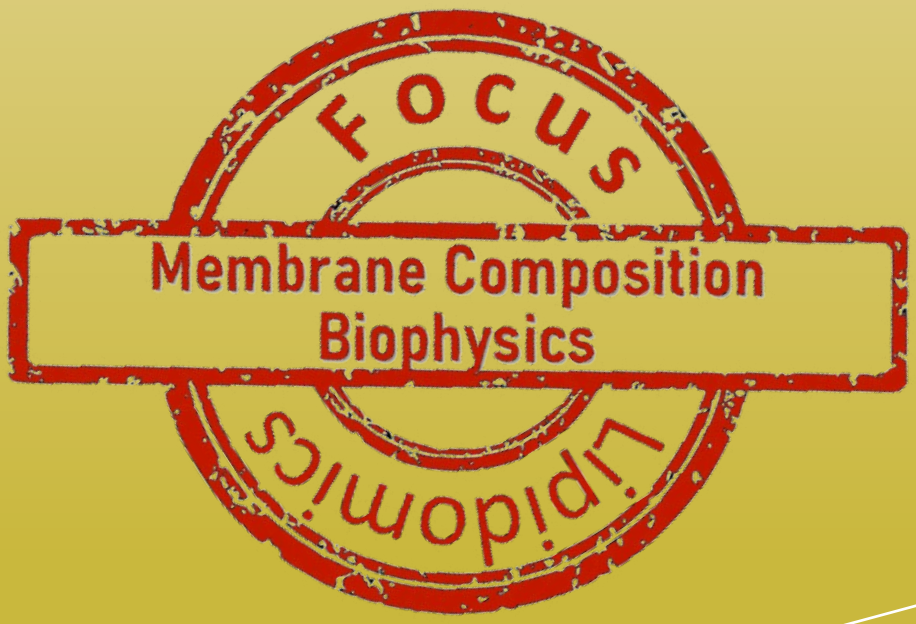
LIPIDOMICS FORUM



Research Center Borstel, Leibniz Lung Center

September 7th-9th, 2026

universität wien

Research Center Borstel
Leibniz Lung Center

DZIF

DGMS
Deutsche Gesellschaft für Massenspektrometrie

iLS

elixir
GERMANY

LIPIDOMICS
Informatics for Life Sciences

de.NBI
GERMAN NETWORK FOR BIOINFORMATICS INFRASTRUCTURE

Platin Sponsor

SCIEX

The power of precision

Gold Sponsors

Avanti Research
A CRODA BRAND

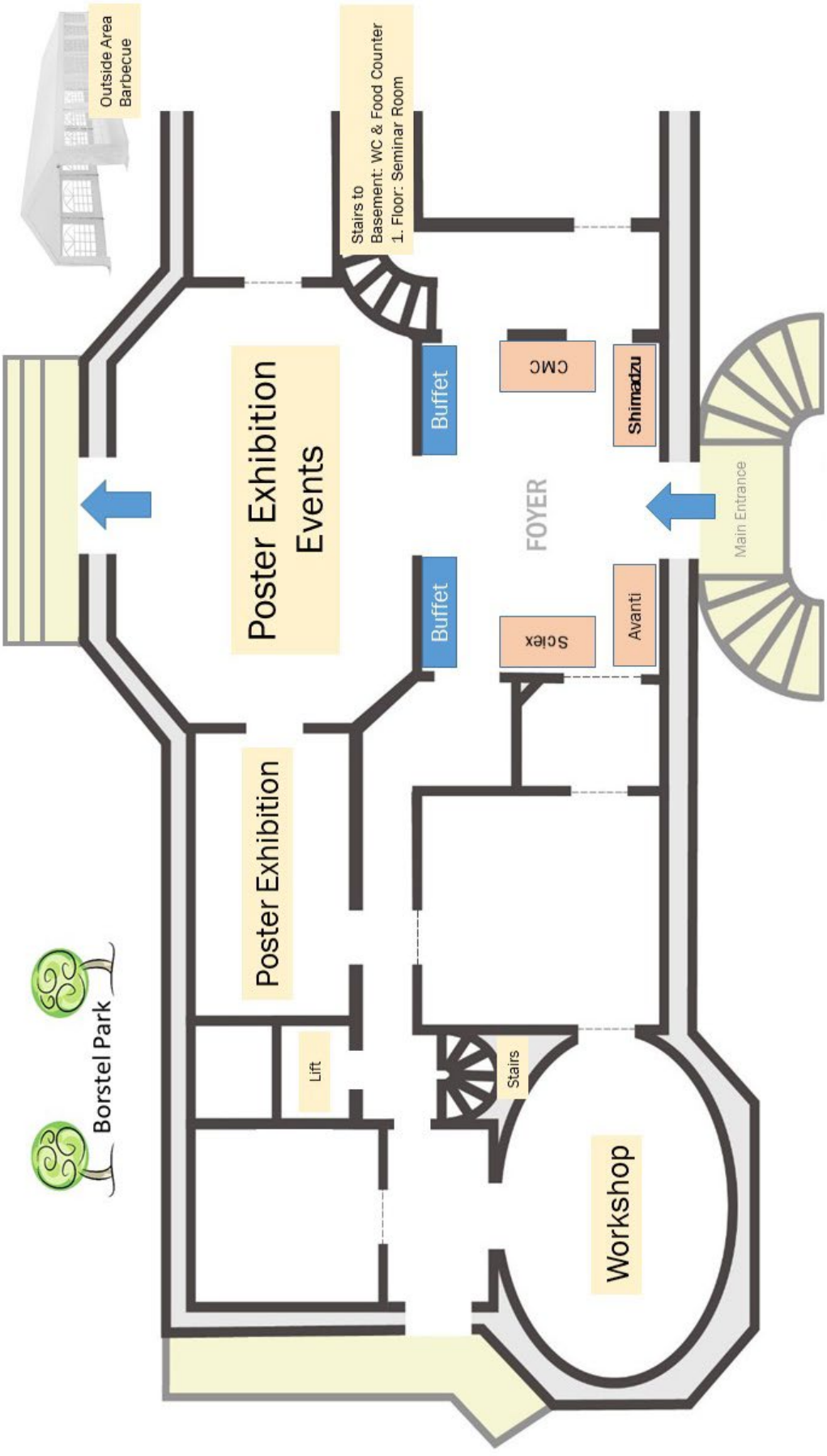
cmc Instruments GmbH

SHIMADZU
Excellence in Science

Organizers

Robert Ahrends, Uni Wien
Nicolas Gisch, RCB
Nils Hoffmann, CeBiTec – FZ Jülich
Dominik Kopczynski, Uni Wien
Dominik Schwudke, RCB

Location


Outside Area
Barbecue
Stairs to
Basement: WC & Food Counter
1. Floor: Seminar Room
Poster Exhibition
Events
Buffet
CMC
Shimadzu
FOYER
Main Entrance
Buffet
Sciex
Avanti
Poster Exhibition
Borstel Park
Lift
Stairs
Workshop

Program

Monday, 7. September 2026

10:00 - 16:30	**LIFS - Workshop**
16:00	Registration opens
18:00 - 19:00	Opening Keynote - **K1** Tracing lipid metabolism using alkyne lipids, click chemistry and mass spectrometry, *Christoph Thiele (LIMES, University Bonn, Germany)*
19:00	Dinner / Come Together **Music: Jazz Trio:** Jörg Hochapfel (Piano), John Hughes (Bass), Björn Lücker (Drums)
21:30	Bus transfer to hotels

Tuesday, 8. September 2026

approx. 8:30	Bus transfers
09:30 - 10:30	**T1** Methods for reconstitution of membranes and analysis of their interactions with antimicrobials, *Christian Nehls (Research Center Borstel, Germany)*
10:30 - 10:50	**ST1*** Developing a toolbox for investigating lipid and cell specificity of membrane active peptides on immune cells, *Aileen Kerfin et al. (Research Center Borstel, Germany)*
10:50 - 11:10	**ST2*** Uptake and incorporation of hydroxy fatty acids into polar lipids in human cells, *Luca M. Wende et al. (University of Wuppertal, Germany)*
11:10 - 11:30	Coffee Break
11:30 - 11:50	**ST3** Deciphering the phospholipidome of human lymphoma cells by LC-MS/MS utilizing mobility-resolved fragmentation, *Heiko Hayen et al. (University of Münster, Germany)*
11:50 - 12:10	**ST4** Lipids, extracellular vesicles and cancer cell aggressiveness, *Kathrin M. Engel et al. (Leipzig University, Germany)*
12:10 - 12:30	**ST5** Comprehensive isomer-resolved ganglioside profiling by HILIC-nanoESI-MS/MS, *Nina Troppmair et al. (University of Vienna, Vienna, Austria)*
12:30 - 13:00	**Sp1** Beyond lipid composition: unlocking lipid structural features using the ZenoTOF 8600 (Sciex), *Amirreza Dowlati et al. (University of Vienna, Austria)*
13:00 - 14:00	Lunch
14:00 - 15:00	**K2** Twists of fat in plasma membranes, *Georg Pabst (University of Graz, Austria)*

Program

15:00 - 15:20	**ST6** 'Lipid-docking' landscape of a membrane active peptide reveals novel targets in NLRP3 inflammasome activation in macrophages, *Andra B. Schromm (Research Center Borstel, Germany)*
15:20 - 15:40	**ST7** Does the Fenton reaction have marked physiological relevance (in the presence of salt)? - An in vitro MS study with unsaturated phospholipids, *Jürgen Schiller et al. (Leipzig University, Germany)*
15:40 - 16:00	Coffee Break
16:00 - 17:00	**K3** Computational toolkits for discovering and analysing lipid binding sites in membrane proteins, *Phillip Stansfield et al. (University of Warwick, United Kingdom)*
17:00 - 19:00	Poster Session
19:00 - 21:30	Come Together, Barbecue **Musical Guests:** Farmer and Firefighter Brass Band Hartenholm
21:30	Bus transfers to hotels

Wednesday, 9. September 2026

approx. 8:30	Bus transfers
09:30 - 10:30	**T2** Visualizing lipid distributions by mass spectrometry imaging – methods, applications, and challenges, *Sven Heiles (ISAS Dortmund, Germany)*
10:30 - 10:50	**ST8*** Characterizing lipid droplet formation in murine C3HeB/FeJ and C57BL/6 macrophages infected with Mycobacterium tuberculosis, *Linda Zemke et al. (Research Center Borstel, Germany)*
10:50 - 11:20	Coffee Break
11:20 - 11:40	**ST9*** The use of liposomes for in-depth assessment of the membrane activity of antimicrobial peptides, *Janina Louisa Nandy et al. (Research Center Borstel, Germany)*
11:40 - 12:40	**K4** Biophysical membrane properties are altered during ferroptosis execution, *Lohans Pedrera et al. (University of Cologne, Germany)*
12:40 - 13:00	**Closing Session**
13:00 - 14:00	**Lunch - Farewells - Transfers**
14:00 - 15:00	DGMS - FG Lipid Analysis and Lipidomics Meeting

* eligible for the Lipidomics Forum 2026 Poster and Talk Price

Index

* eligible for the Lipidomics Forum 2026 Poster and Talk Price

Poster Index

* eligible for the Lipidomics Forum 2026 Poster and Talk Price

K1

Tracing lipid metabolism using alkyne lipids, click chemistry and mass spectrometry

Authors: Christoph Thiele

Affiliations: Life and medical sciences institute (LIMES), University of Bonn, Germany

The high complexity of cellular lipidomes and of the underlying metabolic pathways requires powerful labeling and detection systems for systematic lipid tracing experiments. Alkyne lipids, containing a terminal triple bond, are tracers with favorable biological properties very similar to unlabeled natural counterparts. We have developed a labeling and detection system based on alkyne lipid tracers and specialized reporter molecules that confer high specificity and sensitivity to labeled metabolites. Tracers are added to living cells and metabolites are extracted in pulse-chase setups to achieve time resolution. Copper(I)-dependent click reaction between extracted lipids and dedicated mass spectrometry reporter molecules is followed by mass spectrometrical analysis. The reporter carries a positive charge leading to improved ionization and increased sensitivity. Uniform and predictable neutral loss-type fragmentation in tandem mass spectrometry leads to reliable identification and quantification of labeled metabolites by shotgun analysis followed by LipidXplorer data analysis. Parallel multi-labeling with several precursors, combined with multiplexed analysis enables efficient high-content tracing. The presentation will introduce the basic concepts and some recent applications in the analysis of triglyceride cycling.

T1
Methods for reconstitution of membranes and analysis of their interactions with antimicrobials

Authors: Christian Nehls

Affiliations: Division of Biophysics, Research Center Borstel, Leibniz Lung Center, Borstel, Germany

In membrane biophysics, model membranes have been used for decades to study the fundamental properties and interaction processes of biological membranes. Although technological advancements allow us to gain more and more insight into natural membranes in their beautiful complexity, the molecular basis of many processes can still only be accessed by reducing the complexity of the membrane in vitro. The appropriate level of complexity, the biophysical observation technique and consequently the membrane preparation technique need to be chosen with regard to the specific biological question.

This tutorial will (a) explore the conceptual considerations involved in selecting suitable model systems and (b) provide an overview of membrane reconstitution systems and preparation techniques. These range from classical systems, such as liposomes, monolayers and planar bilayers, to advanced systems, such as asymmetric membranes and microfluidics-driven preparation.

Finally, we will (c) elaborate on how biophysical techniques, such as fluorescence spectroscopy, atomic force microscopy and microfluidics, can elucidate the mode of action of antimicrobial compounds and support their development in combatting antimicrobial resistance.

Sp1
Beyond lipid composition: unlocking lipid structural features using the ZenoTOF 8600

Authors: Amirreza Dowlati Beirami [1,2], Evelyn Rampler [2]

Affiliations: [1] Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Waehringer Str. 42, 1090, Vienna, Austria,
[2] Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Str. 38, 1090, Vienna, Austria

Background: The biological functions of lipids and glycosphingolipids (GSLs) extend beyond their sum composition and are strongly influenced by their structural characteristics. These include headgroup and glycan composition, fatty acyl and ceramide composition, sn-position, and double-bond location. However, a comprehensive understanding of lipid biology remains dependent on analytical workflows capable of resolving this structural complexity and enabling high-confidence structural annotation [1].

Methods: Liquid chromatography–high-resolution mass spectrometry (LC–HRMS) workflows were established on the ZenoTOF 8600 platform to enable comprehensive structural lipidomics and glycolipidomics. Collision-induced dissociation (CID) and electron-activated dissociation (EAD) were employed as complementary fragmentation strategies for structural characterization of GSLs and bulk lipids [2]. The workflow was evaluated using biologically relevant applications, including glycosphingolipid profiling during human stem cell differentiation and comprehensive plasma lipidomics following a single-phase extraction protocol.

Results: EAD generated substantially richer structural information than CID, producing more diagnostically relevant fragment ions and significantly increasing confidence in structural annotation through automated rule-based interpretation. The workflow enabled comprehensive characterization of glycosphingolipid classes, including GM3, GM2, GM1, and GD1, while simultaneously supporting broad coverage of major plasma lipid classes, including PC, LPC, PE, LPE, PI, SM, DG, and TG. The high sensitivity of the ZenoTOF 8600 further enabled the detection of low-abundance GM3 species directly in plasma together with bulk lipids following single-phase extraction. Combined with high-resolution reversed-phase chromatography, EAD enabled structural elucidation of lipid isomers, including localization of double-bond positions in selected lipid species.

Conclusions: These complementary workflows demonstrate how integrating advanced chromatographic separation, EAD fragmentation, and high-sensitivity TOF-MS extends lipidomics beyond molecular composition toward routine structural lipidomics. This facilitates more confident lipid annotation and provides deeper biological insight across both glycosphingolipidomics and plasma lipidomics.

Referennces: [1] Beirami, A. D.; Rampler, E. Listening to cellular whispers through glycosphingolipids: a comprehensive review of recent advancements in glycosphingolipid analysis and annotation. Anal. Chem. 2026, 98 (16), 11577–11602. https://doi.org/10.1021/acs.analchem.5c08130.

[2] Pühringer, M.; Lamp, L. M.; Kolářová, D.; Vošmik, M.; Panzenboeck, L.; Köllensperger, G.; Holčapek, M.; Hartler, J.; Rampler, E. Resolving lipid isomers up to the double bond position level using reversed-phase chromatography, EAD fragmentation, and high-resolution mass spectrometry: application to pancreatic cancer. Anal. Chim. Acta 2026, 1398, 345225. https://doi.org/10.1016/j.aca.2026.34522.

K2
Twists of fat in plasma membranes

Authors: Georg Pabst [1,2,3]

Affiliations: [1] Biophysics, Institute of Molecular Biosciences (IMB), NAWI Graz, University of Graz, Humboldtstr. 50/III, 8010, Graz, Austria,
[2] Field of Excellence BioHealth, University of Graz, Graz, Austria,
[3] BioTechMed-Graz, Graz, Austria

Modern mass spectrometry based lipidomics allows us to catalogue the lipid constituents of biological membranes with remarkable precision, yet we still struggle to explain how these molecules work together to shape membrane organization and function. A central challenge lies in a fundamental duality between lipid chemical specificity and physical collectivity. Individual lipid species interact specifically with proteins as ligands, allosteric modulators, or structural scaffolds, while at the same time lipids act collectively to define bulk membrane properties — such as fluidity, thickness, and curvature stress—that modulate protein function without specific molecular recognition.

We address this challenge by focusing on membrane asymmetry—the unequal distribution of lipids across the bilayer—a defining feature of plasma membranes. Using a bottom up strategy, we construct plasma membrane mimics from a small, chemically well defined set of lipid species, selected on the basis of their headgroup and acyl chain structure. We fabricate organelle sized asymmetric lipid vesicles and quantify their bulk physical properties using small angle scattering and complementary biophysical methods. These measurements reveal that the collective properties of one leaflet can modulate those of the opposite leaflet, sometimes in non intuitive ways, highlighting asymmetry mediated interleaflet coupling.

To link asymmetry to function, we reconstituted the outer membrane phospholipase A (OmpLA) into asymmetric bilayers and quantified its activity. Lipid asymmetry strongly regulates OmpLA: activity is reduced in charge neutral asymmetric membranes but enhanced in charged asymmetric environments. Moreover, ions further tune enzymatic activity via interactions with lipid headgroups. Together, our results are consistent with allosteric modulation of protein function by changes in lateral stress profiles and show how chemically resolved lipid composition and leaflet specific lipid distribution converge to control membrane protein function, underscoring the need to integrate lipidomics with physical measurements of membrane organization.

K3
Computational toolkits for discovering and analysing lipid binding sites in membrane proteins

Authors: Katarina Blow, Matyas Parrag, Phillip Stansfeld

Affiliations: School of Life Sciences & Department of Chemistry, University of Warwick, Warwick, UK

Protein-lipid interactions are fundamental to the structure, dynamics, and function of membrane proteins, influencing processes ranging from folding and assembly to signalling, transport, and catalysis. While experimental characterisation of these interactions in native-like membrane environments remains challenging, computational approaches have become indispensable for identifying lipid-binding sites and elucidating their functional roles. Recent advances in AI-driven co-folding methods now enable prediction of holo protein structures containing bound lipids, small molecules, nucleic acids, and other cofactors, often capturing conformational changes associated with ligand binding. However, these structural predictions typically require substantial post-processing before they can be integrated into established molecular dynamics (MD) simulation workflows.

Here, we introduce CCD2MD, a modular suite of tools designed to bridge the gap between co-folded structural predictions and simulation-ready systems for GROMACS [1]. CCD2MD automates the conversion of co-folded complexes into both coarse-grained (CG) and atomistic representations, supporting simulations in solution and membrane environments. Although demonstrated here using protein–lipid complexes, the framework is readily extensible to systems containing nucleic acids, carbohydrates, metal ions, and small-molecule ligands. By streamlining system preparation, CCD2MD enables researchers to rapidly transform AI-generated structural models into robust MD simulations.

CCD2MD forms part of a broader ecosystem of computational tools developed for membrane protein research. MemProtMD enables automated insertion of membrane proteins into biologically relevant lipid bilayers and has been used to generate large-scale simulation datasets that reveal conserved lipid interaction patterns across diverse membrane protein families [2]. Complementing this, MemPrO and MemProD provide rapid prediction of membrane orientation, distortions and positioning directly from protein structure, facilitating automated membrane embedding of newly predicted protein complexes [3]. Together, these tools support efficient construction of biologically realistic membrane simulation systems.

For multiscale modelling, CCD2MD integrates naturally with CG2AT, allowing equilibrated coarse-grained systems to be converted into atomistic representations for detailed mechanistic investigation [4]. This approach combines the efficiency of coarse-grained simulations with the resolution of atomistic models, enabling exploration of lipid-binding events across biologically relevant timescales.

The resulting simulation trajectories can be analysed using tools such as PyLipID [5] and LipIDens [6], which provide complementary approaches for identifying and characterising lipid-binding sites. These platforms enable quantitative analysis of lipid occupancies, residence times, enrichment profiles, and interaction networks, facilitating the discovery of functionally important lipid-binding regions and the comparison of lipid specificities across protein families.

References:
[1] Blow, JCIM, 2025.
[2] Stansfeld, Structure, 2015.
[3] Parrag, JCTC, 2026.
[4] Vickery, JCTC, 2021.
[5] Song, JCTC, 2022.
[6] Ansell, Nature Communications, 2023.

T2
Visualizing lipid distributions by mass spectrometry imaging – methods, applications, and challenges

Authors: Sven Heiles [1, 2]

Affiliations: [1] Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V., 44139 Dortmund, Germany,
[2] Lipidomics, Faculty of Chemistry, University of Duisburg-Essen, 45141 Essen, Germany

Mass spectrometry imaging (MSI) has transitioned from niche analytic applications to a very active research field primarily being used in the field of life sciences. Whereas MSI workflows are capable to visualize the distribution of many biomolecular compound classes such as peptides and proteins, it is primarily used to obtain metabolic and lipidomic maps of biological samples complementing well-established biochemical imaging methods such as fluorescence microscopy of proteins. The range of MSI applications span from whole animal body imaging to cartograph metabolic/lipidomic maps of organisms, over histopathological fingerprinting of tissues to assist disease characterization, to subcellular MSI to track fundamental biological and biochemical processes.

In this tutorial, basics, applications, and current challenges of selected MSI methods in the field of lipid imaging are detailed. The first part will introduce the basic principles of MSI technologies, primarily focusing on sample preparation, instrumentation, and sampling schemes. Due to the broad range of MSI methods that exist to date, this tutorial will be limited to the main MSI platforms, i.e., desorption electrospray ionization (DESI), matrix-assisted laser desorption/ionization (MALDI), and secondary ion mass spectrometry (SIMS). This will be followed by introducing current data analysis pipelines for MSI, quality control measures, and reporting guidelines for MSI lipid data. In the last part of the tutorial, a subjective selection of current applications of MSI will be highlighted and current challenges will be detailed to motivate discussions.

K4

Biophysical membrane properties are altered during ferroptosis execution

Authors: Lohans Pedrera, Sara Lotfipour Nasudivar, Eric Pérez, Uris Ros, Ana J. García-Sáez

Affiliations: Membrane Dynamics, Max Planck Institute of Biophysics, Frankfurt, Germany

Ferroptosis is a form of regulated necrosis characterized by the iron-dependent accumulation of lipid peroxides in cellular membranes; however, the specific biophysical membrane properties affected during this process and how these alterations integrate into the molecular machinery of ferroptosis remain key unanswered questions. To address this, we combined quantitative live-cell confocal microscopy with several complementary biophysical methods to systematically investigate changes in membrane biophysics upon ferroptosis induction. Our data reveal that ferroptosis execution is a multi-step process driven by a defined sequence of biophysical alterations. We delineated a precise timeline in which initial lipid peroxidation directly increases water permeation into the hydrophobic membrane core, leading to membrane solvation and calcium influx into the cytosol. This osmotic influx drives a cascade of secondary events, including cell rounding and phosphatidylserine externalization. The plasma membrane subsequently detaches from the underlying actin cortex, resulting in the formation of a single massive swelling bleb that is a morphological hallmark of ferroptosis. This stage is accompanied by a reorganization of membrane components into immobile fractions, a reduction in lipid packing density specifically within bleb-associated membrane regions, and a decrease in the mechanical resistance of the plasma membrane to breakthrough. The process culminates in complete cellular collapse, characterized by the irreversible loss of lipid asymmetry and membrane integrity. We propose that this stepwise disruption of membrane integrity is the fundamental driver of the loss of membrane functionality that ultimately defines ferroptotic death.

ST1*

Developing a toolbox for investigating lipid and cell specificity of membrane active peptides on immune cells

Authors: Aileen Kerfin[1], Erik Winzheim[1], Jonas Engelhardt[2], Jochen Behrends[3], Günther Weindl[2], Andra Schromm[1]

Affiliations: [1] Division of Immunobiophysics, Priority Area Infections, Research Center Borstel, Leibniz Lung Center, Borstel, Germany,
[2] Pharmaceutical Institute, Section Pharmacology and Toxicology, University of Bonn, Bonn, Germany,
[3] Fluorescence Cytometry Science & Technology Unit, Research Center Borstel, Leibniz Lung Center, Borstel, Germany

The emergence of antibiotic-resistant bacteria and the lack of new antibiotics reduce therapeutic options to severe diseases such as pneumonia, COPD, and sepsis. Antimicrobial peptides (AMPs) are candidates to replace or supplement antibiotic treatment. Peptides directly target pathogens by binding to microbial membranes and altering the membrane structural and physical properties, ultimately leading to membrane destabilization and cell death. Additionally, to the antimicrobial effect, antimicrobial peptides have an anti-inflammatory effect on host cells. Accordingly, the dual mode of action makes antimicrobial peptides a promising alternative to conventional antibiotic therapies.

Recently, we discovered a new pathway of modulating immune cell activation via an interaction of peptides with the host cell membrane [1]. Here, we present a systematic analysis of the interaction of the LPS-neutralizing peptide Pep19-2.5 with eukaryotic model membranes and human immune cells (PBMCs). In flow cytometry experiments, we demonstrate a binding preference of Pep19-2.5 to the monocyte fraction in PBMCs. To characterize the specificity of membrane interaction we are developing a toolbox to investigate binding, integration, and biophysical effects of Pep19-2.5 by Zetasizer (DLS), fluorescence resonance energy transfer (FRET), fluorescence polarization, and flow cytometry on liposomes and membrane coated microspheres. Cellular peptide localization was investigated by confocal microscopy. Our data demonstrate hydrophobic and electrostatic membrane interaction of Pep19-2.5 resulting in membrane stiffening in reconstituted membranes and life cells. The biophysical changes induced by Pep19-2.5 on the eukaryotic membrane are suitable to effect immune signaling pathways and provide insights into the mechanisms of immune control by membrane active peptides.

References: [1] Schromm, PNAS U S A, 2021.

ST2*

Uptake and incorporation of hydroxy fatty acids into polar lipids in human cells

Authors: Luca M. Wende, Jonas Ditzler, Nils Helge Schebb

Affiliations: Food Chemistry, School of Mathematics and Natural Sciences, University of Wuppertal, Germany

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 (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The enzymatic oxidation of PUFA can be catalyzed by lipoxygenases (LOX), cyclooxygenases or CYP450 monooxygenases leading to a wide range of different biologically active oxylipins such as prostaglandins and leukotrienes. The effects of oxylipins are mainly associated with their free i.e., non-esterified form although the majority in cells occurs esterified, bound in phospholipids or other lipids. However, the biological role of esterified oxylipins is still unknown. In addition, it is not clear in which cellular lipids oxylipins are bound.

Currently, the best option for quantification of esterified oxylipins in biological samples is 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 during the saponification step. The combination of this method with an initial lipid class fractionation enables pinpointing the distribution of oxylipins in different lipid classes [3]. Moreover, direct analysis of oxidized phospholipids allows to uncover the most abundant molecular lipid species in which oxylipins are integrated [4].

In the presentation we show the distribution and uptake of supplemented LOX products in HEK293 cells in comparison to the immune cell line Mono-Mac-6. The results demonstrate a striking structure-specific integration of oxylipins into lipid classes and species. Moreover, the comparison of the two cell lines reveals cell specific differences in oxylipin uptake and lipid integration. These findings raise important new questions about the regulation and biological function of the esterified oxylipin pool in human cells.

References:
[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] Wende, L. M., et al., J. Lipid. Res., 2025, 67, 100950.
[4] Carpanedo, L., et al., Anal. Chim. Acta., 2024, 1326.

ST3

Deciphering the phospholipidome of human lymphoma cells by LC-MS/MS utilizing mobility-resolved fragmentation

Authors: Dominik Wieland [1], Anja Schmitt [2], Stephan Hailfinger [2], Heiko Hayen [1]

Affiliations: [1] University of Münster, Institute of Inorganic and Analytical Chemistry, Corrensstraße 48, 48149 Münster, Germany,
[2] University Hospital Münster, Department of Medicine A, Department of Hematology, Oncology and Pneumology, Münster, Germany

Several diseases can be associated with lipid alterations, spanning from shifts in double bond positions to significant remodeling of the lipidome. Human lymphoma cell lines react differently to induction of ferroptosis, an iron mediated cell death, based on lipid per-oxidation. However, the precise biological processes and lipid species involved still remain unclear. Phospholipids, as major building blocks of the cellular membrane, are the main lipid category contained in human cells. Especially ether-linked phospholipids with highly unsaturated fatty acyl moieties are suspected to play a key role in ferroptotic processes. The molecular diversity and subtle changes between lipid species necessitate the utilization of powerful hyphenated mass spectrometric techniques for their analysis.

Human lymphoma cells were cultivated and lipids extracted according to MATYASH et al. [1] These extracts were analyzed utilizing reversed phase liquid chromatography coupled to trapped ion mobility spectrometry (TIMS), enabling the separation of selected constitutional isomers. Mass spectrometric measurements were conducted on a quadrupole time of flight mass spectrometer (Q-TOF-MS) utilizing mobility resolved fragmentation. The resulting diagnostic MS/MS spectra, along with ion mobility values, facilitated confident lipid assignment. Combined with retention time and accurate mass, this four-dimensional data set was processed with MetaboScape software, enabling unequivocal lipid annotation. Manual validation of the data was conducted via lipid class specific four-dimensional Kendrick mass plots as well as confidence scores generated by MetaboScape software.

This methodology was implemented across eleven different human lymphoma cell lines with varying susceptibility towards ferroptosis. For each cell line, six biological replicates were included to mitigate potential deviations from cell cultivation. The workflow enabled confident identification of 263 individual lipid species spanning 10 phospholipid and 2 sphingolipid subclasses, including the resolution of 63 isomeric species at the fatty-acyl compositional level. Observed variations in ferroptosis susceptibility may be linked to changes in the phospholipidome. Therefore, multivariate data analysis strategies like hierarchical clustering heatmaps, principal component analysis, or volcano plots were applied to explore peculiar changes in species level lipid composition.

References: [1] Matyash, V.; Liebisch, G.; Kurzchalia, T.; Shevchenko, A.; J. Lipid Res. 2008, 49, 1137–1146.

ST4

Lipids, extracellular vesicles and cancer cell aggressiveness

Authors: Gabriel E. Lampert [1], Venkatesh K. Chetty [2], Kathrin M. Engel [1]

Affiliations: [1] Institute of Medical Physics and Biophysics, Faculty of Medicine, Leipzig University, Leipzig, Germany,
[2] Exosome Research Group, Department of Pediatrics III, University Hospital Essen, Essen, Germany

Lipid metabolism is a critical contributor to cancer development and progression, as it influences cell signaling, membrane dynamics, and tumor aggressiveness. Extracellular vesicles (EV), mediators in intercellular communication, carry distinct lipid signatures that may serve as promising biomarkers. As breast cancer is the most frequently diagnosed malignancy in women and one of the leading causes of cancer-related deaths, elucidating the molecular mechanisms underlying its pathogenesis is essential for developing effective interventions.

Two breast cancer cell lines, MCF7 and MDA-MB 231, were used to investigate the lipid composition in non-metastatic and metastatic contexts, respectively, alongside HEK 293 as a non-cancerous control cell line. EV were isolated by size exclusion chromatography. Lipids from cells and EV were extracted by a modified Bligh and Dyer method, separated by high performance thin layer chromatography (HPTLC) and analyzed via electrospray ionization (ESI) MS. Targeted lipid identification was carried out using predefined transition lists in Skyline. Sum peak areas were calculated for each lipid species for relative quantification.

Our approach revealed that (I) the lipid composition of the cells differs among each other and that (II) EV exhibit lipid profiles distinct from their parental cells. PCA analyses showed a clear separation of the two cancer cell lines from HEK cells as well as the respective EV. Differences were detectable in the polar and the apolar lipid fractions of all cell line-derived samples compared to their respective EV. The nature and the fatty acyl composition of lipids differed significantly between HEK, MCF7 and MDA-MB 231 cells and their EV regarding chain length and the degree of saturation.

In conclusion, our lipidomics approach revealed distinct differences across non-metastatic and metastatic breast cancer cell lines and their respective EV in comparison to a healthy control model, underscoring the potential of advanced lipidomics strategies to provide novel insights into cancer biology.

ST5

Comprehensive isomer-resolved ganglioside profiling by HILIC-nanoESI-MS/MS

Authors: Nina Troppmair [1,2], Jonas Santol [3,4,5], Mark Baumert [6], Lukáš Opálka [7], Alice Assinger [3,8], Cristina Coman [1*], Robert Ahrends [1*]

Affiliations:
[1] Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria,
[2] Vienna Doctoral School in Chemistry, University of Vienna, Vienna, Austria,
[3] Department of Vascular Biology and Thrombosis Research, Center of Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria,
[4] Department of Surgery, HPB Center, Vienna Health Network, Clinic Favoriten and Sigmund Freud Private University, Vienna, Austria,
[5] Division of Hepatobiliary and Pancreas Surgery, Department of Surgery, Mayo Clinic, Rochester, MN, USA,
[6] Advion Ltd, Harlow Enterprise Hub, Harlow, United Kingdom,
[7] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic,
[8] Ludwig Boltzmann Institute of Cardiovascular Research, Vienna, Austria,
[*] contributed equally and thus share last authorship

Gangliosides are structurally complex glycosphingolipids that regulate cellular signaling via interactions with extracellular binding partners and by influencing protein function within the membrane.[1, 2] Their comprehensive analysis remains analytically challenging, in part due to low endogenous abundance, extensive structural diversity, and the frequent occurrence of isomeric species arising from both glycan and ceramide moieties.[3] Here, we present a hydrophilic interaction liquid chromatography (HILIC) nanoelectrospray ionization tandem mass spectrometry (nanoESI-MS/MS) method specifically designed to achieve isomer-resolved analysis and molecular species-level quantification of gangliosides. To improve characterization of low-abundance fatty acyl fragments, the workflow was extended by combining nanoESI with online fraction collection and subsequent direct infusion. The method demonstrated stable chromatographic performance, high sensitivity, and reproducible quantitative results across ten mouse tissues. Application of the workflow enabled quantification of 80 ganglioside molecular species belonging to 18 subclasses and revealed pronounced tissue-specific differences in subclass distribution and ceramide composition. Overall, this study establishes a robust and sensitive platform for comprehensive ganglioside analysis, providing new opportunities to investigate ganglioside diversity, regulation, and function in complex biological systems.

References:
[1] Kolter, ISRN Biochemistry, 2012.
[2] Cantù; Del Favero; Sonnino; Prinetti, Chem Phys Lipids, 2011.
[3] Barrientos; Zhang, Analytica Chimica Acta, 2020.

ST6

‘Lipid-docking’ landscape of a membrane active peptide reveals novel targets in NLRP3 inflammasome activation in macrophages

Authors: Andra B. Schromm

Affiliations: Division of Immunobiophysics, Priority Area Infections,
Research Center Borstel, Leibniz Lung Center, Borstel, Germany

Macrophages are first-line innate immune cells and crucial in the recognition of pathogens and infections. Pattern-recognition receptors at the cytoplasmic membrane and in intracellular compartments are highly activated by microbial lipids such as endotoxins (lipopolysaccharide, LPS) from Gram-negative bacteria and by cellular danger-signals (DAMPs). Macrophage activation is part of their physiological defense function, but an overreaction of the cells leads to hyper-inflammation and is a key factor in the pathogenesis of severe diseases.

We are investigating the diverse mechanisms of antimicrobial peptides (AMPs) to balance macrophage activation and have discovered that AMPs can regulate the immune response to endotoxin via direct targeting of the immune cell cytoplasmic membrane [1]. Recent studies on the activation of the intracellular NLRP3 inflammasome have identified unexpected targets of an immune regulatory peptide and revealed a novel mechanism of intracellular NLRP3 inhibition. A step-by step analysis of the NLRP3 inflammasome activation sequence was performed to identify the peptides targets. We employ advanced macrophage mimicry model systems and model membranes with tunable properties (i.e. charge, fluidity, and lipid composition) [1,2] to generate a detailed picture of AMP interactions, selectivity and biophysical consequences on immune cell membranes. These studies revealed binding of the peptide to a marker lipid within cells, leading to membrane alterations and inhibition of a crucial step in inflammasome assembly, putting a break on inflammatory mediator production in vitro and in vivo [3]. Our findings establish AMPs as potent membrane organizers in immune cells, highlighting their potential to orchestrate and fine-tune inflammatory responses at multimodal levels. The presentation will specifically discuss tools to identify peptide lipid-specificity, tracing and biophysical effects on cell membranes in these studies.

References:
[1] Schromm et al., PNAS, 2021.
[2] Liebold et al., Science, 2024.
[3] Engelhardt et al., Advanced Science, 2026 (accepted).

ST7

Does the Fenton reaction have marked physiological relevance (in the presence of salt)? - An in vitro MS study with unsaturated phospholipids

Authors: Johanna Scheiding, Jenny Leopold, Kathrin M. Engel, Jürgen Schiller

Affiliations: Faculty of Medicine, Institute for Medical Physics and Biophysics, Leipzig University, Leipzig, Germany

The pathways of (phospho)lipid oxidation require much more detailed exploration because many significant inflammatory diseases (such as atherosclerosis and arthritis) [1] are characterized by elevated levels of lipid (per) oxidation products. The Fenton reaction, i.e. the decomposition of H_2O_2 catalyzed by transition metals ($Cu1^+$ or Fe^{2+}), is used in many laboratories to generate hydroxyl radicals (HO•), and it is also assumed to play a major role in vivo. Recently, ferroptosis, an iron-dependent form of cell death, has attracted significant interest and is being investigated by many research groups. [2]

We have demonstrated that HO• radicals primarily convert lipids with at least one unsaturated fatty acyl residue into (hydro)peroxides. These transient primary products are subsequently converted into more stable chain-shortened aldehydes and carboxylic acids. In contrast, chlorohydrins are generated by treating unsaturated phospholipids with HOCl leading to the addition of HOCl to the double bond as main product. [3] The current opinion is that increased levels of myeloperoxidase (MPO), the enzyme that produces HOCl from H_2O_2 and chloride, are required for the generation of chlorohydrins. [4]

Using MALDI and ESI MS, we could demonstrate that halogenated products, particularly chlorohydrins and, to a lesser extent, dichlorides, are the primary byproducts of the Fenton reaction when performed in the presence of NaCl. In contrast, (hydro)peroxides and their derivatives are generated in the absence of NaCl. Thus, the choice of initiator ($FeCl_2$ or $FeSO_4$) significantly affects the outcome. These results call into question the in vivo relevance of the Fenton reaction because physiological systems always have an excess of NaCl which leads to the conversion of the generated hydroxyl radicals into HOCl. Thus, HOCl is a more relevant in vivo oxidant. This has been demonstrated already in early radiation experiments. [5]

References:
[1] Leopold, Schiller, Antioxidants (Basel), 2024.
[2] Lee, Roh, Cancer Metastasis Rev., 2025.
[3] Schröter, Schiller, Oxid Med Cell Longev., 2016.
[4] Winterbourn, Toxicology, 2002.
[5] Saran, Bertram, Bors, Czapski, Int J Radiat Biol., 1993.

ST8*

Characterizing lipid droplet formation in murine C3HeB/FeJ and C57BL/6 macrophages infected with *Mycobacterium tuberculosis*

Authors: Linda Zemke [1], Nadine Aboutara [2], Dominik Schwudke [2,3,4], Gareth Prosser [1,4], Norbert Reiling [1,4]

Affiliations:
[1] Microbial Interface Biology, Research Center Borstel, Leibniz Lung Center, 23845 Borstel, Germany,
[2] Bioanalytical Chemistry, Research Center Borstel, Leibniz Lung Center, 23845 Borstel, Germany,
[3] Research Center Borstel, Airway Research North (ARCN), German Center for Lung Research (DZL), 23845 Borstel, Germany,
[4] German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, 23845 Borstel, Germany

Mycobacterium tuberculosis (Mtb), the intracellular bacterium causing tuberculosis (TB), infects about one-fourth of the global population, with 5-10% progressing to active disease. Granuloma formation in the lungs – a hallmark of TB – comprises complex structures of epithelial, innate, and adaptive immune cells centred around infected macrophages. These macrophages serve as the primary host cell for *Mtb* and often exhibit a foamy phenotype due to the increased accumulation of lipid droplets (LD), which are composed of neutral lipids such as cholesterol esters and triacylglycerols. While lipids are a key carbon source for *Mtb*, LD biogenesis, dynamics, and their composition in primary macrophages are not well-studied.

Animal models remain crucial for studying disease mechanisms. No animal model fully replicates human TB disease and lung pathology and commonly used mouse strains differ markedly in their susceptibility and pulmonary responses to an infection with *Mtb*: C57BL/6 mice show relative resistance and exhibit a pathology that differs from that observed in humans, whereas C3HeB/FeJ mice are highly susceptible and show a prominent increase in bacterial numbers associated with centralized necrotic granulomas, hypoxia, and cavity formation.

In this study, we compared the responses of primary macrophages derived from C3HeB/FeJ and C57BL/6 mice following infection with *Mtb*. Analysis revealed that early macrophage activation is closely linked to an increased presence of neutral lipids within the first day of infection, while prolonged infection at lower bacterial burdens also promoted lipid droplet accumulation. Application of high content imaging revealed macrophages from C3HeB/FeJ mice to exhibit an inherently higher presence of neutral lipids compared to cells from C57BL/6 mice. Additionally, shotgun lipidomics identified distinct differences in the global lipidome of both macrophage populations. Ongoing work focuses on detailed phenotypic and metabolomic characterization to identify the molecular mechanisms underlying these prominent strain-specific differences in lipid metabolism and their potential impact on *Mtb* pathogenesis.

ST9*

The use of liposomes for in-depth assessment of the membrane activity of antimicrobial peptides

Authors: Janina Louisa Nandy [1], Rieke Bruns [1], Marcus Fletcher [2], Kareem al Nahas [3], Niels Röckendorf [4], Thomas Gutsmann [1], Christian Nehls [1]

Affiliations: [1] Research Center Borstel, Leibniz Lung Center, Division of Biophysics, Borstel, Germany,
[2] Imperial College London, Department of Chemical Engineering, London, UK,
[3] Max Planck Institute of Biochemistry, Munich, Germany,
[4] Research Center Borstel, Leibniz Lung Center, Division of Immunology and Cell Biology, Borstel, Germany

Antimicrobial resistance is a rapidly increasing global concern and has the potential to become a significant public health crisis in the future. Antimicrobial peptides (AMPs) offer a promising alternative to conventional antibiotics. We utilized an evolutionary algorithm to systematically design, rank, and optimise a series of synthetic AMPs targeting specific bacterial strains with a single-liposome assay to investigate their membrane permeabilization activity. Giant unilamellar vesicles (GUVs) mimicking bacterial membranes were generated by octanol-assisted liposome assembly, immobilized in a microfluidic device and exposed to AMPs under continuous flow. Membrane permeabilization was monitored using a dye-release assay with single-vesicle resolution. Beyond conventional rupture-based analysis, we implemented a rate-resolved approach by quantifying fluorescence loss rates from time-resolved intensity traces, revealing heterogeneous permeabilization kinetics across liposome populations. This allowed us to precisely classify compound-specific permeabilization kinetics into a kinetic fingerprint.

With this approach, we a) evaluate the permeabilizing efficacy of AMPs, b) classify their mode of action in combination with other methods, and c) demonstrate the potential of individual liposome trapping for standardized compound testing.

P1*

Improving lipid identification and quantification: chromatogram deconvolution for LCMS/MS workflows

Authors: Felix Niedermaier [1, 2], Denise Wolrab-Frühauf [1], Robert Ahrends [1], Dominik Kopczynski [1]

Affiliations: [1] Institute of Analytical Chemistry, University of Vienna, Vienna, Austria,
[2] Doctoral School of Chemistry (DoSChem), University of Vienna, Vienna, Austria

Lipidomics relies on mass spectrometry-based workflows to identify and quantify complex lipid species. Due to the modular architecture of lipids, including headgroups, backbones, and fatty acyl chains, distinct precursor ions often produce isobaric or identical fragment ions. This problem is amplified in data-independent acquisition (DIA), where wide isolation windows (e.g., 25 Da) allow co-eluting precursors with different *m/z* values to generate highly chimeric MS/MS spectra. Consequently, fragments originating from multiple precursors, including isobars, isomers, and lipid-class-specific ions, are merged into a single MS/MS spectrum. Current lipid identification strategies often process such chimeric spectra in an uncontrolled manner, assigning them to one or more candidate lipids, thereby increasing false-positive identifications. Here, we introduce an algorithm that deconvolutes chimeric MS/MS spectra and chromatograms by exploiting their temporal correlation with associated precursor chromatographic profiles, independent of elution peak shape. Using simulated and real experimental lipidomics data, we demonstrate that this approach substantially improves lipid fragment assignment, reduces false-positive identifications, and enables more reliable fragment-level quantification, leading to more robust downstream statistical analyses and biological interpretations.

P2
Enabling FAIR comparative lipidomics with LipidCompass

Authors: Nils Hoffmann [1,2,3], Dominik Kopczynski [2], Nina Troppmair [2], Magdalena Figlmüller [2], Alexander Wenger [2], Felix Niedermaier [2], Denise Wolrab [2], Cristina Coman [2], Franziska Nicolaus [1,3], Sanjay Srikakulam [1], Yu Xia [4], Judith Simcox [5], Federico Tesio Torta [6], Xianlin Han [7], Erin Baker [8], Ruth Welti [9], Jeffrey McDonald [10], Oscar Pastor Rojo [11], Peter Meikle [12], Christian Klose [13], Christer Stenby Ejsing [14], Gerhard Liebisch [15], Kim Ekroos [16], Dominik Schwudke [17], Robert Ahrends [2]

Affiliations:
[1] Forschungszentrum Jülich, Jülich, Germany,
[2] Universität Wien, Vienna, Austria,
[3] BIBI, Bielefeld University, Bielefeld, Germany,
[4] Tsinghua University, Beijing, China,
[5] University of Wisconsin-Madison, Madison, WI, USA,
[6] Yong Loo Lin School of Medicine, Singapore,
[7] UT Health San Antonio, TX, USA,
[8] University of North Carolina, Chapel Hill, NC, USA,
[9] Kansas State University, Manhattan, KS, USA,
[10] UT Southwestern Medical Center, Dallas, TX, USA,
[11] Hospital Ramón y Cajal, Madrid, Spain,
[12] Baker Heart and Diabetes Institute, Melbourne, Australia,
[13] Lipotype GmbH, Dresden, Germany,
[14] University of Southern Denmark, Odense, Denmark,
[15] University Hospital Regensburg, Regensburg, Germany,
[16] Lipidomics Consulting Ltd., Espoo, Finland,
[17] Research Center Borstel – Leibniz Lung Center, Borstel, Germany

Lipids are essential to life, and disturbances in lipid homeostasis underlie many diseases, from metabolic and cardiovascular disorders to cancer and neurodegeneration. Yet despite rapid methodological progress, lipidomics still lacks a central, comparable reference for where lipids occur and at what concentrations. We present LipidCompass (https://lipidcompass.org), an open, community-driven repository built on the FAIR principles (Findable, Accessible, Interoperable, Reusable) [1] that makes lipidomic data from many sources easy to discover, compare, and interpret.

LipidCompass ingests quantitative results in the community standard mzTab-M [2], mapping metadata to controlled vocabularies such as NCIT and PSI-MS [3,4] so that species, tissues, diseases, and analytical methods are described consistently. The embedded Goslin converter normalizes lipid names to current shorthand nomenclature and cross-links them to LIPID MAPS [5], SwissLipids [6], and ChEBI [7]. Because lipids are represented along the hierarchical levels of the shorthand nomenclature, studies reported at different structural resolutions can be compared at their shared, lowest common level — a prerequisite for meaningful cross-study analysis. The current version already spans 15 species and 40 tissues across more than 80 studies and 140 datasets, comprising over 11,000 distinct lipids at all hierarchical levels and more than 3.67 million individual lipid quantities.

Beyond storage, LipidCompass is intended for interactive exploration. Users can search and filter by lipid class, organism, tissue, and disease, and compare quantitative profiles across datasets through informative visualizations with bar charts, heatmaps, treemaps, and sunburst plots. Those are complemented by lipidome

similarity analysis via LipidSpace. [8] For example, a researcher can retrieve reported concentration ranges for a lipid of interest across healthy and diseased tissues, place their own measurements in that context, and identify studies that used comparable analytical methods. All public data can be browsed without registration and downloaded as CSV, JSON, or mzTab-M, while a documented OpenAPI interface enables programmatic access from R, Python or other programming languages. Registered users can submit their own mzTab-M studies through a guided, validating wizard, feeding into a community curation workflow prior to of after publication.

As a central component of the Lipidomics Informatics for Life Sciences (LIFS) [9] project, LipidCompass integrates with tools such as LipidXplorer [10,11] and LipidSpace. A pipeline now under development further assembles mzTab-M results together with their underlying raw data into complete MetaboLights [12] submissions, linking LipidCompass to the broader metabolomics data ecosystem. Together with the International Lipidomics Society and the Lipidomics Standards Initiative, it adopts the lipidomics checklist and a community-guided review and curation model, establishing LipidCompass as a shared reference resource for comparative, quantitative lipidomics.

References:
[1] Wilkinson, Sci. Data, 2018.
[2] Hoffmann, Anal. Chem, 2015.
[3] Coronado, MEDINFO, 2004.
[4] Mayer, Database, 2013.
[5] O'Donnell, Sci. Signal, 2019.
[6] Aimo, Bioinformatics, 2015.
[7] Hastings, Nucleic Acids Research, 2016.
[8] Kopczynski, Anal. Chem. 2023.
[9] Herzog, PLOS ONE, 2012.
[10] Yurekten, Nucleic Acids Research, 2024.

P3
LipidLens: Making sense of the soup

Authors: Sanjay Kumar Srikakulam [1], Nils Hoffmann [1,2]

Affiliations: [1] IBG-5, Forschungszentrum Jülich GmbH, Jülich, Germany,
[2] Analytical Chemistry, Universität Wien, Vienna, Austria

Lipidomics knowledge is scattered across tens of thousands of papers, in supplementary files, tables, and figure captions, where lipid identities, biological context, and quantitative measurements are recorded inconsistently and in a non-machine-readable way. This fragmentation makes published findings hard to reuse at scale and runs counter to the FAIR principles the community increasingly depends on. We present LipidLens, a literature-extraction-first pipeline that converts full-text articles into ontology-grounded entities, measurements, and relations, carrying explicit provenance on every assertion.

LipidLens retrieves the best available open-access full text given a DOI, PMID, or PMCID, parses it into a normalized document (text, tables, structure) that is machine- and AI-readable, and detects candidate mentions by combining a lexical index built directly from prebuilt ontologies with a neural recognizer. Lipid mentions are normalized deterministically with Goslin [1] before grounding, so shorthand such as „PC 34:1“ is reconciled to a canonical form. A grounding cascade then resolves mentions against versioned ontology databases, including ChEBI [2], SwissLipids [3], MONDO [4], UBERON [5], Cell Ontology [6], NCBITaxon [7], and others. A curated equivalence layer, expressed in SSSOM [8], reconciles fragmented identifiers that routinely split the same lipid across databases. Measurements are extracted, and their units are normalized to the Units Ontology [9], with PSI-MS [10] filling the mass-spectrometry gap, and relations between entities are recovered via an agentic AI step. Every entity, measurement, and relation retains a pointer back to the exact manuscript span it came from, and outputs are exported in community-standard formats such as BioC-JSON, W3C Web Annotation, SSSOM, and PROV-O rather than a bespoke schema.

As a proof of concept, the pipeline was run end-to-end on a few select open-access (CC BY 4.0) publications using local ontology snapshots. On a small, curated evaluation set, the grounding layer resolves lipid and biological entities with high precision, and targeted precision criteria measurably suppress spurious grounding of common English words as taxonomic names without sacrificing recall for genuine mentions. Systematic benchmarking against a larger annotated corpus is ongoing.

Looking ahead, we are extending LipidLens into a complete agentic system, in which language-model agents leverage its retrieval, parsing, grounding, and provenance capabilities as tools for planning acquisition, adapting extraction strategies to individual documents, and iteratively verifying and self-correcting their outputs against the ontologies and provenance record. Our goal is a reproducible, auditable assistant that curates lipidomics knowledge across the literature at scale while keeping a human in the loop for the assertions that matter.

References:
[1] Kopczynski, Analytical Chemistry, 2022.
[2] Malik, Nucleic Acids Research, 2026.
[3] Aimo, Bioinformatics, 2015.
[4] Vasilevsky, Genetics, 2026.
[5] Mungall, Genome Biology, 2012.
[6] Tan, Scientific Data, 2026.
[7] Federhen, Nucleic Acids Research, 2012.
[8] Matentzoglu, Database, 2022.
[9] Gkoutos, Database, 2012.
[10] Mayer, Database, 2013.

P4*

Towards standardized and reproducible lipidomics workflows for FAIR mass spectrometry data analysis

Authors: Franziska Nicolaus [1,2], Bo Burla [4], Philippine Louail [5], Fatemeh Mirzadeh Sarcheshmeh [6], Wout Bittremieux [6], Vannuruswamy Garikapati [7], Alexander Sczyrba [1,2,8], Nils Hoffmann [1,2,3]

Affiliations:
[1] IBG-5, Forschungszentrum Jülich GmbH, Jülich, Germany,
[2] Bielefeld Institute for Bioinformatics Infrastructure (BIBI), Faculty of Technology, Bielefeld University, Bielefeld, Germany,
[3] Analytical Chemistry, Universität Wien, Vienna, Austria,
[4] Singapore Lipidomics Incubator, Life Sciences Institute, National University of Singapore, Singapore,
[5] Institute for Biomedicine, Eurac Research, Bolzano, Italy,
[6] Department of Computer Science, University of Antwerp, Antwerpen, Belgium,
[7] Medical School OWL, Bielefeld University, Bielefeld, Germany,
[8] Center for Biotechnology - CeBiTec, Bielefeld University, Bielefeld, Germany,

Modern mass spectrometry-based lipidomics is routinely generating datasets of large size and high complexity, making standardized and reproducible data analysis as well as quality assessments of these high-dimensional datasets increasingly difficult. To address this, methods and workflows need to be developed with the purpose of enabling reliable identification, qualification, and provenance tracking of lipids, while following FAIR (Findable, Accessible, Interoperable, Reusable) principles [1]. The standardized workflows are intended to facilitate data sharing, interoperability, and future multi-omics integrative analysis.

Community efforts in enabling mass spectrometry-based metabolomics standards have advanced considerably, creating a need for further development and integration into lipidomics mass spectrometry data analysis workflows. The efforts in integrating community-driven standards into workflows include contributions to the implementation of the Human Proteome Organization (HUPO) Proteomics Standards Initiative (PSI) file standards. mzQC [2] is a reporting and exchange format for mass spectrometry quality control data developed by the HUPO-PSI Quality Control (QC) Working Group. As a proof of concept, quality control metrics have been exported from a lipidomics workflow [3] developed at the Singapore Lipidomics Incubator (SLING) to demonstrate the applicability of the mzQC format in lipidomics workflows. Next to the quality control reporting, mzTab-M [4] serves as a reliable and standardized file format for metabolomics and lipidomics mass spectrometry metadata and result reporting, developed by the HUPO-PSI Proteomics Informatics (PI) Working Group, in collaboration with the Metabolomics Standards Initiative (MSI).

In addition to the integration of the standard formats into the workflows, the implementation of automated exports to openly accessible platforms such as EMBL-EBI BioStudies [5], Metabolights [6], or LipidCompass will support data exchange and multi-omics integration. The FAIR methods and workflows will contribute to the aim of reproducible data analysis and facilitate lipidomics mass spectrometry research while creating the foundation for advanced AI-supported biological analysis.

References:

[1] S. R. Wilkinson et al. (2025). Applying the FAIR Principles to computational workflows. Sci Data. 12(1), 328. doi: 10.1038/s41597-025-04451-9.

[2] C. Bielow et al. (2024). Communicating Mass Spectrometry Quality Information in mzQC with Python, R, and Java. J. Am. Soc. Mass Spectrom. 35 (8), 1875-1882. doi: 10.1021/jasms.4c00174.

[3] B. Burla et al. (2025). MRMhub: one-stop solution for automated processing of large-scale targeted metabolomics data. bioRxiv. doi:10.64898/2025.12.20.695370. Github URL: https://github.com/SLINGhub/MRMhub

[4] N. Hoffmann et al. (2019). MzTab-M: A Data Standard for Sharing Quantitative Results in Mass Spectrometry Metabolomics. Analytical Chemistry. 91 (5), 3302-3310. doi: 10.1021/acs.analchem.8b04310.

[5] U. Sarkans et al. (2018). The BioStudies database—one stop shop for all data supporting a life sciences study. Nucleic Acids Research, 46(D1), D1266–D1270. doi: 10.1093/nar/gkx965.

[6] O. Yurekten et al. (2023). MetaboLights: open data repository for metabolomics. Nucleic Acids Research. gkad1045, doi:10.1093/nar/gkad1045.

P5*

HILIC-MS/MS reveals general lipid depletion in TMPro mice cardiac tissues

Authors: Laura Lehmicke [1], Ahmed Elwakiel [1], Philipp Deist [2], Uta Ceglarek [1]

Affiliations: [1] Section Clinical Mass Spectrometry, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig, Germany,
[2] Mass Spectrometry for Biology, Institute Pasteur, Paris, France

Introduction: Endothelial dysfunction and vascular aging are central contributors to age-related cardiovascular pathology and can be studied with TMPro mice. These mice carry a defect in thrombomodulin (TM)-dependent activation of protein C, resulting in accelerated vascular aging. The resulting pro-oxidative environment is expected to impact mitochondrial membrane composition and function. Cardiolipins (CL), mitochondria-enriched phospholipids (PLs), are promising markers of mitochondrial state. The diversity of fatty acyl chain combinations in CLs leads to significant isobaric overlap, posing challenges for mass spectrometric analysis. Additionally, CL are 100- to 1000-fold lower abundant than other PL classes. We developed a rapid HILIC method for reliable CL analysis and applied it to evaluate lipid changes in TMPro mouse cardiac tissue.

Methods: Method development was conducted using a standard mixture of 7 CL standards (50 ng/ml each) and the SPLASH Lipidomix (diluted 1:180, all: Avanti Polar Lipids, USA) in MeOH. 1 mg aliquots of murine heart tissue of six wild-type (WT) and six TMPro mice (all aged 13 months) were extracted with *n*-hexane/*iso*-propanol 60/40 v/v with CL $(14:1)_4$ as internal standard by incubation for 1 h at 4 °C.

HILIC separation was performed on a zwitterionic iHILIC-Fusion (50 x 2.1 mm, 3.5 µm, Umeå, SW) with eluents A (aq. ammonium acetate buffer (pH 5.8, 15 mM)) and B (ACN). The gradient program was carried out on a Nexera HPLC system (Shimadzu, JP) with a total method runtime of 8.5 minutes. MS/MS acquisition was conducted on a QTRAP 6500 mass spectrometer (SCIEX, CA) in negative ion mode. Using multi-reaction monitoring (MRM) up to 124 MRMs transitions were monitored, with 85 dedicated to CL, oxidized CL and monolyso-CL.

Results: A rapid separation of 10 PL classes including CL was achieved with HILIC retention of doubly deprotonated CLs at a pH of 5.8 and minimal water content. CL species had a reproducible mean retention time of 2.2 ± 0.01 minutes in standard and biological samples.

Application of the method to the WT and TMPro mice cardiac tissues resulted in detection of 51 CL and PL species, demonstrating the polarity coverage of the extraction procedure. The peak area of the CL internal standard had a coefficient of 10% over all sample injections assuring reproducible sample preparation and enabling relative quantitative analysis of murine samples.

Measurement of heart tissue revealed the anticipated CL $(18:2)_4$ as the main CL in both WT and TMPro mice. WT mice had a slightly higher per-lipid variance compared to the TMPro mice with a mean per-lipid variance of 38% compared to 35%. Comparing both groups, the TMPro mice exhibited a general depletion of all lipid species included in the targeted measurement.

Conclusion: After sucessful development the application of a rapid and robust HILIC-MS/MS method revealed general lipid depletion in the cardiac tissue of TMPro mice, following their accelerated vascular ageing. Depletion of CL species could have been caused by a lower mitochondrial density in TMPro mice. In the future, hyphenation of the HILIC to an orthogonal reversed phase chromatography will lessen the isobaric overlap via CL species separation before mass spectrometric detection and increase identification confidence.

P6*

Incorporation of epoxy-fatty acids in phospholipids of human cells

Authors: Jonas Ditzler, Laura Carpanedo, Luca Wende, Nils Helge Schebb

Affiliations: Food Chemistry, School of Mathematics and Natural Sciences, University of Wuppertal, Germany

Several oxylipins are potent lipid mediators. They can be formed by non-enzymatic or enzymatic oxidation of polyunsaturated fatty acids (PUFA). Cytochrom-P450 monooxygenases catalyze the formation of epoxy-PUFA which are involved in the regulation of vascular tone and inflammation. These biological roles are described for their non-esterified i.e. free form. Less is known about the biological role of esterified epoxy-PUFA although they are more abundant in cells e.g. bound to phospholipids (PL) in membranes. Moreover, it is unknown in which PL classes they are bound.

Esterified oxylipins such as epoxy-PUFA are currently quantified by targeted LC-MS/MS as free oxylipins following saponification [1,2]. However, this leads to a loss of information on the lipid class where those oxylipins were bound. Here, we directly analyzed oxylipin-bearing PL in HEK293 cells by non-targeted LC-HRMS [3]. It enables the detection of 422 different oxPL species of different classes i.e. PC, PE, PI, PS, PC-P, PC-O and PE-P. Isobaric compounds are efficiently separated by liquid chromatography, while the data-dependent MS2 analysis allows oxPL characterization to molecular species level. Different oxPL species could be characterized based on retention time, precursor ion and specific product ions. The bound oxylipins were assessed via the α-cleavage fragment of the epoxy group and PL-classes were determined by specific head group fragments or neutral losses. Using the abundance of the neutral losses of the bound fatty acyls the PL sn-1 and sn-2 isomers were characterized.

For the experiment, HEK293 cells were supplemented with epoxy-fatty acids such as 9(10)-EpOME and 12(13)-EpOME for different incubation times. This revealed a time dependent cellular incorporation of epoxy-fatty acids and an oxylipin-structure dependent incorporation pattern into the PL of the cells. While 9(10)-EpOME, 12(13)-EpOME were found to be primarily bound to PC, 14(15)-EpETrE was primarily bound in different PI species. Interestingly positional isomers like 8(9)-EpETrE or 11(12)-EpETrE were dominantly found in PC. This demonstrates a highly structure dependent incorporation of epoxy-PUFA, suggesting a distinct biological role of the esterified epoxy-PUFA in cell membranes.

References:
[1] Hartung, N. M., et al., Anal. Bioanal. Chem., 2023, 415, 913.
[2] Ostermann A. I., et al., Prostaglandins Other Lipid Mediat. 2020, 146, 106384.
[3] Carpanedo, L., et al., Anal. Chim. Acta., 2024, 1326.

P7*

LC-MS Analysis of triacylglycerols bearing epoxy fatty acids

Authors: Katja Mosel, Apimanju Uthayaraja, Kathrin Plitzko, Nils Helge Schebb

Affiliations: Food Chemistry, School of Mathematics and Natural Sciences, University of Wuppertal, Germany

Polyunsaturated fatty acids (PUFAs) are vulnerable to oxidation, resulting in the formation of a wide range of oxidation products (oxylipins), including epoxy-PUFAs. While non-esterified (free) oxylipins have been studied extensively, most oxylipins in biological systems are present as esterified species in complex lipids, such as triacylglycerols (TAGs). However, analytical methods for the characterization of TAGs bearing epoxy-PUFA remain limited.

The aim of this project is to develop mass spectrometric approaches for the analysis of epoxy-TAGs. To generate standards for LC-MS method optimization, we synthesized epoxy-PUFAs and incorporated them into triacylglycerols. These standards were subsequently used to optimize the liquid chromatography and mass spectrometric parameters. In the next step, edible oils were epoxidized using *meta*-chloroperoxybenzoic acid (*m*CPBA), and the resulting epoxy-TAGs were characterized. Samples were analyzed by reversed-phase liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) using a Q Exactive HF Orbitrap operated in positive electrospray ionization mode (ESI(+)) with full MS/data-dependent MS^2 acquisition. Epoxy-TAG precursor ions were detected as ammonium adducts ($[M+NH_4]^+$). The resulting fragmentation data were used to investigate characteristic fragmentation pathways and support the structural annotation of epoxy-TAG species.

Analysis of vegetable oils showed that several isobaric epoxy-TAG species were not chromatographically separated. To increase analytical specificity, we therefore developed a targeted LC-MS/MS method operating in multiple reaction monitoring (MRM) mode. With this specificity the quantitative pattern of TAG bearing epoxy-PUFA were characterized in edible oils.

P8

Comprehensive quantitation and structural characterization of oxylipin isomers via high-resolution QTOF mass spectrometry utilizing CID and EAD

Authors: Cagakan Ozbalci [1], Paul R. S. Baker [2], Sahana Mollah [2], Paul Norris [2], Thomas Horvath [3,4]

Affiliations: [1] SCIEX, UK,
[2] SCIEX, USA,
[3] Dept of Pathology, Texas Children's Hospital, Houston,
[4] Dept of Pathology & Immunology, Baylor College of Medicine, Houston, TX

Background: Oxylipins are crucial bioactive lipid mediators that regulate inflammation, immune responses, and diverse physiological pathways. Analyzing these molecules presents a dual challenge: distinguishing closely related structural isomers and detecting them at trace physiological levels. Here, we demonstrate the use of a highly sensitive, hybrid quadrupole time-of-flight (QTOF) mass spectrometer for the simultaneous quantitative and qualitative profiling of oxylipins.

Methods: Analysis was performed using a ZenoTOF 8600 system. External calibration curves ranging from 0.00864 to 494.4 pg/µL were generated for 70 oxylipins. The method utilized both Collision-Induced Dissociation (CID) and Electron Activated Dissociation (EAD) to acquire full product ion spectra without compromising duty cycle or throughput. To ensure rigor, data were processed strictly according to newly established oxylipin research community guidelines, which require evaluating $n \geq 5$ replicates at lower concentrations, ensuring precision (CV < 15 – 20%) and accuracy (±15 – 20%), and calculating Signal-to-Noise ($S/N \geq 5$) using a peak-to-peak algorithm without data smoothing.

Results: The platform achieved quantitative sensitivity rivaling high-end Triple Quadrupole Mass Spectrometry (TQMS). The Lower Limit of Quantitation (LLOQ) was determined to be 0.0864 pg/µL for 20-OH-LTB4 (0.43 pg on-column; 5 µL injection; S/N = 10.5) and 0.0329 pg/µL for 14,15-DiHETrE (1.645 pg on-column; S/N = 8.32). Qualitatively, EAD fragmentation successfully generated structurally diagnostic ions to differentiate isomers. For example, 11-HETE yielded unique high-intensity fragments at m/z 151.0350 and 245.1152, whereas 11,12-EET produced a distinct fragment at m/z 231.0984. The Extracted Ion Chromatograms (XICs) for these EAD-derived ions showed clean, isomer-specific peaks at the correct retention times—a level of structural specificity not achievable with CID alone.

Conclusions: Coupling high-resolution mass spectrometry with EAD-based fragmentation provides a powerful, single-platform solution for oxylipin research. It enables robust quantitation with TQMS-level sensitivity while delivering enhanced structural confirmation for complex lipid isomers.

P9*

Characterization of lipid metabolic landscape of T-cell differentiation

Authors: Nikolai Kureev [1,3], Christer Ejsing [2], Peter Dittrich [3], Alba Diz-Muñoz [1]

Affiliations: [1] Cell Biology and Biophysics Unit, EMBL, Heidelberg, Germany,
[2] Department of Biochemistry and Molecular Biology, SDU, Odense, Denmark,
[3] Faculty of Mathematics and Informatics, FSU, Jena, Germany

The human immune system is a sophisticated network capable of defending the human organism against different pathogens and diseases. T-cells play a pivotal role in orchestrating immune responses, and their dysfunction can affect the ability of the immune system to resist external pathogens or lead to the progression of cancer and autoimmune diseases. Many biomechanical properties of cells, including T-cells, are controlled by the properties of the membrane, which in turn is in part determined by lipid composition. Lipids have a broad range of chemical structures, and their diversity is controlled by a complex network with a large number of reactions. In many cases, lipid composition changes during a cell's life cycle, for instance, in the process of differentiation. T-cell activation is a remarkable example of a cell differentiation that causes lipidome remodeling. During the course of this project, we aim to unravel lipid flux during T-cell differentiation. We are using mathematical modeling approaches based on the Flux Balance Analysis (FBA) and Kinetic Flux Profiling (KFP) supported with mass spectrometry measurements of T-cells' lipid profiles, and validating such models using different perturbation methods. Together, this approach will unravel how the metabolic state drives T-cell function.

References:
[1] Kanno, Communications Biology, 2024.
[2] Chapman, Nature Reviews Immunology, 2020.
[3] Howden, Nature Immunology, 2019.
[4] Lim, Nature Chemical Biology, 2022.

P10
Differentiation of isomeric phospholipids by fragmentation of lipid adduct ions

Authors: Nicole Zehethofer

Affiliations: Thermo Fisher (Bremen, Germany) GmbH

The structural characterization of phospholipids remains a major challenge in lipidomics, particularly when distinguishing isomeric species that differ only in double bond position. Recent studies have demonstrated that information on lipid double bond location can be critical for biological interpretation and disease identification. For example, identification of breast and brain cancer tissues has been shown to rely on the ability to resolve lipid isomers that are indistinguishable at the sum composition level. Consequently, analytical approaches capable of providing double bond positional information are increasingly important for translational lipidomics applications.

In this study, we investigate the fragmentation of phospholipid-metal adduct ions for the differentiation of isomeric phospholipids using a direct infusion workflow. Lipid standards were introduced via an Advion NanoMate® nanoelectrospray source coupled to a high-resolution mass spectrometer. Barium was selected as an adduct-forming reagent due to its ability to alter lipid fragmentation pathways and promote the formation of structurally informative product ions for fatty acids.

Initial experiments demonstrated that MS2 measurements of phospholipid-metal adduct ions alone were insufficient to correctly identify the double bond position in lipid isomers due to the spectral complexity. To overcome this limitation, in-source CID experiments were performed to generate acyl group product ions from bariated phospholipid precursors. Subsequent fragmentation of these product ions (acyl group + barium) resulted in spectra containing diagnostic fragment ions that were absent in conventional collision-induced dissociation (CID) experiments.

Preliminary results indicate that fragmentation of bariated fatty acid ions provides access to structural information related to double bond position, enabling differentiation of isomeric lipid species that cannot be resolved by CID alone.

Ongoing work focuses on expanding the approach to additional phospholipid classes and other cations.

P11*

Mechanistic investigation of the influence of unsaturated fatty acids on platelet activation using direct infusion and LC-MS/MS

Authors: Alexander Eder [1,2], Philipp Mourikis [3], Stefanie Rubenzucker [1,2], Cristina Coman [1], Amin Polzin [3,4], Robert Ahrends [1]

Affiliations: [1] University of Vienna, Faculty of Chemistry, Department of Analytical Chemistry, Währinger Str. 38, 1090 Vienna, Austria,
[2] University of Vienna, Vienna Doctoral School in Chemistry (DoSChem), Währinger Str. 42, 1090 Vienna, Austria,
[3] Department of Cardiology, Pulmonology, and Vascular Medicine, Medical Faculty, Heinrich Heine University Medical Center Düsseldorf, 40225 Düsseldorf, Germany,
[4] Cardiovascular Research Institute Düsseldorf (CARID), 40225 Düsseldorf, Germany.

Cardiac disease is one of the most relevant causes of death in modern society. In particular, due to a rising average age of the population, cardiovascular deaths have been increasing more than expected from the population growth alone, which underlines the necessity of further research into understanding and combating such diseases. [1] One of the possible approaches is focusing on the prevention of cardiac events. Here, recent clinical studies have shown a positive effect of polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) on plaque formation and occurrence of cardiac events when supplemented in high-risk patients. [2,3]

Even though clinical guidelines have already been updated to recommend the supplementation of EPA in certain cases, the underlying mechanisms behind the effects described above are still not fully understood. [4]

Hence, there is a strong need to support clinical decision-makers with mechanistic explanations of the interaction of EPA with the platelet lipidome. Previously, a comprehensive LC-MS methods for the analysis of signaling lipids has been developed. [5] Direct-infusion experiments can reveal shifts in bulk membrane lipids using an untargeted approach. Together they offer a complete picture of the changes in the platelet lipidome when exposed to stimulants and can be used to track the effect of the treatment of platelets with PUFAs.

First exploratory studies of the platelet lipidome have already been published. [6] To go a step further, ex vivo trials have been performed on isolated platelets to deepen our understanding of platelet activation, looking at the influence of different stimulants and their interaction with omega-3 fatty acids such as EPA or DHA.

Platelet activation leads to characteristic changes in the lipid profile, both at the level of very low abundant signaling lipids such as oxylipins as well as in the composition of the cell membrane. Those changes are conserved amongst multiple stimulating agents, confirming the current theory that the different agonists converge into a common pathway.

However, while the concentration of many signaling lipids rises during platelet activation, only very few, such as thromboxane A2 seem to be influenced by the treatment with omega 3 fatty acids. This allows further insight into the mechanisms of platelet activation providing a more complete descriptions of the chemical and enzymatic reactions at play and could potentially lead to novel targets for antiplatelet drugs.

References:

[1] G. A. Roth et al., „Demographic and Epidemiologic Drivers of Global Cardiovascular Mortality“, N. Engl. J. Med., Bd. 372, Nr. 14, S. 1333–1341, Apr. 2015, doi: 10.1056/NEJMoa1406656.

[2] D. L. Bhatt et al., „Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia“, N. Engl. J. Med., Bd. 380, Nr. 1, S. 11–22, Jan. 2019, doi: 10.1056/NEJMoa1812792.

[3] M. J. Budoff et al., „Effect of icosapent ethyl on progression of coronary atherosclerosis in patients with elevated triglycerides on statin therapy: final results of the EVAPORATE trial“, Eur. Heart J., Bd. 41, Nr. 40, S. 3925–3932, Okt. 2020, doi: 10.1093/eurheartj/ehaa652.

[4] X. Wang, S. Verma, R. P. Mason, und D. L. Bhatt, „The Road to Approval: a Perspective on the Role of Icosapent Ethyl in Cardiovascular Risk Reduction“, Curr. Diab. Rep., Bd. 20, Nr. 11, S. 65, Okt. 2020, doi: 10.1007/s11892-020-01343-7.

[5] S. Rubenzucker, M.-C. Manke, R. Lehmann, A. Assinger, O. Borst, und R. Ahrends, „A Targeted, Bioinert LC–MS/MS Method for Sensitive, Comprehensive Analysis of Signaling Lipids“, Anal. Chem., Bd. 96, Nr. 23, S. 9643–9652, Juni 2024, doi: 10.1021/acs.analchem.4c01388.

[6] B. Peng et al., „Identification of key lipids critical for platelet activation by comprehensive analysis of the platelet lipidome“, Blood, Bd. 132, Nr. 5, S. e1–e12, Aug. 2018, doi: 10.1182/blood-2017-12-822890.

P12*

Building a faster, future-ready LipidXplorer ecosystem for multidimensional MS-based lipidomics

Authors: Md Ballal Hossen [1], Daniel Krause [1], Nils Hoffmann [2], Dominik Schwudke [1,3,4]

Affiliations: [1] Division of Bioanalytical Chemistry, Research Center Borstel, Leibniz Lung Center, Borstel, Germany,
[2] Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences (IBG-5), Jülich, Germany,
[3] German Center for Infection Research, Thematic Translational Unit Tuberculosis, Borstel, Germany,
[4] German Center for Lung Research (DZL), Airway Research Center North (ARCN), Borstel, Germany

MS-based lipidomics requires data-processing tools that can keep up with the chemical diversity of lipid species and with modern instruments that provide data in multiple dimensions. We addressed this in two subsequent phases. First, we improved the existing LipidXplorer ecosystem by making lxPostman more accurate and standardizing its outputs, complemented by a QC strategy for filtering low-confidence lipid identifications. We also upgraded LipidXplorer's codebase to Python 3.12 and added parallel processing that resulted in cutting the processing time by 2.79 times. Second, to prepare the platform for multidimensional data, we completed the architectural design of LipidXplorerNext, centered on a new database structure built to deal with multiple dimensions alongside conventional direct-infusion data, with extensibility built in for future MFQL development. As a first step toward multidimensional acquisition, we present a 2D data acquisition pipeline using cyclic ion mobility spectrometry (IMS) for shotgun lipidomics. Both the lxPostman QC strategy and the cyclic IMS pipeline were evaluated on blood plasma data. Implementation of the redesigned LipidXplorerNext architecture is currently underway. Together, the completed upgrades already improve reliability and throughput today, while the LipidXplorerNext redesign lays the groundwork for scalable support of next-generation, high-throughput MS technologies.

P13

Dynamic lipidome remodeling during neutrophil extracellular trap formation

Authors: Patrick Münzer [1,2], Cristina Coman [3], Gundula Lingens [1,2], Nina Troppmair [3], Stefanie Rubenzucker [3], Ferdinand Kollotzek [1,2], Shane R. Ellis [4], Oliver Borst [1,2,5], Robert Ahrends [3]

Affiliations: [1] DFG Heisenberg Group Cardiovascular Thrombo-Inflammation and Translational Thrombocardiology, Tübingen, Germany,
[2] Department of Cardiology and Angiology, University of Tübingen, Tübingen, Germany,
[3] Institute of Analytical Chemistry, University of Vienna, Vienna, Austria,
[4] Molecular Horizons and School of Science, University of Wollongong, Wollongong, NSW, Australia

Neutrophil extracellular trap (NET) formation (NETosis) contributes to the pathogenesis of numerous inflammatory and thrombotic diseases. Although membrane remodeling is essential for NET release, the temporal dynamics of the neutrophil lipidome during NETosis remain poorly understood. Here, we established a comprehensive quantitative lipidome of human neutrophils, comprising 1,048 lipid species spanning nine orders of magnitude, and characterized its remodeling during NETosis. NET formation induced extensive changes in phosphatidylinositols, phosphatidic acids, diacylglycerols (DGs), and lysoglycerophospholipids. Calcium- and reactive oxygen species–dependent NETosis pathways exhibited distinct lipid remodeling trajectories but converged on phospholipid lipase–driven signaling networks. Pharmacological inhibition of these lipid remodeling pathways markedly impaired NET formation, while exogenous DG restored NETosis. These findings identify dynamic phospholipid remodeling and DG-dependent signaling as central regulators of NETosis and highlight phospholipid lipase networks as promising therapeutic targets for NET-associated diseases.

P14*

Culture-dependent remodeling of the *M. tuberculosis* virulence lipids phthiocerol dimycocerosates and strategies for quantification

Authors: Wiebke Gauda [1], Lisa Niwinski [2], Gareth Prosser [2], Nicolas Gisch [1], Norbert Reiling [2], Dominik Schwudke [1,3,4]

Affiliations: [1] Division of Bioanalytical Chemistry, Priority Area Infections, Research Center Borstel, Borstel, Germany,
[2] Division of Microbial Interface Biology, Priority Area Infections, Research Center Borstel, Borstel, Germany,
[3] German Center for Infection Research, Partner Site: Hamburg-Lübeck-Borstel-Riems, Borstel, Germany,
[4] Airway Research Center North, Member of the German Center for Lung Research (DZL), Site: Research Center Borstel, Borstel, Germany

Approximately a quarter of the world's population is infected with *Mycobacterium tuberculosis* (*Mtb*) [1], underscoring the pathogens ability to establish persistent infections inside the host. This success is partially attributed to virulence-associated lipids like phthiocerol dimycocerosates (PDIMs) [2,3]. These hydrophobic lipids are located on the mycobacterial outer membrane (MOM). Structurally, they are composed of a hydrophobic chain with either a methoxy- or keto group, classifying the PDIMs into DIM A or DIM B, respectively. To this chain, aliphatic mycocerosic acids are attached.

PDIMs can be spontaneously lost during in-vitro cultivation, altering the cell envelope in terms of cell wall permeability and surface hydrophobicity. Consequently, experimental outcomes are affected as PDIM-deficient mutants exhibit also a reduced virulence. To overcome PDIM loss, propionate supplementation of the culture media has been shown to restore PDIM production by providing precursor molecules for mycocerosic acid biosynthesis [4]. Another major challenge in studying these lipids on a deeper structural level with mass spectrometry is the lack of suitable deuterated standards, necessitating alternative quantification strategies.

PDIM quantities of the genetically defined laboratory strain H37Rv 2010 WT were successfully determined in MS1 mode using the the Q Exactive plus (Thermo Fisher Scientific, Bremen, Germany) coupled to the TriVersa NanoMate (Advion, Ithaca, USA) for direct infusion. With the developed method we could clearly discriminate between DIM A and DIM B. In the absence of authentic PDIM standards, 15:0-18:1-d7 triacylglycerol was successfully applied as a surrogate standard for PDIM quantification. Propionate supplementation showed no effect on total PDIM amounts; however, species-level analysis revealed a shift toward higher-mass PDIMs with longer mycocerosic acid chains. This effect was also observed in vivo during infection in the lungs of *Mtb*-infected mice [5]. To further characterize the functional consequences of PDIM remodeling, we performed infection experiments using human-derived macrophages to investigate the impact of longer-chain PDIM species and the associated changes in cell envelope properties. Despite the observed shift towards longer-chained PDIMs, no impact on macrophage infection was observed.

The MS2 fragmentation pathways in the positive ion mode enabled structural elucidation on the species level, whereby several isomers can be detected for one precursor, characterized by its core chain length and two mycocerosic acids. With help of lxPostman, the fragment ratios from a defined DIM B molecule derived from *M. marinum* was applied for improving the structural analysis with a computational tool.

References:

[1] World Health Organization. Global tuberculosis report 2025. Geneva: World Health Organization (2025).

[2] Onwueme, K. C., Vos, C. J., Zurita, J., Ferreras, J. A. & Quadri, L. E. N. The dimycocerosate ester polyketide virulence factors of mycobacteria. Prog. Lipid Res. 44, 259–302 (2005).

[3] Rens, C., Chao, J. D., Sexton, D. L., Tocheva, E. I. & Av-Gay, Y. Roles for phthiocerol d imycocerosate lipids in Mycobacterium tuberculosis pathogenesis. Microbiology 167, 001042 (2021).

[4] Mulholland, C. V. et al. Propionate prevents loss of the PDIM virulence lipid in Mycobacterium tuberculosis. Nat. Microbiol. 9, 1607–1618 (2024).

[5] Jain, M. et al. Lipidomics reveals control of Mycobacterium tuberculosis virulence lipids via metabolic coupling. Proc. Natl Acad. Sci. USA 104, 5133–5138 (2007).

P15*

Analysis of the glycosyldiacylglycerols of *Streptococcus canis*

Authors: Anna Nehrmann [1], Miriam Katsburg [2], Ursula Schombel [1], Dominik Schwudke [1,3,4], Marcus Fulde [2,5], Nicolas Gisch [1]

Affiliations:
[1] Division of Bioanalytical Chemistry, Research Center Borstel, Borstel, Germany,
[2] Institute of Microbiology and Epizootics, Centre for Infection Medicine, Consulting Laboratory for beta-haemolytic streptococci in Veterinary Clinics and Practice, Freie Universität Berlin, Berlin, Germany,
[3] German Center for Infection Research, Thematic Translational Unit Tuberculosis, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany,
[4] German Center for Lung Research (DZL), Airway Research Center North (ARCN), Research Center Borstel, Leibniz Lung Center, Borstel, Germany,
[5] Institute of Microbiology, University of Veterinary Medicine Hannover, Germany

Streptococcus canis is an opportunistic pathogen observed in animals, especially dogs and cats, but occasionally also in humans. Usually, this pathogen is classified into the Lancefield group G. Recently, two strains of *S. canis* belonging to Lancefield group C (IMT52870 and IMT56394) were identified. Whole genome sequencing analysis pointed towards a horizontal gene transfer between *S. canis* and *Streptococcus dysgalactiae*. [1]

The aim of this study is to analyze the glycosyldiacylglycerols (GLs) in *S. canis* and to determine whether there are variances in the molecular species distribution among individual strains. We here compare two methods for analysing GLs. On the one hand, we investigated the GLs by nano spray ionisation and subsequent bioinformatic analysis following our established workflow for lipid characterisation. [2] Six selected *S. canis* strains, four group G strains (G361, IMT49926, AGF1074 and AGF1293) and the two group C strains, were analyzed with the lipidomics approach and structures were further specified by NMR analysis. All examined strains express two GLs, α-D-glucose-3-(1,2-diacyl)-glycerol and α-D-glucose-(1→2)-α-D-glucose-3-(1,2-diacyl)-glycerol. In all strains, the fatty acid analysis showed a high amount of palmitic acid as well as 18:1 and 16:1. We compare these results with those obtained using desorption electrospray ionization (DESI), which is an ionization technique that enables high-spatial-resolution mass spectrometry (MS) imaging of surface samples, from an analytical TLC plate as matrix.

References:
[1] Katsburg et al., Vet. Microbiol., 2023
[2] Eggers & Schwudke, Clin. Met. MIMB, 2018.

P16

Tandem mass spectrometric behavior of ammonium and lithium adducts of acetate esters

Authors: Dominik Schwudke [1,2,3], Wiebke Gauda [1], Marcellina Przybylska [1], Thorben Gehle [4], Alexander Haack [4]

Affiliations:
[1] Division of Bioanalytical Chemistry, Research Center Borstel, Borstel, Germany,
[2] German Center for Infection Research, Thematic Translational Unit Tuberculosis, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany,
[3] German Center for Lung Research (DZL), Airway Research Center North (ARCN), Research Center Borstel, Leibniz Lung Center, Borstel, Germany,
[4] Institute of Electrical Engineering and Measurement Technology, Leibniz University Hannover of Microbiology, Germany

Ammonium and Lithium adducts are used in shotgun lipidomics and have gained prominence in analyzing lipids [1,2]. The analysis of cholesterol via acetylation [3] initiated several methodical developments to include derivatization procedures to attain acetic acid esters of hydrophobic and/or poorly ionizable molecules. Yet, gaining structural details like double bond position or methylation/hydroxylation sites remains challenging. Recent reports on the fragmentation mechanisms of protonated primary alcohols [4] and acetates [5] present new opportunity to structurally characterize long aliphatic chains.

Acetylation of long chain alcohols was performed as previously described [3]. Tandem-MS analysis was conducted on a Q Exactive Plus (Thermo Fisher Scientific, Bremen, Germany) coupled with the TriVersa NanoMate (Advion, Ithaca, US) for the chip-based nano-ESI measurements in direct infusion mode. Measurements are complemented by density-functional theory (DFT) modeling of the fragmentation pathways, including barriers and threshold energies. Model analytes, such as octyl acetate, pentyl acetate isomers, and unsaturated isomeric dodecyl acetates, as well as lipid extracts from *M. tuberculosis* are studied.

Conclusions: The fragmentation mechanisms unraveled here, especially their carbocation chemistry, seem to allow isomer discrimination specifically when using $[M+Li]^+$ ions. $[M+NH_4]^+$ showed complex rearrangements which is reflecting the high mobiity of the charge in this adduct. This basic fragmentation study lay important groundwork for alternative structural characterization method of long aliphatic chains.

References:
[1] PLoS One 2009, 4, e6261.
[2] JLR 2003, 44, 1071-1079.
[3] Biochim Biophys Acta 2006, 1761, 121.
[4] JASMS, 2025, 10.1021/jasms.5c00348.
[5] Anal Chem 2024, 96, 30, 12433.

P17

The host-microbe interactome: decoding lipid metabolism and membrane dynamics during mycobacterial infection

Authors: Sylvana V. Huettel [1,2,3,4], Aby Anand [1,2,4], Lena Gonner [1,2,3], Mélanie Foulon [5], Wiebke Gauda [6], Paulo Glatz [1,2,3], Rico Franzkoch [7], Leonhard Breitsprecher [7], Béla Boumazy [1,2,4], Lizdany Florez [8], Ulrike Laugks [8,9], Anna-Carina Mazur [1,2,3,4], Miriam Quentin [1,2,3], Thierry Soldati [5], Carolin Seuring [8,9], Dominik Schwudke [6,10,11] and Caroline Barisch [1,2,3,4,12]

Affiliations:
[1] Centre for Structural Systems Biology, Hamburg, Germany,
[2] Host-Microbe Interactome, Research Center Borstel, Leibniz Lung Center, Borstel, Germany,
[3] Biology Department, University of Hamburg, Hamburg, Germany,
[4] Molecular Infection Biology, Department of Biology/Chemistry & Center of Cellular Nanoanalytics, University of Osnabrück, Osnabrück, Germany,
[5] Department of Biochemistry, Faculty of Science, University of Geneva, Geneva, Switzerland,
[6] Division of Bioanalytical Chemistry, Research Center Borstel, Borstel, Germany,
[7] iBiOs–integrated Bioimaging Facility, Center of Cellular Nanoanalytics, University of Osnabrück, Osnabrück, Germany,
[8] Multi-user CryoEM Facility, Centre for Structural Systems Biology, Hamburg, Germany,
[9] Department of Chemistry, University of Hamburg, Hamburg, Germany,
[10] German Center for Infection Research, Thematic Translational Unit Tuberculosis, Partner Site Hamburg-Lübeck-Borstel-Riems, Borstel, Germany,
[11] German Center for Lung Research (DZL), Airway Research Center North (ARCN), Research Center Borstel, Leibniz Lung Center, Borstel, Germany,
[12] German Center for Lung Research, Airway Research Center North, Borstel, Germany

A central focus of the group is how mycobacteria exploit host lipid resources. We study host lipid droplet interactions with intracellular bacteria, trace lipid flux using clickable fatty-acid probes, and examine how bacterial lipid metabolism contributes to intracellular adaptation. In particular, our work on the long-chain fatty acyl-CoA synthetase FACL6 indicates that bacterial fatty-acid activation is critical for lipid homeostasis, cell envelope integrity, intracellular growth and antibiotic susceptibility.

Beyond lipid acquisition, we investigate membrane damage and repair as key determinants of infection outcome. We analyze how mycobacteria interact with host organelles, including lipid droplets and the endoplasmic reticulum, and how organelle contacts contribute to membrane remodelling, bacterial containment or intracellular escape. These questions are addressed through a multidisciplinary imaging pipeline spanning live and fixed fluorescence microscopy, correlative approaches, expansion microscopy and cryo-electron microscopy.

Together, our projects define the host-microbe interface as a dynamic lipid and membrane environment that can be exploited therapeutically. By linking lipid metabolism, membrane damage and antimicrobial targeting, we aim to uncover new vulnerabilities in mycobacteria and establish lipid-based strategies for host-directed therapy, drug delivery and improved antimicrobial susceptibility

References: [1] Mélanie Foulon, Sylvana V. Hüttel, Leonhard Breitsprecher, Wiebke Gauda, Lyudmil Raykov, Hendrik Koliwer-Brandl, Miriam Ohlhagen, Roland Thünauer, Dominik Schwudke, Hubert Hilbi, Thierry Soldati, Caroline Barisch. bioRxiv 2026.02.10.704997; doi: https://doi.org/10.64898/2026.02.10.704997

[2] Anand A, Mazur A, Rosell-Arevalo P, Franzkoch R, Breitsprecher L, Listian SA, Hüttel SV, Müller D, Schäfer DG, Vormittag S, Hilbi H, Maniak M, Gutierrez MG, Barisch C. 2023. ER-dependent membrane repair of mycobacteria-induced vacuole damage. mBio 14:e00943-23. https://doi.org/10.1128/mbio.00943-23