metabolic profiles can be seen resulting from physical activity, and the study of lipid metabolism is especially important in understanding if and how physical activity affects the health of sedentary subjects[1]. Although it has become clear that resistance and endurance exercises use lipids as an energy source[2], the role of lipidome has not been fully defined.

Study design

A randomized controlled trial was conducted to investigate changes in the plasma lipidome after a 24-week supervised exercise training program in young adults. After baseline examination, participants were assigned to one of three groups:(i) a control group (no exercise), (ii) a moderate-intensity exercise group, and (iii) a vigorous-intensity exercise group. After 24-week exercise(resistance and endurance training), plasma samples were collected after an overnight (10 hours) fast, and samples were aliquoted and stored at −80°C.

Methods

Lipidomics profiling analysis was done using a previously published method.[3] Plasma lipids were extracted using the Matyash method and analyzed with Hydrophilic interaction liquid chromatography (HILIC)-MS/MS. Acquired data were evaluated using SCIEX OS Software and an in-house developed software tool (mzQuality) was used for batch correction and assessing data quality. The data was statistically analyzed using partial least square discriminant analysis (PLS-DA), volcano plot, one-way ANOVA, correlations, enrichment analysis and pathway analysis in R(4.2.2).

Results

After conducting a quality control check, we could report 794 lipids across 18 subclasses.

Compared to control and vigorous-intensity exercise group, many lipids undergo significant changes after moderate exercise. Phosphatidylethanolamines (PE), Phosphatidylcholines (PC) and Triglycerides (TG), were significantly increased in the plasma lipidome after moderate exercise. This may reflect increased lipolysis and fatty acid mobilization from adipose tissue leading to elevated circulating triglyceride levels. Gender differences in the increase of TG levels after exercise were observed and this may be related to hormonal variations, insulin sensitivity and metabolic rate. In addition, several lipids exhibited a strong correlation with handgrip strength, brown adipose tissue(BAT), glucose and Apolipoprotein AI(APOA1). In conclusion, our study offers valuable insights into the complex interplay between exercise, lipid metabolism, and physiological parameters.

1. Muscella A,et.al. The Regulation of Fat Metabolism During Aerobic Exercise. Biomolecules. 2020 Dec 21;10(12):1699.

2. Motiani, P.et al. Exercise training alters lipoprotein particles independent of brown adipose tissue metabolic activity. Obes. Sci. Pract. 2019, 5, 258–272

3. Zhang Z.et.al. Development of a targeted hydrophilic interaction liquid chromatography-tandem mass spectrometry based lipidomics platform applied to a coronavirus disease severity study. J Chromatogr A. 2023 Oct 11;1708:464342.

P12 Conversion and integration of OMICS data from a prototype, benchtop multi-reflecting time-of-flight (MRT) platform with third-party informatic workflows

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Introduction

OMIC studies typically generate large and complex data sets, derived from various sample types such as biofluids. As mass spectrometry (MS) technology rapidly advances and with data acquisition methods such as data independent analysis (DIA) progressing, the ability to delve deeper into the metabolome and lipidome is significantly improved, whilst at the same time generating high-dimensional datasets. In this study, we describe a data processing pipeline which converts MS data that was collected using a novel, multi-reflecting ToF into a generic file format, that can be readily processed by a variety of third-party informatic tools.

Methods

Metabolomic and lipidomic data relating to samples from a range of sources, including plasma, serum and urine were collected using LC-MS. HILIC and reversed-phase chromatographic conditions were used for metabolite and lipid analysis respectively, with gradients consisting of standard UPLC™ (~10min) and rapid (<3min) timescales. MS data were acquired using DDA and DIA approaches with a prototype, benchtop multi-reflecting time-of-flight, capable of providing high resolving power and with ppb mass accuracy. Data were collected using high acquisition rates to coincide with the rapid gradient conditions, allowing for adequate sampling and ensuring sufficient data points per chromatographic peak. Subsequent data were converted into generic mzML format via the in-house data conversion tool for processing with multiple third-party informatics.

Preliminary Data

Metabolomic data originating from urine and lipidomic data in the form of extracts from human plasma samples were collected using waters_connect™ software, prior to being converted into mzML format using the Data Convert tool. The tool performs several steps to generate mzML outputs, firstly performing a data conditioning step to lockmass correct mass spectra before stripping lockmass spectra from the data. Specifically, for DDA data, SetMass values are lockmass corrected and MS/MS scans for the same SetMass are merged. Dependent on the intended third-party informatics, the resulting mzML was also centroided. Following data conversion, the processing pipeline for the metabolomic and lipidomic datasets consisted of using MZmine 3 and MS-DIAL, whilst Skyline was used for data visualization. To provide ultimate flexibility with data format streams, the ability to seamlessly connect from waters_connect to commercially available software using the application program interface (API) is also demonstrated for both OMIC datasets. Lipostar 2 and MARS demonstrate this additional flexibility and were used for interrogating the lipid and metabolite datasets respectively. The results ascertained via both routes (i.e., conversion to mzML or API), highlight the instrument capabilities in all cases for polar metabolites and lipids, exhibiting mass accuracies in the region of 200 ppb, enabling greater confidence in compound identification(s) and maintaining high resolution across the acquired mass range(s). Based on the lipidomic analyses (EquiSPLASH™ dilution series constructed in a plasma matrix), high sensitivity and dynamic range is highlighted with 5 ng/mL levels routinely achieved. In the case of rapid chromatographic separation, a 3 min metabolomic analysis yielded comparable results with equivalent samples, which were acquired using 'standard' chromatographic conditions. For example, based on extracted compounds; mass accuracy in the region of 200 ppb, quantitative accuracy (>25 points per peak), spectral quality and mass resolution were readily achieved for high throughput analyses.

Novel Aspect

Sophisticated OMIC data conversion workflows relating to multiple acquisition formats acquired using a novel, benchtop multi-reflectron ToF to generic format(s).

P13 HIGH THROUGHPUT PLASMA PROFILING OF HUMAN LIVER DISEASE SAMPLES USING RAPID CHROMATOGRAPHY AND A MULTI-REFLECTING TIME-OF-FLIGHT MASS SPECTROMETER

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Introduction

Acute-on-chronic liver failure (ACLF) is a serious condition which develops based on acute decompensation (AD) of cirrhosis and is characterized by intense systemic inflammation, multiple organ failure, and high short-term mortality. However, there are still no validated biomarkers for the diagnosis of ACLF. Understanding the role of metabolites and lipids in the pathogenesis of ACLF would help the development of new diagnostic and therapeutic strategies. Investigating biomarkers for large scale studies requires robust, high-throughput analytical methods. Rapid microbore metabolic profiling (RAMMP) methods and conventional high-resolution mass spectrometry have previously been shown to considerably reduce analysis time. Here we compare these methods with vacuum jacketed technology (VJC) and ultra high-resolution mass spectrometry for the analysis of plasma from liver disease patients.

Methods

Human plasma samples from four patient groups, healthy control (n=25), cirrhosis (n=25), ACLF (n=25), and acute liver failure (ALF) (n=25), underwent extraction for lipids and small molecules. Quality-control (QC) sample was prepared by combining aliquots of each study sample and phenotypic pools were created by combining aliquots within the study groups. Chromatographic separation was performed using the RAMMP methodology with small molecules analysed using the HILIC ACQUITY ™ BEH ™ amide (1.0 x 50 mm) and lipids profiled using a ACQUITY ™ BEH ™ C8 (1.0 x 50 mm) column. Mass spectrometry data was acquired on the SELECT SERIES ™ MRT using the data independent acquisition (DIA) mode MS ^{E} in both positive and negative ESI mode. All data were processed using MARS and Lipostar software.

Preliminary Data

Conventional LC-MS analysis using chromatographic methods between 10-30 min per sample provides detailed characterisation of biological matrices. However, analysing large collections of samples using these analysis regimes places a burden on costs, resourcing and can lead to batch response variation, impacting data quality. The need for fast, high throughput methods has increased in recent years to tackle these burdens.Deploying RAMMP and VJC chromatographic methodologies affords much shorter analysis times but increases the need for faster scanning MS acquisitions to maintain peak fidelity. Whilst traditional Tof based mass analysers enable fast acquisition speeds, generally their mass resolution capabilities are limited (10-90K FWHM). In comparison ion trap-based analysers offer higher mass resolution (>100K FWHM) but require longer scan times to achieve this elevated resolution making them less compatible with rapid analysis. The SELECT SERIES MRT system overcomes the limitations of orthogonal Tof and trapping mass analysers. Using Multi Reflecting Time-of-flight it achieves mass resolution of >200K FWHM at scan rates compatible with RAMMP and VJC methodologies.Data analysis of the entire batch of 112 injections was performed in a single polarity using both a RAMMP method and with VJC technology with the same LC parameters. VJC produced narrower chromatographic peaks (typical peak width 0.6s). The fast MS scanning for this acquisition enabled collection of >20 data points across these peaks, fully defining the analytical peak.Interrogation of the HILIC small molecule data by OPLS-DA determined significant differences in amino acids and biogenic amines, in particular depletion of branched chain amino acids and an increase in aromatic amino acids. For the lipid data, much lipid dysregulation was seen in the diseased patients with both increases and decreases seen particularly for LPA, LPC and PC lipid species.

Novel Aspect

Sophisticated OMIC data conversion workflows relating to multiple acquisition formats acquired using a novel, benchtop multi-reflectron ToF to generic format(s)

P14 Comparative analysis of lipid droplet accumulation in murine C3HeB/FeJ and C57BL/6 macrophages upon infection with *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (*Mtb*) is the intracellular bacterium responsible for tuberculosis (TB), infecting about one-fourth of the world's population, with 5-10% progressing to active disease. A hallmark of TB is granuloma formation in the lungs, a complex structure of epithelial, innate, and adaptive immune cells with infected macrophages at its core. These macrophages are the preferred host cell of *Mtb* and often exhibit a foamy phenotype due to increased lipid droplet (LD) accumulation, composed of neutral lipids, cholesterol esters, and triacylglycerols (TAG). While lipids are a key carbon source for *Mtb*, LD biogenesis, dynamics and their composition in primary macrophages are not well-studied.

No animal model fully replicates human TB disease and lung pathology because *Mtb* has co-evolved with humans and is adapted accordingly. However, animal models are crucial for studying TB. The commonly used C57BL/6 mouse model and its genetic knockouts show relative resistance to *Mtb*, differing in lung pathology. In contrast, the C3HeB/FeJ mouse strain is highly susceptible, rapidly developing active TB with centralized necrotic granulomas, hypoxia, and cavity formation.

This study compared LD formation in primary macrophages of C3HeB/FeJ and C57BL/6 mice infected with *Mtb* H37Rv. Bone-marrow-derived macrophages (BMDMs) from both strains were differentiated and infected with *Mtb* H37Rv at varying multiplicities of infection (MOI). Using a multi-mode quantitative microscopic analyser, we phenotypically analysed neutral lipids stained with Nile red in murine macrophages. Our data suggest that macrophage activation appears to be an early key event in neutral lipid accumulation, which commences within the first 24-hours post-infection. Additionally, macrophages infected with a low MOI for a longer duration also showed an increase in Nile red mean fluorescence intensity, pointing to an infection-dependent lipid droplet formation in murine macrophages during *Mtb* infection. While C3HeB/FeJ macrophages appear to have an inherently higher Nile red fluorescence compared to C57BL/6 macrophages, both mice principally react with an increase in Nile red fluorescence upon *Mtb* infection, although it varies in strength.

P15 Illuminating the Host-Microbe Interactome - Advanced Ultrastructural and Live Imaging of Membrane Damage and Lipid Flows in Mycobacterial Infections

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Tuberculosis (Tb) is caused by *Mycobacterium tuberculosis* and remains one of the most deadly infectious diseases. The World Health Organization (WHO) estimates that in 2021, Tb killed 1.6 million people emphasizing the importance to develop new drugs, vaccines and diagnostic tools to reduce this burden in the future.

M. tuberculosis employs multiple strategies to survive intracellularly. One of its most striking adaptations is its ability to utilize host lipids such as fatty acids and sterols to: (i) generate energy, (ii) build its characteristic lipid-rich cell wall and (iii) produce storage lipids during infection. To be constantly in a fatty acid-rich environment, the pathogen actively contributes to generate the “foamy” phenotype in host macrophages, for which the accumulation of host lipid droplets (LDs) is characteristic. Using the *Dictyostelium discoideum/M. marinum* infection system, we found that mycobacteria access host LDs to build up their own lipid storage organelles and exploit ER-derived phospholipids when LDs are lacking.

The Barisch lab aims to unravel the molecular mechanisms by which pathogenic mycobacteria acquire lipids from their host to support chronic infection. Combining the application of functionalized lipid probes with mass spectrometry-based lipidomics and advanced microscopy techniques, the group analyses metabolic lipid flows between mycobacteria and their host at the subcellular and ultrastructural level.

In addition, the lab investigates the role of endoplasmic reticulum (ER)-dependent membrane repair and other repair machineries during mycobacteria infection. For example, to investigate the formation of membrane contact sites between the ER and the *Mycobacterium*-containing vacuole (MCV), the group employs techniques such as 3D-correlative light and electron microscopy (CLEM) that include high-pressure freezing and transmission electron microscopy (EM)-tomography as well as serial block-face scanning EM. This, together with spinning disc live cell imaging and flow cytometry, uncovered that ER-dependent repair constitutes a host defence mechanism against intracellular pathogens such as *M. tuberculosis.*

P17 ANALYSES OF THE MYCOBACTERIAL LIPIDOME DURING INFECTION USING SHOTGUN LIPIDOMICS

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The infectious disease Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), has afflicted humans for thousands of years and continues to be a major health threat, causing over a million deaths worlwide every year. The fact that approximately one-third of the world's population is infected with *Mtb* highlights the pathogen's well-adapted interaction with its human host. Especially *Mtb*-specific lipids play a direct role in intracellular survival, host-pathogen interaction and virulence[1,2].

Tracing the alteration of the mycobacterial lipidome, especially during *Mtb* infection, contributes to the development of new diagnostic tools and treatments targeting the lipid components or its biochemical pathways. In this study, intracellular bacteria deriving from macrophage culture system are of interest to monitor adaptations in their lipidome. Four different lipid classes which are unique cell wall lipids of *Mtb* were investigated via a shotgun lipidomics approach: Phosphatidyl-*myo*-inositol mannosides, phenolic glycolipids, phthiocerol dimycocerosates and sulfolipids. Initial results focus on the establishment of the method, for which *M. bovis* BCG was used. For the experimental setup, it was also important to determine the number of bacteria required to measure a lipid profile using shotgun lipidomics. To this end, bacterial suspensions with different bacterial counts were prepared using Fluorescent Activated Cell Sorting and extracted using a one-pot extraction method. The mass spectrometric analysis was performed with an adapted semi-targeted method using the Q-Exactive Hybrid quadrupole-orbitrap mass spectrometer (Q Exactive Plus, Thermo Scientific).

1. Lerner, T. R., Queval, C. J., Fearns, A., Repnik, U., Griffiths, G., & Gutierrez, M. G. (2018). Phthiocerol dimycocerosates promote access to the cytosol and intracellular burden of Mycobacterium tuberculosis in lymphatic endothelial cells. *BMC Biology*, *16*(1). https://doi.org/10.1186/S12915-017-0471-6

2. Mishra, A. K., Driessen, N. N., Appelmelk, B. J., & Besra, G. S. (2011). Lipoarabinomannan and related glycoconjugates: structure, biogenesis and role in Mycobacterium tuberculosis physiology and host-pathogen interaction. *FEMS Microbiology Reviews*, *35*(6), 1126–1157. https://doi.org/10.1111/J.1574-6976.2011.00276.X

P18 Enhancing Data Quality in Untargeted Lipidomics: A Class-Specific Curation Approach Using LC-TIMS-HRMS

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Untargeted lipidomics analysis has recently seen significant advancements with the advent of Trapped Ion Mobility Spectrometry, increased availability of High-Resolution Mass Spectrometry instruments, and the development of sophisticated data processing packages. While these have democratized lipidomics, enabling many new scientists to take their first steps in this exciting research field, the inherent complexity of preparation, analysis, data processing and evaluation, can easily lead to under utilization of applied tools and unreliable results. Especially commonly applied (semi-)automated data processing tools, such as Bruker MetaboScape, msDial, and XCMS, claim comprehensive analysis from raw data to results, but produce datasets rife with over-annotations, duplicate entries, and low-quality signals. We believe, generated lipid compound tables, require a rigorous post-processing data curation process to ensure the integrity and utility of measured lipidomics data.

Our here presented workflow begins with rigeriously controlled UHPLC-tims-ToF Pro2 (Bruker) mass spectrometry data and a common pre-processing method in MetaboScape (Bruker), handling feature detection in both positive and negative ionization modes, as well as compound grouping (considering isotopes, adducts, MS1 and MS2 spectra, and mobilograms). Extracted lipid signals are then annotated using an in-house spectral library, Bruker's LipidSpecies detection, and LipidBlast.

The resulting crude lipid feature table requires a subsequent curation to remove false annotations, duplicates and low quality signals. We perform a quality control (QC) sample based filtering, annotation polishing and lipid class curation, in an custom R programming language-based Shiny App. Signals are filtered using Signal-to-Noise Ratio (S/N), Limit of Detection (LOD), and Precision (measuring variability across replicate injections). Additionally, a Dilution Correlation is used to retain signals that show consistent behavior across a dilution series. Lipid annotations are normalized and parsed against the LIPID MAPS database, followed by a cleanup of lipid nomenclature and shorthand notations.

Further, a class-specific curation method is employed. Kendrick Mass Defect (KMD) Analysis is used to create KMD plots for each lipid class to identify and confirm class-specific mass defects. Reference KMD Plots are calculated based on lipid head groups to identify discrepancies between the assigned class and measured accurate mass. Finally, an Retention Time-Correlation is analyzed to determine the correlation between retention times and a lipid's tail carbon content and double bond number to validate the assignment to a lipid class. Lipid signals that deviate from expected behavior are subjected to manual re-annotation or removal, polishing the initial compound table.

This class-specific curation method addresses common misannotations, such as distinguishing between phosphatidylethanolamine (PE) and phosphatidylcholine (PC), and corrects for processing artifacts. The curated dataset is robust, high-quality, and reliable, facilitating accurate and reproducible lipidomics research. Following data curation, our services can be tailored to provide actionable insights and drive scientific discovery, whether in lipidomics alone or in combination with other 'omics' approaches such as metabolomics, metagenomics, metatranscriptomics, and proteomics.

P19 Testing Novel Polypropylene Material for use in Shotgun Lipidomics

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Strong background signals can make shotgun lipidomics analysis extremely challenging and suppress less abundant lipid species. Therefore, a stable and reproducible background is essential for shotgun lipidomics experiments. In cooperation with Eppendorf, we tested new material for prototype reaction tubes used in analytical workflows for shotgun lipidomics and GC-MS.

In a first shotgun lipidomics experiment 1 mL of MS electrospray ionization solution, consisting of $CHCl_3$/MeOH/isopropanol 1/2/4 (v/v/v) with 0.05 mM ammonium acetate, were incubated at room temperature in prototypes as well as in standard Eppendorf Safe-Lock tubes as reference. Shotgun lipidomics was performed with nanoESI-MS/MS using a Q Excative Plus with a Triversa Nanomate as ion source. The fatty acid background in the prototypes and reference tubes was quantified and analyzed by alkaline hydrolysis and subsequent GC-MS measurement. For fatty acid quantification an Agilent Technologie GC-MS (5975) with XL Mass Selective Detector was used.

First shotgun lipidomics measurements indicate that in comparison to the reference tubes less background was detected. GC-MS measurements showed similar chemical background for standard fatty acid analysis between prototype and reference tubes.

P22 MASS SPECTROMETRY IMAGING GUIDED SEARCH FOR LIPID BIOMARKERS IN HUMAN SERUM SAMPLES

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Arteriosclerosis, the hardening and narrowing of arteries, is among the deadliest diseases in westernized countries and of fast growing importance in other nations. The disease starts from “fatty streaks”, which are caused by intracellular lipid accumulation that can develop to fibrotic and calcific layers, to the last stage of “complicated lesions” that show surface defects and lead to thrombosis.

Mass spectrometry imaging (MSI) showed the accumulation of specific lipids around these calcifications. This study extends these findings and shows that lysophosphatidylcholine (LPC) and other lipid oxidation products are localized in the core of atherosclerotic plaques.

Molecular biomarkers are essential for the diagnosis of human diseases, in the monitoring of recuperative processes and research applications.

In this work, we have established a method for the quantification of human serum lipids using UHPLC coupled with TIMS-TOF mass spectrometry, employing the Ultimate SPLASH ONE serum lipid standard from Avanti Polar Lipids. In addition, we have adapted and expanded the various pre-established methods (e.g. switching from C18 to C8 column material as well as the maintenance and cleaning of C18 and C8 UHPLC columns used for serum lipidomics) for this kind of analysis to accommodate the specific needs and requirements of our equipment.

In the future, we plan to apply this method to the global analysis of local lipid accumulations in atherosclerotic plaques from patients with advanced peripheral arterial occlusive disease. These lipids have been identified through MALDI imaging and will be globally searched in serum.

The goal is to identify specific lipids that could serve as biomarkers for the calcification status of the plaques, potentially facilitating the treatment of this condition.

P23 LipidSpace: Simple Exploration, Reanalysis, and Quality Control of Large-Scale Lipidomics Studies

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Currently, lipid analysis is gaining tremendous importance. Discoveries are made, resulting in a better understanding of biological membranes and signaling functions. With new mass spectrometers moving closer to 100 Hz, lipidomics has increased speed, so lipid data acquisition is way faster than data interpretation. As a result, thousands of datasets including lipidomes (sets of lipid species from individual samples) are generated. However, they cannot be that quickly analyzed, digested, and interpreted anymore without a proper analysis tool. To overcome this bottleneck, we developed LipidSpace [1], designed to mine lipidomes structural space, fostering the rapid comparison of hundreds of lipidomes. When considering the molecular similarity of all lipids, we can better understand which classes have the potential to compensate for changed lipid classes to sustain membrane homeostasis functionally. Similarities of complete lipidomes are computed to cluster them together. An entire hierarchical relationship of the lipidomes is reported. Additionally, study variables related to the samples, such as age, body mass index, or condition, can be added to the analysis. LipidSpace offers a function to quickly determine subsets of lipids across all lipidomes that can describe these study variables well. These lipids may act as putative biomarkers, helping to understand underlying mechanisms, or serving for quality control. Four built-in tutorials give an introduction to functions and methods such as feature analysis or quality control. We used this suite to reanalyze and combine already published datasets and made additional discoveries besides the published conclusions. To summarize, it is now possible to easily compare similar or distinct lipidomes and gain quick knowledge of which lipids are shaping a distinct lipidome, which lipids can be used to separate conditions or to identify the insufficient quality of measured datasets during an early stage of a study.

[1] Kopczynski, D., Hoffmann, N., ... & Ahrends, R. (2023). LipidSpace: Simple Exploration, Reanalysis, and Quality Control of Large-Scale Lipidomics Studies. Analytical Chemistry, 95(41), 15236-15244.

P24 Improving the Specificity of Tuberculostearic Acid Detection with Ion Mobility driven Tandem Mass Spectrometric Experiments (IMS^N) to Investigate Host Pathogen Interaction in Tuberculosis

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The cell wall of *Mycobacterium tuberculosis* (Mtb) contains the unique fatty acid (FA) 10-methylstearic acid called tuberculostearic acid (TSA) in different lipid classes but is absent in eukaryotic cells. Phosphatidylinositol (PI) 35:0 (16:0_19:0) was identified as the major phosphoglycerolipid of several Mtb-lineages [1] and it was utilized as a marker for mycobacterial burden in preclinical model systems and metabolic tracer [1,2]. In the course of animal experiments and the clinical study, we detected molecules containing FA19:0 in healthy controls. In-depth structural elucidation is not possible with our commonly applied shotgun lipidomics platform using nano-ESI-MS/MS (Q Exactive™ Plus Hybrid Quadrupol-Orbitrap™, Thermo, Bremen Germany, Nanomate Triversa, Advion, Ithaca, US). To decipher what isomeric forms of FA 19:0 are present we used the capabilities of ion mobility array of the Cyclic MS (Waters, Manchester, UK) [3]. It allows collision induced dissociation, before, within and after the IM array and enables high separation power for selected ions. We used several strategies to build multi-pass sequences (IMSn) to improve specificity and retain sensitivity for targeted FA19:0 analysis with and without precursor selection. To discriminate between lipids comprising mycobacterial derived FA19:0 (TSA) and isomeric FA19:0 we chose biochemically reasonable isomers 16-Me-18:0, 17-Me-18:0, 2-Me-18:0 and nonadecanoic acid (NDA), which collision cross sections only vary slightly from TSA (max Δ<0.7%). Using multipass Cyclic experiments with a total separation time of about 830 ms and exclusion of major abundant ions (*m/z* 255 – FA 16:0 and 283 FA 18:0) isomers could be partially separated.

To investigate the PI 16:0_19:0 (TSA) stability and its recycling into the host lipidome a cell culture system was used as model. We incubated bone marrow derived murine macrophages (BMDM) for 24, 72 and 144 h with rifampicin-inactivated Mtb H37rv. Afterwards, we performed a semi-targeted analysis for likely PI species built from FA 19:0 (TSA) that could be metabolic products of the macrophages or originate from the mycobacteria.

The FA19:0 from PI 16:0_19:0 and PI 20:4_19:0 in the control samples could be annotated to 17-Me-18:0 or NDA isomeric forms. In cell cultures where Mtb was added, the FA19:0 from PI 16:0_19:0 is identified as mainly TSA. All ion fragmentation showed the presence of 17-Me-18:0 or NDA as well.

Next, we will infect HMDM with fluorescently labeled Mtb H37ra allowing Fluorescent Activated Cell Sorting (FACS) (ARIAIII, BD) to select macrophages that phagocytized bacteria and the cells that did not. Aim is to investigate the lipidome of the macrophages after 4 h and 3 days of infection, with focus on lipid species containing FA19:0 with our continuously developing approaches.

1. Brandenburg, J, Heyckendorf J, Marwitz F et al., Tuberculostearic acid-containing phosphatidylinositols as markers of bacterial burden in tuberculosis. ACS Infect Dis, 2022

2. Brandenburg, J. et al. WNT6/ACC2-induced storage of triacylglycerols in macrophages is exploited by *Mycobacterium tuberculosis*. J Clin Invest, 2021

3. Giles, K et a l. A Cyclic Ion Mobility-Mass Spectrometry System. Anal Chem, 2019

P25 CRITICAL SHIFTS IN LIPID METABOLISM PROMOTE MEGAKARYOCYTE DIFFERENTIATION AND PROPLATELET FORMATION

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During megakaryopoiesis, megakaryocytes (MKs) undergo cellular morphological changes with strong modification of membrane composition and lipid signaling. Here, we adopt a lipid-centric multiomics approach to create a quantitative map of the MK lipidome during maturation and proplatelet formation. Data reveal that MK differentiation is driven by an increased fatty acyl import and de novo lipid synthesis, resulting in an anionic membrane phenotype. Pharmacological perturbation of fatty acid import and phospholipid synthesis blocked membrane remodeling and directly reduced MK polyploidization and proplatelet formation, resulting in thrombocytopenia. The anionic lipid shift during megakaryopoiesis was paralleled by lipid-dependent relocalization of the scaffold protein CKIP-1 and recruitment of the kinase CK2α to the plasma membrane, which seems to be essential for sufficient platelet biogenesis. Overall, this study provides a framework to understand how the MK lipidome is altered during maturation and the effect of MK membrane lipid remodeling on MK kinase signaling involved in thrombopoiesis.

P26 MATERNAL VITAMIN D-RELATED METABOLOME AND OFFSPRING RISK OF ASTHMA OUTCOMES

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Background
Gestational vitamin D deficiency is implicated in development of respiratory diseases in the offspring, but the mechanism underlying this relationship is unknown.

Objectives
To study the link between gestational vitamin D exposure and childhood asthma phenotypes using maternal blood metabolomics profiling.

Methods
Untargeted blood metabolomic profiles were acquired using liquid chromatography-mass spectrometry at one week postpartum from 672 women in the $COPSAC_{2010}$ mother-child cohort, and at pregnancy week 32-38 from 779 women in the VDAART mother-child cohort. In $COPSAC_{2010}$, we employed multivariate models and pathway-enrichment analysis to identify metabolites and pathways associated with gestational vitamin D blood levels and investigated their relationship with development of asthma phenotypes in early childhood. The findings were validated in VDAART and in cellular models.

Results
In $COPSAC_{2010}$, higher vitamin D blood levels at one week postpartum were associated with distinct maternal metabolome perturbations with significant enrichment of the sphingomyelin pathway ($p<.01$). This vitamin D-related maternal metabolic profile at one week postpartum containing 46 metabolites was associated with decreased risk of recurrent wheeze (Hazard Ratio (HR)=0.92 [95% CI, 0.86-0.98], p=.01) and wheeze exacerbations (HR=0.90 [0.84-0.97], p=.01) at age 0-3 years. The same metabolic profile was similarly associated with decreased risk of asthma/wheeze at age 0-3 in VDAART (OR=0.92 [0.85-0.99], p=0.04). Human bronchial epithelial cells treated with high-dose vitamin D3 showed an increased cytoplasmatic sphingolipid level ($p<0.01$).

Conclusion
This exploratory metabolomics study in two independent birth cohorts demonstrates that the beneficial effect of higher gestational vitamin D exposure on offspring respiratory health is characterized by specific maternal metabolic alterations during pregnancy, which involves the sphingomyelin pathway.

Keywords
Vitamin D, metabolomics, pregnancy, childhood asthma, sphingomyelin

P27 Exploring and Comparing Lipidomics Studies with LipidCompass

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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 metabolomics-platforms based on chromatography and high-resolution mass spectrometry, the need has increased for a central, well connected and comprehensive resource for both experimental and computational scientists.

With LipidCompass, we want to offer a FAIR resource to simplify the exploration of quantitative lipidomics data from different angles, following the structural hierarchy induced by the shorthand nomenclature, as established by LIPID MAPS [1, 2] and SwissLipids [3], refined by the recent update to the shorthand nomenclature for lipids. Lipidomics and Metabolomics tools that support mzTab-M [4] as an output format can submit their data to LipidCompass. Usually, additional reviewing and curation will be needed to map tool-specific CV parameters to canonical ones from NCIT, PSI-MS, NCBITaxon and other commonly used controlled vocabularies. Lipid names are being parsed using Goslin [5] to simplify mapping against LIPID MAPS, SwissLipids and other external databases. Automatic conversion allows for easier import and mapping of legacy data and reduces the risk of manual translation errors. LipidCompass provides comprehensive data exploration, comparison and interactive visualization features that simplify the detection of differences between samples within the same study, but, for the first time, also allow analysis and visualization of similarities and differences on a large scale between studies.

LipidCompass will be the central integration hub for multiple lipid-related web services, such as LipidXplorer [6] and LipidSpace [7] as part of the Lipidomics informatics for life-science (LIFS) project [8]. Further collaboration with the International Lipidomics Society interest groups on standardization and clinical lipidomics will integrate support with the upcoming lipidomics checklist. (Anonymized) results from clinical lipidomics ring trials may also be integrated into the database for interactive comparison of results.

[1] V. B. O'Donnell, E. A. Dennis, M. J. O. Wakelam, and S. Subramaniam, Sci Signal, vol. 12, no. 563, p. eaaw2964, Jan. 2019, doi: 10.1126/scisignal.aaw2964.

[2] G. Liebisch et al., J Lipid Res, vol. 61, no. 12, pp. 1539–1555, Dec. 2020, doi: 10.1194/jlr.S120001025.

[3] L. Aimo et al., Bioinformatics, vol. 31, no. 17, pp. 2860–2866, Sep. 2015, doi: 10.1093/bioinformatics/btv285.

[4] N. Hoffmann et al., Anal Chem, vol. 91, no. 5, pp. 3302–3310, Mar. 2019, doi: 10.1021/acs.analchem.8b04310.

[5] Kopczynski et al., Goslin 2.0 Implements the Recent Lipid Shorthand Nomenclature for MS-Derived Lipid Structures, Anal. Chem., pp. 6097–6101, Apr. 2022, doi: 10.1021/acs.analchem.1c05430

[6] R. Herzog, D. Schwudke, and A. Shevchenko, Curr Protoc Bioinformatics, vol. 43, p. 14.12.1-30, Oct. 2013, doi: 10.1002/0471250953.bi1412s43.

[7] D. Kopczynski et al., LipidSpace: Simple Exploration, Reanalysis, and Quality Control of Large-Scale Lipidomics Studies, Anal. Chem., pp. 15236-15244, Oct. 2023, doi: 10.1021/acs.analchem.3c02449.

[8] D. Schwudke, A. Shevchenko, N. Hoffmann, and R. Ahrends, Journal of Biotechnology, vol. 261, pp. 131–136, Nov. 2017, doi: 10.1016/j.jbiotec.2017.08.010.