IRTG Retreat 2025

Youth hostel DJH Hamburg „Horner Rennbahn“

24 to 26 November 2025

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Talk Abstracts

Session I

Keynote Lecture

Blanche Schwappach-Pignataro

Medical Faculty, University Medical Center Hamburg-Eppendorf, Germany

AI and the Scientific Literature – where do we stand?

The use of AI tools is now common in personal and professional contexts. How does this affect the craft of following and contributing to the scientific literature? How will contributions be indicated and assessed? Which driving forces will shape scientific fields and careers? Building on a recent opinion article (https://doi.org/10.1038/s44319-025-00522-5) and a workshop held by IRTG/SFB 1557 (Navigating PhD Life in the Age of AI: Tools, Strategies, Considerations (by Dr. Vanessa von Kortzfleisch)), I would like to engage into a discussion with PhD students and PIs.

Biochemical characterization of regulation and function of the Rab23 GTPase and the formin-related protein multiple wing hairs

Nada Elagouri

Biochemistry and Structural Biology Division, Institute for Biochemistry, University Münster

The Rab family of small GTPases plays an important role in the regulation of intracellular trafficking and membrane organization. They are activated by guanine nucleotide exchange factors (GEFs). Rab23 was previously shown to be required for the membrane trafficking at mature cilia during ciliogenesis and for planar cell polarity (PCP) [1]. We have previously investigated the Rab23 activation by its cognate tri longin domain (TLD) GEF Inturned-Fuzzy (IntuFuz), revealing molecular adaptations in the catalytic mechanism and recruitment of the complex [2].
However, the intracellular trafficking pathways controlled by Rab23 as well as its downstream effectors are yet unknown. To understand this, we aim to investigate the interaction networks of Rab23. Therefore, hTERT-RPE1 stable cell lines expressing human tagged *Hs*Rab23 and the active mutant *Hs*Rab23Q68L were generated, and subsequent pull-downs and proteomics analysis were performed. The proteomics data revealed specific interactors that implicate Rab23 in cytoskeleton regulation and remodeling.
In addition, the GEF for Rab23 IntuFuz was previously found to regulate the localization of the atypical formin-related protein multiple wing hairs (Mwh) during PCP [1] , which in turn regulates the actin polymerization in *Drosophila* wing cells [3]. To investigate this, co-immunoprecipitation and pull-down experiments were performed. Our results do not show any specific interaction of Mwh with the GEF complex. but a transient interaction with Rab23. This could indicate that the regulation of Mwh and in turn the actin remodeling is achieved via Rab23 and not via its GEF during PCP. As a first step towards understanding the molecular basis of Rab23-Mwh interplay, we determined the crystal structure of the formin homology domain (FH3) of Mwh and now work towards the reconstitution of the Rab23-Mwh complex.

1. A. Gerondopoulos *et al.*, “Planar Cell Polarity Effector Proteins Inturned and Fuzzy Form a Rab23 GEF Complex”, doi: 10.1016/j.cub.2019.07.090.
2. Wilmes, Stephan et al. “Mechanistic adaptation of the metazoan RabGEFs Mon1-Ccz1 and Fuzzy-Inturned”, doi:10.1126/sciadv.adx2893
3. Q. Lu, D. A. Schafer, and P. N. Adler, “The Drosophila planar polarity gene multiple wing hairs directly regulates the actin cytoskeleton”, doi: 10.1242/dev.122119.

The Rab7-like GTPase Ypt7 hypervariable domain contributes to GEF recognition and effector binding

Tunde Lawal

Biochemistry division, Department of Biology/Chemistry, University of Osnabrück, Germany

Rab-GTPases function as organelle-specific identity markers, directing proteins to distinct membranes in eukaryotic cells. They are membrane-anchored via a C-terminal prenyl group attached to a highly conserved GTPase domain through a flexible, hypervariable domain. Rabs cycle between inactive (GDP-bound) and active (GTP-bound) states. In their inactive state, they associate with GDI and are soluble; upon encountering a cognate guanine nucleotide exchange factor (GEF) on a membrane, GDP is exchanged for GTP, enabling binding to effector proteins and their recruitment to target membranes.
While the GTPase domain is generally considered the primary determinant of specificity in Rab-GEF and Rab-effector interactions, recent studies demonstrate a role for the hypervariable domain in GEF recognition. Here, we present evidence that the hypervariable domain of the Saccharomyces cerevisiae Rab7-like GTPase, Ypt7, is crucial for proper GEF-mediated activation and subsequent effector binding, thereby influencing effector function in vivo and in vitro.

VPS13C initiates lipid transfer and membrane remodeling for efficient lysosomal repair (Poster Index 13)

Elisabeth Südhoff

Molecular Cell Biology division, Center for Cellular Nanoanalytics, Department of Biology/Chemistry, University of Osnabrück, Germany

Perturbations in lysosome integrity are frequently linked to neurological disorders and ageing. Using an unbiased proteomic approach, we identified the Parkinson's disease- associated bridge-like lipid transport protein VPS13C/PARK23 as a core component of an early lysosome damage response pathway. VPS13C readily binds lysosomes under mechanical or osmotic stress in anticipation of membrane lesions. The latter triggers a conformational change in the protein's *C*-terminus, involving its ATG2C domain acting as a sensor of damage-induced lipid packing defects. We show that ER-lysosome contacts formed by VPS13C serve as critical binding platforms for OSBP and ORP9 to enable efficient ER wrapping of damaged lysosomes. A chemical approach to assess directional ER-to-lysosome lipid transport revealed that VPS13C is essential for large-scale lipid delivery to acutely damaged lysosomes to facilitate their repair. Our findings reinforce the notion that loss-of-function mutations in *VPS13C* enhance the risk of Parkinson's disease by disrupting an adequate lysosome damage response in cell types vulnerable to lysosome dysfunction.

Session II

Keynote Lecture

Lorenz Adlung

Adipose Systems Immunology, University Medical Center Hamburg-Eppendorf, Germany

From Signal Variability to Tissue Plasticity: Quantitative Modelling Across Scales in Cell Fate Dynamics

Biological systems maintain function across vast spatial and temporal scales, from molecular signalling in single cells to tissue-level metabolic adaptation. My research combines mechanistic modelling, single-cell multi-omics and machine learning to reveal how such multi-scale organisation influences inflammatory and metabolic outcomes.
As a PhD student, I employed quantitative experiments and mathematical modelling to demonstrate that protein abundance alone can dictate how haematopoietic cells interpret growth factor signals. This revealed predictive rules governing the usage of the AKT/ERK network and proliferation control. Building on this approach, our single-cell modelling of JAK2/STAT5 signalling revealed that survival decisions in erythroid progenitors arise from stochastic variation in pathway components and cell volume. This defines a molecular threshold for life and death.
Building on these principles, the Adlung Lab now establishes and applies similar modelling frameworks to metabolic inflammation and adipose tissue plasticity. Using single-nucleus RNA sequencing and spatial transcriptomics, we recently identified distinct lipogenic and thermogenic adipocyte populations in murine brown fat, uncovering a Wnt–ChREBP axis that controls their dynamics during cold adaptation. By integrating deterministic and data-driven approaches, including neural ordinary differential equations (neural ODEs), we aim to bridge the gap between molecular signalling heterogeneity and emergent tissue behaviour in inflammatory and metabolic diseases.
Together, these efforts outline a quantitative framework for understanding how variability carries rather than distorts biological information, and how predictive modelling across scales can inform strategies to restore tissue homeostasis in chronic inflammation and metabolic dysfunction.

The isolation of intact lysosomes from whole flies (Poster Index 1)

Franziska Flottmann

Zoology and Developmental Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

We are utilizing *Drosophila melanogaster* as a model system to study endomembrane-related disorders, such as lysosomal storage diseases and endosomal maturation failure to study organelles' lipid and protein composition and their cargo in detail. The focus is on the whole animal, to cover the systemic relevance, and specific organs and tissues, such as adipocytes and nephrocytes. The aim is to analyze the content and composition of intracellular membrane compartments under clinically relevant genetic, dietary or aging conditions in the *Drosophila* system.
Our first lysosome-specific bait protein, RpH-ILV-ALFA, is a novel, minimally-interactive, ratiometric lysosomal pH sensor that is targeted to intralumenal vesicles and works in both mammalian cells and *Drosophila* (Cheetham-Wilkinson et al., 2024; PMID: 39752501).
Here, we present our latest progress in establishing a protocol for affinity tag-mediated pull-down to isolate intact lysosomes from *Drosophila* tissues. Using a human LAMP1:HA bait protein resulted in a significant enrichment of intact lysosomes from tissue for reproducible protein identification by mass spectrometry.

Oncogenic mutations differentially dysregulate stoichiometry and structural organization of G CSFR signaling complexes

Mia Mönnig

Biophysics Division, Center for Cellular Nanoanalytics, Department of Biology/Chemistry, University of Osnabrück, Germany

Class I/II cytokine receptors (CR) signal via the JAK/STAT pathway, which is initiated by ligand binding to the respective CR. The spatiotemporal organization and dynamics of CR assembly in the plasma membrane (PM), associated JAKs, and downstream signaling remains incompletely understood and is actively debated in the field. By employing live-cell micropatterning and phosphoflow cytometry binding of JAK2, TYK2 and JAK1 to G-CSFR and downstream signaling activity with all three JAKs was confirmed. Building on these findings, the oncogenic mutations T618I and T640N in G-CSFR were characterized, both exhibiting constitutive downstream signaling with all binding JAKs in the absence of ligand. To further characterize both mutations in G-CSFR, alternating laser excitation (ALEX) combined with single-molecule Förster resonance energy transfer (smFRET) was applied. This revealed enhanced dimerization levels and distinct receptor complex conformations for G-CSFR T618I compared to T640N. Three-color co-tracking experiments of G-CSFR WT, T618I, and T640N in the presence of ligand revealed the formation of trimeric receptor complexes. The findings reveal mutation-specific effects on G-CSFR dimerization and complex conformation, demonstrating T618I and T640N promote autoactivation through distinct structural changes. The formation of trimers upon ligand stimulation suggests a previously underappreciated complexity in CR assembly, contributing to the understanding of G-CSFR signaling dynamics.

Meet me at the contact site – The role of Tmem43 at ER-mitochondria contact sites

Lena Menzel

Zoology and Developmental Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Arrhythmogenic right ventricular cardiomyopathy type 5 (ARVC5) is a severe genetic disorder that is characterized by progressive fibrofatty replacement of heart muscle cells, severe arrhythmias, and sudden cardiac death. It has been linked to mutations in the transmembrane protein TMEM43 - particularly the p.S358L variant.
Our research uses a Drosophila model expressing either the wild-type (Tmem43wt) or mutant (Tmem43p.S333L) ortholog to further investigate Tmem43/ TMEM43 function.
We propose that the Tmem43p.S333L mutation results in impaired interaction with Porin, leading to disrupted ER-mitochondria contact sites (ERMCS) and mitochondrial dysfunction, ultimately resulting in heart failure.
This project further explores the role of Tmem43 at ERMCS, combining the application of artificial ER-mitochondria linkers (mitoER-Tethers), and proteome analyses. These findings contribute to the understanding of Tmem43 function and how its mutation and disrupted ERMCS contribute to the development of cardiac disease.

Session III

Keynote Lecture

Christian Löw

Membrane Protein Structural Biology, Centre for Structural Systems Biology Hamburg, Germany
European Molecular Biology Laboratory (EMBL) Hamburg, Germany

Cryo-EM on Solute Carriers – Basic Research and Drug interaction

Nutrient uptake across the lipid bilayer is essential for life. Out of more than 900 known membrane transport systems in the human body, almost 450 belong to the solute carrier (SLC) family of secondary active transporter. They play a central role in controlling the compartmentalization of metabolism and many members of this superfamily are linked to human disease. Despite being attractive therapeutic targets, many SLCs have been poorly characterized on a functional and structural level.
My research group studies the remarkable substrate promiscuity of peptide transporters (SLC15 family) and tries to understand how vitamins and drugs are recognized and transported (SLC19 family). Furthermore, we use structural biology and biochemical tools to identify substrates of currently orphan SLC transporters (e.g. MFSD1). During this presentation I will highlight our endeavor towards the structural characterization of human SLCs. The resolution revolution in cryo-EM combined with biochemical tools allows us to investigate the functions of SLCs and their relevance in physiology on a molecular level and explore the SLC ‘drugability’.

Nanodisc assembly with no additional lipids enables membrane protein delipidation: the MsbA example

Polina Zotova

Structural Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

The lipid environment plays a crucial role in defining the structural and functional properties of membrane proteins. Lipid binding and release events can cause conformational changes and thereby modulate protein activity. In addition to non-specific, solvent-like interactions with the membrane many proteins contain tightly bound lipids that are essential for their conformational stability and biological function. Characterizing these specific protein-lipid interactions remains challenging. Lipid-binding sites can sometimes be examined through targeted mutations, however, this approach may disturb the native protein structure. A more relevant strategy is to delipidate the wild-type protein, i.e., to remove tightly bound lipids from their pockets, and investigate structural and functional consequences.

Here, we present a new approach for membrane protein delipidation based on assembling nanodiscs in a lipid-free environment. Cryo-EM 2D class averages of lipid-free nanodiscs containing the ABC transporter MsbA revealed particles lacking micelle density, with the MSP1D1 belt protein wrapped directly around the protein transmembrane domain. The high-resolution structure of this sample shows disordered helices forming an empty cavity, which in lipid-containing nanodiscs is usually occupied by density resembling a lipid. One possible explanation is that the scaffold protein may extract lipids from their binding pocket during nanodisc assembly. This approach therefore provides a tool to get and characterize delipidated membrane proteins, allowing to investigate structural and functional roles of specific lipids.

MsbA in Inside-Out vesicles

Alischa Scholz

Structural Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

MsbA is an ATP-binding cassette transporter (ABC) found in gram-negative bacteria. Due to its similarity to the human ABC transporter P-glycoprotein, a multidrug efflux pump, MsbA is intensely researched as a model system. MsbA transports a precursor of lipopolysaccharides (LPS) which is sitting in the outer membrane of the bacteria by ATP hydrolysis through the inner membrane of Ecoli.
Previous cryo-EM studies have provided valuable insights into the structure of proteins and the changes in their conformation during transport. Detergents, nanodiscs, peptidiscs and a few others demonstrate different levels of ability to show the transporter in various conformations. It is therefore concluded that the environment for protein purification and structural analysis is essential.
The aim of this project is to study MsbA in a more native environment. One of the methods used is the formation of proteoliposomes. In this method, MsbA is introduced to an E. coli polar lipid mixture to form proteoliposomes. This method can produce high-resolution structures, as demonstrated for the multidrug-resistant transporter AcrB and the ion channel ELIC in recent years. Preliminary data, obtained by collecting data via cryo-EM, training an AI to recognise liposomes in the micrographs, and picking particles via a Python program, shows an inside-out orientation of MsbA in an open, inward-facing conformation. Future analyses of membrane composition, structure and conformation in proteoliposomes and, subsequently, in cell-derived vesicles could also provide new insights into MsbA.

Dynamics and structure of Gasdermin E pores at mitochondrial membranes during cell death

Nadine Gehle

Molecular Cell Biophysics Division, Center for Cellular Nanoanalytics, Department of Biology/Chemistry, University of Osnabrück, Germany

Gasdermins (GSDMs) are a family of pore-forming proteins that induce pyroptosis by creating pores in the plasma membrane (PM), leading to PM permeabilization and the subsequent release of inflammatory cytokines, which trigger an immune response. Gasdermin E (GSDME) is particularly noteworthy as it is activated by the apoptotic caspase-3, positioning it as an essential protein in the transition from apoptosis to pyroptosis. Previous studies have suggested that GSDME disrupts both the PM and mitochondrial membranes during apoptosis; however, whether GSDME preferentially targets mitochondria over the PM remains unresolved.
To address this gap, we investigated the real-time sequence of events involving GSDME membrane permeabilization during apoptosis, focusing on its localization preference in the presence and absence of BAX and BAK proteins that facilitate the permeabilization of the outer mitochondrial membrane. Our approach included live-cell microscopy to monitor these processes and super-resolution microscopy to study the nanostructures of GSDME in cellular contexts.
Our findings reveal that during apoptosis, fragmentation of mitochondria occurs prior to PM permeabilization. Importantly, we demonstrated that GSDME targets both the outer and inner mitochondrial membranes, suggesting its role in the release of mitochondrial DNA. Using dual-color DNA-PAINT microscopy, we provided the first evidence of GSDME forming pores at the mitochondrial membranes with nanometer resolution. These insights underscore the significant involvement of GSDME in mitochondrial permeabilization during apoptosis and suggest a potential link to inflammatory responses. Further research into the interactions between GSDME, BAX, and BAK could expand our understanding of the intricate crosstalk underlying cell death programs.

Session IV

Keynote Lecture

Sabrina Jabs

Host Microbe Interactions, Institute of Clinical Molecular Biology Kiel, Germany

Novel regulators of the Mannose 6-phosphate pathway

Degradation of macromolecules delivered to lysosomes by processes such as endocytosis or autophagy is crucial for cellular function. Lysosomes require more than 60 soluble hydrolases in order to catabolize such macromolecules. These soluble hydrolases are tagged with mannose 6-phosphate (M6P) residues by the Golgi-resident GlcNAc-1-phosphotransferase (GNPT) complex, recognized by M6P receptors, and transported to lysosomes. Defects in this pathway result in the hypersecretion of hydrolases and lead to lysosomal storage disorders in patients, such as mucolipidosis II. We and others previously identified a novel membrane protein, LYSET, as essential for maintaining the Golgi localization and preventing degradation of GNPT, thus regulating proper M6P pathway function. LYSET plays a crucial role not only in viral infection, but also in facilitating the utilization of extracellular nutrients to promote cancer cell growth. Furthermore, we found that Golgi phosphoproteins 3 and 3L (GOLPH3/3L) interact with both LYSET and GNPT, regulating their steady state cis Golgi localization by mediating retrograde transport within the Golgi apparatus. GOLPH3/3L therefore represent novel regulators of the M6P pathway. We are now dissecting the interactions between LYSET, GNPT and GOLPH3/3L to better understand the pathogenesis of diseases related to the M6P pathway and to target cancer development and viral infections depending on lysosomal degradative functions.

Structural and Cellular Mechanisms of ABCA2 in Cholesterol Trafficking

Shi Min Tan

Structural Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

The ATP-binding cassette transporter ABCA2 plays a vital role in regulating cholesterol homeostasis in the brain1. Dysregulation of cholesterol homeostasis has been linked to several chronic sterol-dependent diseases, including Alzheimer's disease2. ABCA2 is involved in intracellular cholesterol trafficking at the late endosomes/lysosomes. Within the ABCA family of type II exporters, ABCA1 and ABCA2 are responsible for lipid metabolism in the brain. Unlike ABCA2, ABCA1 transports cholesterol across the plasma membrane to lipid-poor apolipoproteins1,2. While the structure and function of ABCA1 are well-established, the mechanism of ABCA2 remains to be characterized.
This study aims to elucidate the structural and cellular mechanisms of ABCA2 in cholesterol trafficking using single-particle cryogenic electron microscopy (cryo-EM). We resolved different states of the full-length ABCA2 structure at 2.5 Å and 3 Å, revealing features that are distinct from other available ABCA family structures. The transmembrane domain and the extracellular domain (ECD) form a charged and hydrophilic molecular basket on the upper membrane leaflet that could facilitate the transfer of cholesterol from the membrane to the ECD. Notably, multiple cholesterol molecules are tightly bound to the ECD, a finding that has not been observed in other type II family structures. These structures provide valuable insights into how ABCA2 sequesters cholesterol into the endosome/lysosome compartment, which might aid in developing new therapeutic strategies against sterol-dependent diseases.

1. Davis, W., Jr, & Tew, K. D. (2018). ATP-binding cassette transporter-2 (ABCA2) as a therapeutic target. Biochemical pharmacology, 151, 188–200. https://doi.org/10.1016/j.bcp.2017.11.018
2. Bossaerts, L., Cacace, R., & Van Broeckhoven, C. (2022). The role of ATP-binding cassette subfamily A in the etiology of Alzheimer's disease. Molecular neurodegeneration, 17(1), 31. https://doi.org/10.1186/s13024-022-00536-w

Yeast Fat1 is targeted to lipid droplets to activate free very long chain fatty acids

Carolin Willner

Bioanalytical Chemistry Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Fatty acids must first be activated by conjugation to coenzyme A (CoA) before they can serve as the building blocks for other membrane lipids. A special class of fatty acids are the very long chain fatty acids (VLCFAs) harboring 22 to 26 carbon atoms. The sole yeast VLCFA-CoA synthetase is the protein Fat1. However, the localization of free VLCFAs and that of Fat1 remain unclear. In our project, we show that free VLCFAs preferentially partition into the neutral core of lipid droplets (LDs). Fat1 is also targeted to LDs via an N-terminal amphipathic helix but can also localize to the endoplasmic reticulum in the absence of LDs. Structural predictions of Fat1 suggest a hydrophobic cavity expanding from the proteins active site to the membrane-targeting amphipathic helix. Together our results suggest that VLCFAs can reach the Fat1 active site directly from the core of LDs. Our model offers a molecular explanation for how very hydrophobic VLCFAs can reach a cytosolic active site of a protein. Our data support a model in which organelle targeting motifs co-evolved with the biophysical properties of its metabolite substrate.

Subversion of lipid asymmetry and its impact on plasma membrane plasticity and mechanics (Poster Index 14)

Katharina Sommer

Molecular Cell Biology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

A fundamental feature of cellular plasma membranes (PM) is an asymmetric lipid distribution across the bilayer, with sphingolipids like sphingomyelin (SM) concentrated in the exoplasmic leaflet and phosphatidylserine (PS) confined to the cytosolic leaflet. This energetically costly, out-of-equilibrium arrangement has been linked to a variety of physiological processes. In many cases, it is not lipid asymmetry per se but rather its subversion that mediates vital cellular responses. For instance, damaged lysosomes display a transient release of SM asymmetry that is integral part of a mechanism that drives their repair. Moreover, subversion of lipid asymmetry by Ca2+-activated scramblases plays a role in PM repair after pore formation, in the generation of apoptotic bodies, and in the biogenesis of extracellular matrix vesicles during bone mineralization. However, deciphering the direct consequences of a scrambled lipid distribution on the biophysics and plasticity of cell membranes is challenged by the pleiotropic nature of Ca2+ signaling pathways and a highly adaptable lipid metabolic network. To overcome this complexity, we took advantage of scramblase variants that bypass the need for Ca2+ for activity and found that PM lipid scrambling suffices to drive formation of extracellular vesicles. To elucidate the mechanism underlying scramblase-induced PM vesiculation, we will subject PMs and extracellular vesicles isolated before and after subversion lipid asymmetry to a comprehensive lipidome and proteome analysis. These efforts will be combined with live cell imaging of vesicle-enriched proteins and lipids during vesicle biogenesis.

Session V

Keynote Lecture

Sebastian Springer

Molecular Mechanisms of the Immune Response and Immuno-Biotechnology, Constructor University Bremen gGmbH, Germany

Intracellular trafficking and endocytosis of MHC class I proteins, the central agents of the cellular adaptive immune response

Major histocompatibility complex (MHC) class I proteins are peptide receptors that present the intracellular proteome to the T cells of the immune system. Since viral infections or tumorigenic aberration lead to a change in the proteome, the T cells can detect diseased cells via the novel presented peptides. MHC class I proteins have very interesting life histories: they are made and loaded in the Endoplasmic Reticulum, quality-controlled in the Golgi apparatus, and their cell surface transport and even their endocytosis are governed by their peptide occupancy. We have discovered that MHC class I proteins also form oligomers (clusters) at the cell surface, and we are investigating the biological meaning of these clusters.

Dynamic manipulation of functional plasticity of the host cell endosomal system by an intracellular pathogen

Shirley Helms

Microbiology Division, Department of Biology/Chemistry, University of Osnabrück, Germany

Salmonella enterica is a pathogen causing gastroenteritis with more than 1.3 billion cases worldwide causing major impact on public health. As facultative intracellular pathogen *S. enterica* is capable to modify and remodel the host cell endosomal system resulting in the biogenesis of the *Salmonella*-containing vacuole (SCV) and formation of *Salmonella*-modified membranes (SMMs). This endosomal remodelling is mediated by trans-location of effector proteins by the *Salmonella* pathogenicity island 2 (SPI2) type 3 secretion system (T3SS).
In this project, we investigate the lipid modification and organization of the SMMs after infection by *S. enterica* serovar Typhimurium (STM). We hypothesise that certain SPI2-T3SS effector proteins manipulate the lipid transport towards the SMMs. There are effector proteins proposed to have an impact on the lipid supply but the exact composition of the SCV and SMMs is not known so far. Therefore, we established an immunoprecipitation approach to purify lysosomes/SCVs/ SMMs of cells infected by STM for subsequent extraction of lipids and analysis of their composition. With this method, we aim to analyse the impact of STM and specific effector proteins on the SCV/SMM membrane structure, and the different lipid classes located in these membranes.
Moreover, we are interested in the intraluminal changes of the SCV and their interconnected network. A live cell pH sensor system specifically targeted to the intracellular lumen of the lysosomes is applied for time-resolved analysis of pH dynamics after infection with STM.

Structural Diversity of Rhodobacter capsulatus CobS - the AAA+ ATPase Motor of the Aerobic Vitamin B12 Biosynthesis Pathway

Ana Moura

Structural Biology of Photosynthetic Microorganisms, Department of Biology/Chemistry, University of Osnabrück, Germany

Vitamin B12 or cobalamin is one of the most structurally complex natural small molecules, acting as a cofactor, coenzyme and gene regulator in prokaryotes and eukaryotes. Cobalamin is synthesized in bacteria through two different pathways: aerobic and anaerobic. In the aerobic pathway used by the purple photosynthetic bacterium R. capsulatus, Co(II) insertion into hydrogenobyrinic acid a,c-diamide (HBAD) is catalyzed in an ATP-dependent manner by the cobalt chelatase complex (CobNST) to generate cobyrinic acid a,c-diamide [1]. CobNST comprises three subunits, an AAA+ ATPase (CobS) that provides the energy from ATP hydrolysis for cobalt insertion, a bridging subunit (CobT) and a catalytic subunit (CobN). Although CobNST has been functionally characterized [2], high-resolution structural data lacks to fully understand the reaction mechanism for cobalt insertion.
Here, we determined the structures of CobS AAA+ motor by cryo-EM. Our data shows that R. capsulatus CobS can assemble into helical filaments in vitro, resembling a staircase conformation. Moreover, we observed three extra conformations of the protein. Firstly, a spiral ring, possibly the initial step of the filament formation, and a flat hexameric closed ring, both conformations similar to those previously observed for other AAA+ ATPases [3]. Secondly, a unique double back-to-back spiral ring. Based on these findings, we further aim to understand the role of the different CobS conformations and relate them with the protein ATPase activity and the full cobalt chelatase reaction.

1. McGoldrick, H., Deery, E., Warren, M., & Heathcote, P. (2002).
2. Debussche, L., Couder, M., Thibaut, D., Cameron, B., Crouzet, J., & Blanche, F. (1992).
3. Jessop, M., Arragain, B., Miras, R., Fraudeau, A., Huard, K., Bacia-Verloop, M., Catty, P., Felix, J., Malet, H., & Gutsche, I. (2020).

Structure of a Surface-layer protein covering Anammox cells

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The discovery of anammox bacteria in the 1990s changed our understanding of the global nitrogen cycle (1). These extraordinary microorganisms with unusual morphology derive their energy from the oxidation of ammonium coupled with nitrite reduction, which relies on highly toxic intermediates such as hydrazine and nitric oxide (2). Approximately 50% of the dinitrogen gas released is produced by anammox bacteria. In biotechnology, the Anammox process is being used as a sustainable alternative to current wastewater treatment systems for the removal of nitrogen compounds.

We have made significant contributions to elucidating the nature of the catabolic pathway and characterizing the key soluble enzymes. Currently, using single-particle cryo-electron microscopy and cryo-electron tomography, we solved the complete atomic structure of the Surface-layer protein (SLPs), which form a para-crystalline layer covering the entire anammox cell. SLPs are the most abundant macromolecules in anammox bacteria and play several roles such as membrane scaffolding and external cell protection. In the context of anammox bacteria, we believe that SLPs also play a role in nutrient uptake by sequestering ammonium and nitrite ions inside the cells, which are essential for the anaerobic ammonium oxidation process.

1. Kuypers, M. M. M., Marchant, H. K. & Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* 16, 263-276 (2018)
2. Kartal, B., de Almeida, et al (2013) How to make a living from anaerobic ammonium oxidation. *FEMS Microbiol. Rev.* 37, 428-461
3. Akram, M., et al(2019) A 192-heme electron transfer network in the hydrazine dehydrogenase complex. Sci Adv 5, eaav4310
4. Chicano, T. M., et al(2021) Structural and functional characterization of the intracellular filament-forming nitrite oxidoreductase multiprotein complex. *Nat. Microbiol.* 6, 1129-1139

Optogenetic regulation of organelle contact sites

Pascal Höhne

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Contact sites are pivotal structures for inter-organellar communication, lipid metabolism, calcium homeostasis, structural integrity of organelles and stress-related responses. Currently, they are increasingly characterized as highly dynamic structures, reacting quickly to changes in metabolic conditions such as nutrient availability. However, tools to investigate and manipulate contact site formation and physiology with a high spatiotemporal resolution remain rare.
Therefore, we took an optogenetic approach, by tagging organelle membrane proteins with the optogenetic dimer pair iLID and nano. With one half of the opto-tether system linked to one organelle and the second half on another organelle, we are able to reversibly induce tethering, similar to what we would observe at a contact site, via blue light illumination. We are currently creating and characterizing an optogenetic toolkit for the manipulation of contact sites between different organelles, tuned to match the specific requirements of the respective interfaces.

Poster Abstracts

Poster Session I

Poster numbers 1 – 10

24 November, 16:00 – 17:30

Neprilysin 4 controls acrosome structure and male fertility in *Drosophila melanogaster* (Poster Index: 2)

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The acrosome is a highly conserved organelle located at the anterior tip of the mature sperm. It is essential to successful fertilization across species. Herein, we provide evidence that the peptidase Neprilysin 4 localizes to the acrosome during Drosophila sperm maturation and that activity of this enzyme is essential to proper acrosome structure, sperm tip partitioning and male fertility. In contrast to controls, sperm from animals with impaired Nep4 function exhibited a severely disturbed tip structure that affected intracellular and sperm surface components. These effects were secondary to structural defects in the acrosome morphology. Corresponding sperm were quickly discarded by the females, exhibited a reduced ability to fertilize eggs, and did not initiate embryonic development, even if sperm entry had occurred. This study demonstrates an essential role for a neprilysin in the structural integrity of the acrosome and the sperm tip, and thus introduces a new and crucial function of neprilysins in ensuring male fertility.

Coumarin-Based Photoactivable Lipid Probes for Investigating Lysosomal Lipid Trafficking (Poster Index: 3)

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Lysosomes are essential catabolic organelles responsible for the degradation and recycling of cellular macromolecules. Their dysfunction has been implicated in a wide range of diseases, from rare lysosomal storage disorders to common neurodegenerative conditions such as Parkinson's and Alzheimer's disease. Many of these disorders are associated with abnormal lipid accumulation inside lysosomes. While the mechanisms governing cholesterol export from lysosomes are relatively well understood, the pathways involved in the efflux of sphingosine and other lipids remain largely unclear.

To address this gap, we synthesize multifunctional chemical probes derived from sphingosine and cholesterol, termed lyso-pacSph and lyso-pacChol, respectively (Altuzar et al., 2023). These lysosome-targeted probes enable precise tracking of lipid localization, metabolism, and protein interactions within cells. Each probe integrates four functional modules: a photocleavable coumarin cage that allows controlled release of the active lipid upon blue-light irradiation, a photo-crosslinkable diazirine for covalent capture of nearby proteins, a clickable alkyne handle for post-labelling with fluorescent or biotin tags, and a lysosome-targeting amine to ensure subcellular specificity.

Together, these features enable three key applications: (i) metabolic tracking, to monitor lipid conversion through TLC or lipid mass spectrometry; (ii) visualization studies, where photo-crosslinking followed by fluorescent click labelling allows imaging of lipid localization; and (iii) protein interaction profiling, in which click labeling with biotin and avidin pulldown facilitate identification of lipid-binding proteins by mass spectrometry.

Overall, lyso-pacSph and lyso-pacChol constitute versatile tools to study lysosomal lipid trafficking and to uncover how disruptions in lipid exchange contribute to disease pathology.

Quantitative analysis of persister formation of typhoidal, non-typhoidal and invasive non-typhoidal *Salmonella enterica* strains (Poster Index: 4)

Comfort Yeboaa

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Antibiotic resistance poses a significant complication in the treatment of bacterial infections. However, bacterial persister cells, a dormant and tolerant subpopulation, further complicate eradication. This study shows the quantitative analysis of persister cell formation of Salmonella, both in vitro and intracellularly and explores potential strategies to target these cells.
Infection was done in murine macrophage cells following the methods outlined in Schulte et al., (2021). The use of fluorescent reporter systems allowed the determination of the persister status of Salmonella on a single cell level.
Strains used in this study were resistant to one or more classes of antibiotics. The strains include standard S. Typhimurium (STM) lab strain (SL1344), invasive non-typhoidal STM from sub-Sahara Africa (STM ST 313), non-invasive STM strain from Africa (STM 4/74), epidemic S. Infantis from Israel (SIN 119944), pre-epidemic S. Infantis (SIN 335-3), and reference STM strain (STM NCTC 12023 WT). Our results revealed quite diverse persister frequencies among different strains. In-vitro persistence was high in stationary phase cultures (0.1-1%), compared to exponential phase cultures (0.01-0.1%) across all strains, similar to reports of previous studies. Intracellular replicating STM showed higher stress response compared to non-replicating (NR) STM WT. The proportion of intracellular NR population ranged from approximately 0.4-0.6% between 16 -24 h p.i.

Conclusion
These findings provide new quantitative insights into Salmonella's persistence mechanisms and suggest the need for better therapeutic regimens and treatment options to effectively overcome chronic infections.

Identification of genes of *Salmonella* Typhimurium for hydroponic infection of lettuce roots (Poster Index: 5)

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Salmonella are major pathogens causing gastrointestinal infections. They often enter human hosts via contaminated food of animal origin. However, Salmonella is able to attach to agricultural grown plant species 3,5. Here, the type of irrigation is a crucial point in terms of hygiene and food safety. A frequently used method is growth in hydroponic culture, whereby only the roots come into contact with the irrigation water 1,2. During contamination, pathogens are transferred to the roots via the hydroponic medium, from where they can spread throughout the entire plant 4. However, the exact mechanisms by which Salmonella attach to the roots are poorly characterised 3,6.

We created a mTn5 library of Salmonella Typhimurium mutant strains, used the library to carry out root infection in order to identify genes affecting roots colonisation during hydroponic culture via Transposon-Sequencing. By direct sequencing of the transposon-flanking regions, potentially relevant features can be identified. Therefore, input (mutants before infection) and output (mutants from the root) were compared and the fold change was determined. Here we consider genes that show a disadvantage in root colonisation, as well as genes whose defect leads to an advantage in root colonisation. By screening the whole genome, we were able to identify factors required for root colonisation.

These factors were then validated using gene specific deletion mutants. Since individual mutants of the mTn5 library cannot be directly isolated and analysed, gene-specific mutagenesis of individual candidate genes was performed. These mutants were then pooled together, used for hydroponic infection and analysed via sequencing. For further characterisation of the genes regarding their role in root colonisation we investigated motility, biofilm formation and long-term survival in hydroponic media. Understanding the factors that influence root colonisation can help us to improve the safety of plant-based food production and prevent contamination.

1. Chen, Z., Han, Y., Ning, K., Luo, C., Sheng, W., Wang, S., Fan, S., Wang, Y., and Wang, Q. (2019). Assessing the performance of different irrigation systems on lettuce (*Lactuca sativa* L.) in the greenhouse. PLOS ONE 14, e0209329.
2. Dukes, M.D., Zotarelli, L., and Morgan, K.T. (2010). Use of Irrigation Technologies for Vegetable Crops in Florida. HortTechnology 20, 133-142

3. Hernández-Reyes, C., and Schikora, A. (2013). *Salmonella,* a cross-kingdom pathogen infecting humans and plants. FEMS Microbiology Letters *343*, 1-7.
4. Ilic, S., Moodispaw, M.R., Madden, L.V., and Lewis Ivey, M.L. (2022). Lettuce Contamination and Survival of *Salmonella* Typhimurium and Listeria monocytogenes in Hydroponic Nutrient Film Technique Systems. Foods 11, 3508.
5. Krtinić, G., Đurić, P., and Ilić, S. (2010). Salmonellae in food stuffs of plant origin and their implications on human health. European Journal of Clinical Microbiology & Infectious Diseases 29, 1321-1325.
6. Wheatley, R.M., and Poole, P.S. (2018). Mechanisms of bacterial attachment to roots. FEMS Microbiology Reviews.

Endocytosis and Membrane Trafficking in the Regulation of Notch Signaling (Poster Index: 6)

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The Notch signaling pathway plays central roles in controlling cell fate decisions during tissue development and homeostasis. Receptor activation occurs at Notch synapses, where direct contact between Delta ligand and Notch receptor expressed on neighboring cells, leads to sequential proteolytic cleavages of the receptor and the release of the Notch intracellular domain (NICD). NICD then translocates to the nucleus and activates the expression of Notch target genes. Despite knowing the molecular steps of the signaling cascade, the role of the plasma membrane lipid environment in organizing and regulating Notch signaling remains poorly understood. This project, focusses on investigating, how the lipid microenvironment influences the localization and activation of the receptor. Using advanced fluorescence microscopy, including Total internal reflection, Spinning Disc and lattice light-sheet microscopy, together with quantitative lipidomics and proteomics we aim to characterize the lipid composition and dynamics as well as the local neighborhood at Notch synapses. In complementary approaches, we take advantage of plasma membrane derived vesicles containing endogenous Notch receptors and Delta ligands to investigate the molecular abundance, lipid identity, and timing to induce a productive signaling response. These vesicles serve as a controlled platform to probe receptor–ligand interactions and endocytic mechanisms involved in Notch signal transduction. Together, these studies aim to establish a mechanistic framework linking lipid organization, membrane structure, and receptor activation, to uncover how biophysical membrane properties modulate Notch signaling and intercellular communication.

Reconstitution of the Mon1Ccz1 Rab GEF on model lipid bilayer (Poster Index: 7)

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The structure of the Rab GEF (guanine nucleotide exchange factor) Mon1Cczz1 in solution was determined using cryo-electron microscopy and helped to understand the mechanistic basis for membrane interaction of the complex. Combined with biochemical studies, a model for the Rab7 activation was developed. However, how the complex is oriented on the membrane, how the full-length Rab7 is bound, and how Rab5 binding promotes membrane recruitment and enhances catalytic activity of the complex remains elusive at a molecular level. To address these questions, we aim for the reconstitution of the endosomal and autophagosomal tetrameric complex of Mon1Ccz1 with their respective GTPases and/or with the autophagosome recruiter Atg8 on membranes for structural analysis by cryo-electron microscopy. As Rab7 has the highest affinity towards Mon1Ccz1 in the nucleotide-free state and Rab5 only interacts with Mon1Ccz1 in the GTP-bound state, getting both Rab-GTPases in their respective nucleotide loading states on one membrane represents the primary challenge for which different click techniques are needed. So far, the reconstitution of the *ct*Ypt7/*ct*MC1, *ct*Vps21b/*ct*MC1, and the *Sc*Atg8/*Sc*Ypt7/*Sc*MC1 complexes on DO/PIP+MPB liposomes was achieved. For the autophagosomal complex comprising of *Sc*Atg8/*Sc*Ypt7/*Sc*MC1 first cryo-electron microscopy conditions were screened and protein decorated liposomes were observed.

Cryo-EM structure of catalytic magnesium chelatase subunit ChlH bound to protoporphyrin IX(Index: 8)

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The biosynthesis of chlorophyll is initiated by the activity of magnesium chelatase, a multi-subunit protein complex that catalyzes the insertion of a magnesium ion into the macrocycle of protoporphyrin IX (PP_{IX}). The interplay between the ChlI motor, the bridging subunit ChlD, and the catalytic subunit ChlH is essential for the function of the complex [1].
Previous studies have characterized the function of magnesium chelatase and structures are available for some of the individual subunits [1-4]. High-resolution structures for cyanobacterial ChlH have been solved by X-ray crystallography, allowing characterization of the architecture of the protein and its putative active site [3, 4]. However, the mode of substrate binding and the mechanism by which ChlH inserts a magnesium ion into PP_{IX} remain unknown.
Here, we used single particle cryogenic electron microscopy (cryo-EM) to determine the structure of ChlH from the filamentous cyanobacterium *Nostoc* sp. PCC 7120. Our data reveal the high-resolution structure of PP_{IX}-bound ChlH. We report that the binding of PP_{IX} to ChlH induces a strong conformational change from an open to a closed state. Our results provide a deeper structural insight into ChlH and pave the way for further cryo-EM analysis of ChlH and the whole magnesium chelatase complex.

1. Jensen, P.E., et al. *JBC*. (1996)
2. Shvarev, D., et al. *mBio*. (2023)
3. Chen, X., et al. *Nat Plants*. (2015)
4. Adams, N.B.P., et al. *Nat Plants*. (2020)

Turnover and lateral distribution of membrane transporters in the yeast plasma membrane (Poster Index: 9)

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The *S. cerevisiae* plasma membrane (PM) contains a large number of small molecule transporters that are often strongly regulated in response to nutrient changes or environmental stresses. In a large scale microscopy-based screen, we found that most transporters are removed from the PM upon glucose starvation, resulting in a 40% reduction of the PM proteome within 2h of glucose removal, and similar change occuring with nitrogen starvation. We now seek to understand the interplay between secretory pathways, endocytic turnover and lateral compartmentalization during transporter homeostasis and adaptational response, utilizing microscopy methods such as TIRF and FRAP.
We have thus implemented the CATCHFIRE system for yeast cells to study trafficking and lateral distribution of various transporters. We demonstrate that the CATCHFIRE method, a controlled dimerizer approach, is compatible with *S. cerevisiae*, using the MCC/eisosome as reference target. With this new tool, we now intend to examine non-conventional secretion and recycling of transporters.

Structural insights into phosphoglucomutase 1 as a glycogen metabolism regulator (Poster Index: 10)

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Glycogen is an important source of energy and carbon, conserved through evolution from prokaryotes to eukaryotes[1]. During glycogen metabolism, phosphoglucomutase1 (PGM1) controls glucose utilization by interconverting glucose 1-phosphate and glucose 6-phosphate, enabling adaptation to environmental stress[2]. Using *Synechocystis* (Syn) as a model organism, a previous study showed that a post-translational modification (PTM) of the S47 residue is a key feature in regulating the activity of *Syn*PGM during nitrogen starvation[3]. In this study, Cryo-EM (cryogenic electron microscopy) was used to understand the structural mechanism by which phosphorylation and dephosphorylation of the S47 residue modulate the activity of *Syn*PGM with different substrates. The conformational variations resulting from the PTM at S47 were investigated by comparing the structures of wild-type and S47A mutant *Syn*PGM with and without substrates (glucose 1-phosphate as an activator and fructose 1,6-biphosphate as an inhibitor). The results present newly determined structures of PGM with the inhibitor, and reveal a conformation **distinct from previous studies** upon glucose 1-phosphate binding. Together with biochemical studies, these structural findings provide a deeper understanding of how PGM plays a key role in glycogen metabolism.

1. Wilson, W. A. et al. FEMS Microbiology Reviews 34, 952–985 (2010).
2. Ortega-Martínez, P., Roldán, M., Díaz-Troya, S. & Florencio, F. J. Journal of Experimental Botany 74, 1532–1550 (2023).
3. Doello, S., Neumann, N. & Forchhammer, K. The FEBS Journal 289, 6005–6020 (2022).

Poster Session II

Poster numbers 11 – 20

25 November, 19:30 – 21:00

ER-mitochondria contact site stability as an essential regulator in human cardiomyopathy (Poster Index: 11)

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TMEM43 encodes a four-transmembrane protein located in the outer membrane of the endoplasmic reticulum. A single point mutation, p.S358L, causes inherited cardiomyopathies characterized by arrhythmia and sudden cardiac death. Despite its clinical relevance, the molecular mechanisms underlying TMEM43 function have long remained unclear. Using *Drosophila melanogaster* as a model, we generated knock-in, knock-out, and tagged transgenic lines to study TMEM43-associated disease mechanisms. Flies carrying the *Tmem43*p.S333L mutation, equivalent to the human variant, reproduce major aspects of the human pathology, including cardiac arrhythmia, impaired energy metabolism, mitochondrial alterations, and shortened lifespan, highlighting the conserved role of TMEM43 in cardiac physiology. Our recent work links TMEM43 to endoplasmic reticulum–mitochondria contact sites (ERMCS) and identifies its interaction with Porin/VDAC as a potential mechanism regulating lipid composition and ion exchange between the two organelles. Lipidomics and proteomics analyses revealed changes in phosphatidylethanolamine and phosphatidylserine metabolism, suggesting that the mutation alters the structural and functional integrity of mitochondrial membranes. Together with super-resolution imaging, pulldown assays, and electron microscopy, these findings outline how TMEM43 disruption affects energy balance and organelle communication. Overall, our work establishes *Drosophila* as a robust model for TMEM43-linked cardiomyopathies and provides new mechanistic insight into how TMEM43 contributes to cellular homeostasis, offering an optimistic outlook for understanding and ultimately targeting this genetic heart disease.

Fluorescent-based *in vitro* detection of phosphoinositides for kinetic analysis of kinases and phosphatases (Poster Index: 12)

Jana Milach

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Phosphoinositides (PtsInsPs) are important for membrane identity and signaling processes. Several kinases and phosphatases are involved in converting the different forms of PtsInsPs according to the nutritional and metabolic requirements. In autophagy for example, the generation of phosphatidylinositol 3-phosphate (PtdIns3P) on the autophagosomal membrane is critical during the biogenesis of the autophagosome. However, at late stages its depletion is necessary to ensure the maturation of this organelle. Thus, the tight coordination of the phosphorylation and dephosphorylation reactions is crucial in autophagy as also in many other metabolic pathways. The knowledge on how the enzymes are regulated to ensure proper turnover is limited, in part due to difficulty to detect PtsInsPs *in vitro*. Here, we aim to optimize a lipid-coated silica-beads assay to monitor PtsInsPs production/consumption by specific fluorescently labelled biosensors. As a starting point for the kinetic analysis of kinase and phosphatase activities, we focus on the PtdIns3P conversion into phosphatidylinositol (PtdIns) by the phosphatase Ymr1, involved in autophagy and endocytosis, both described as pathways highly dependent on PtdInsPs turnover.

Unraveling the working mechanism of a tumor suppressor lipid (Poster Index: 15)

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Ceramides are central intermediates of sphingolipid metabolism that can activate a variety of tumor-suppressive cellular programs, including cell cycle arrest, senescence and apoptosis. Consequently, the potential of ceramide-based therapeutics in the treatment of cancer has become a major focus of interest. While a growing body of evidence indicates that ceramides can act directly on mitochondria to trigger Bax-mediated cell death, molecular details of the underlying mechanism are scarce. Combining a computational approach with functional studies in cancer cells, we previously identified the voltage-dependent anion channel VDAC2 as a direct effector of ceramide-induced apoptosis. VDAC residues involved in ceramide binding are also required for mobilizing hexokinase type I (HKI) to mitochondria, a condition promoting cell growth and survival in hyperglycolytic tumors. Coarse-grain molecular dynamics simulations and localization studies in cells revealed that HKI residues 1-15 are both necessary and sufficient to mediate VDAC-HKI binding. Importantly, a membrane buried glutamate essential for ceramide binding by VDACs also establishes a direct and critical contact with the N-term of HKI. Based on these data, we postulate that ceramides exert their tumor suppressor activities in part by acting as modulators of VDAC-based platforms to control mitochondrial recruitment of pro-apoptotic Bax and anti-apoptotic HKI. Our ongoing efforts focus on challenging this model using switchable ceramide transfer proteins and mitochondria-specific release of photocaged ceramides in live cell imaging and functional studies.

Lipid Droplet – Mitochondria contact sites in yeast (Poster Index: 16)

Louis Percifull

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Lipid droplet mitochondria contact sites are key regulators of long-term lipid storage and mobilization in mammalian cells. They have been shown to directly couple the lipid storage organelle with the place of bulk beta-oxidation, the mitochondria. However, the contact site also exists in yeast cells, which do not perform beta-oxidation in mitochondria. This raises the question which functions this contact site might have beyond fatty acid transfer.
We are analysing the yeast lipid droplet-mitochondria contact site by the use of genome-wide screening approaches. With the help of a split-Venus reporter we can visualise the contact site location and expanse. Genome-wide screens uncovered contact site residents and proteins that affect contact site formation. We are working toward resolving the lipid droplet-mitochondria tethering machinery and unravelling functions of this contact site besides fatty acid transfer for beta-oxidation. Our findings will hopefully not only broaden our understanding of the cellular contact site network in yeast, but also help in understanding overlooked features of this contact site in mammalian cells.

In vitro reconstitution and kinetics of the yeast serine palmitoyl transferase (Poster Index: 17)

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Sphingolipids (SL) are a major class of lipids found in cellular membranes. Sphingolipid biosynthesis begins in the endoplasmic reticulum (ER) with the condensation of serine and palmitoyl-CoA to form 3-ketodihyrosphingosine (3-KDS). This first and rate-limiting step is mediated by the **s**erine-**p**almitoyl transferase, an enzyme built from the two catalytic subunits Lcb1 and Lcb2 and a small supportive subunit called **T**sc3 in *Saccharomyces cerevisiae* (yeast*)*. Together these three subunits form a complex, called **SPOTS,** containing also one of the negative SPT regulators **O**rm1 or **O**rm2 and the Phosphatidylinositol-4-phosphate phosphatase **S**ac1.

Dysregulation of sphingolipid metabolism has been implicated in neurodegenerative disorders, diabetes, cardiovascular disease, and cancer progression. Therefore, understanding the molecular mechanisms governing the first step in sphingolipid synthesis is critical for elucidating their role in disease.

Recently, the structure of the yeast SPOTS complex was solved by our group, showing binding sites for ergosterol and ceramide as potential regulatory lipids. Also, the structure of mammalian homologous complex has been solved. While the subunits and structure and of those two complexes differ, the binding of ceramide and the enzyme activity are similar. To further analyze the mode of function and the regulation of the yeast SPT in a near-native environment, we reconstituted the SPOTS complex into ER membrane-mimicking liposomes and established a mass spectrometry-based *in vitro* activity assay.

This enables us to study the kinetics of the reconstituted SPT reaction and compare it to the in-solution kinetics. Future work will focus on further unraveling the multiple regulatory mechanisms, influencing the SPT kinetics with special focus on Orm protein phosphorylation via Ypk1 and lipid composition.

Mapping protein-specific lipid environments (Poster Index: 18)

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Biological membranes not only act as barriers but also provide a highly dynamic platform for proteins that are either embedded as integral transmembrane (TM) proteins or interact transiently as peripheral proteins. The lipid bilayer, as one of the major structural components of the cell, contributes actively to these interactions by shaping the local environment of proteins and thereby influencing their function. The three major lipid classes are glycerophospholipids, sphingolipids and sterols. Glycerophospholipids and sphingolipids feature different head groups, different acyl chain length, saturation and hydroxylation resulting in a large variety of different lipids and lipid classes.
The exact lipid composition for different proteins in their nanoenvironment is not yet known. It therefore remains an open question which lipid groups, acyl chain lengths, and saturations are present in the direct surroundings of specific proteins.
Here we present a method combining three approaches to investigate whether proteins within the same organelle are embedded in distinct lipid environments. These strategies include: (i) extracting lipids from detergent-purified membrane proteins to identify co-purified lipid species; (ii) analysing lipids in lysates of strains overexpressing target proteins to detect changes in overall lipid composition; and (iii) isolating organelles via bait proteins without detergents using the MemPrep method to preserve the surrounding native lipids. Together, these approaches aim to reveal protein-specific lipid compositions within organelles.

Spatiotemporal control of lipid nanodomains in the plasma membrane of live cells (Poster Index: 19)

Marvin Wortmann

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The plasma membrane of mammalian cells is highly heterogenous in nature due to dynamic lateral segregation into nanodomains with distinct and characteristic composition and collective membrane properties. The determinants for nanodomain formation and their biophysical and biochemical properties, however, remain poorly understood, as current analytical methodology is severely limited by the nanoscale dimension and high dynamics of membrane nanodomains. In this project, functionalized nanodot arrays (NDAs) will be leveraged to spatially and temporally control the assembly of different types of membrane nanodomains to enable robust interrogation of their properties by microscopy techniques. To this end, NDAs will be designed to efficiently capture and locally enrich different types of membrane scaffold-proteins (caveolins, tetraspanins), lipid-binding domains (Ostreolysin A, Choleratoxin B) or lipid-modifying enzymes (PI3K) in the plasma membrane of cells adhering on the substrate surface. Membrane properties will be probed using fluorescence reporters (D4H, PH domains, BAR domains) as well as putative interaction partners such as cytokine receptors (IL-6R, IL-10R, IFNGR) and downstream effector and regulator proteins (JAKs, RAS). Nanoscale spatial organization as well as diffusion and interaction dynamics inside different types of membrane NDAs will be probed by advanced and single-molecule fluorescence microscopy techniques.

tba (Poster Index: 20)

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tba