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1998 - 2000	PostDoc - Memorial Sloan Kettering Cancer Center, New York, USA (Prof. James E. Rothman)
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## Britta Brügger

### Roles of lipids in biological systems

#### Goal

**We aim to elucidate roles of lipids in modulating activities of proteins, including enzymes, receptors and proteins involved in transport processes. To study protein-lipid interactions and to understand the functional consequences of these interactions we follow an interdisciplinary approach combining cell biology, chemical biology, biochemistry and lipidomics.**

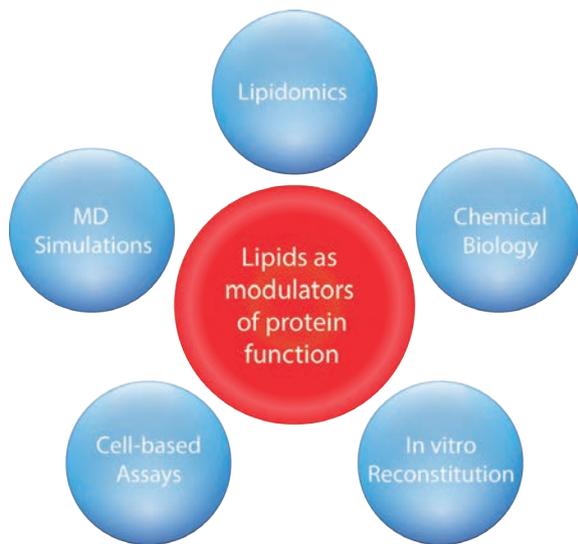
#### Background

Lipids serve multiple tasks within cells, ranging from structural to signalling functions. Hydrophobic by nature lipids usually appear within cells as integral parts of cellular membranes and storage organelles or associated with cytosolic carriers such as lipid transfer proteins.

As opposed to the classical view of membrane lipids being solely structural components to build a lipid mono- or bilayer and to serve as solvent for membrane proteins, we now appreciate that lipids fulfil multiple roles beyond these functions. This is reflected by the complexity of individual lipid species present in a membrane, with hundreds of different species from at least ten different lipid classes typically present in a mammalian membrane. Although lipids are continuously exchanged between subcellular organelles, medi-

ated by vesicular carriers, transfer proteins or membrane contact sites, there must be regimens to ensure that each cellular compartment maintains its specific lipid profile. To achieve this, alterations in membrane lipid profiles must be sensed, evaluated and appropriate responses have to be initiated. Changes of membrane lipid compositions can be global, organelle-specific or restricted to a hotspot within a membrane, and can occur as an adaptation to environmental extrinsic or intrinsic signals, such as circadian rhythmic, cell cycle, signalling cascades, vesicle biogenesis and consumption, to name a few. To understand the consequences of lipid alterations within membranes we focus on elucidating structural and functional aspects of interactions of lipids with proteins within membranes. To achieve this, we follow an interdisciplinary approach, combining cell biology with chemical biology, biochemistry and lipidomics (Figure 1).

Our interest in protein-lipid interactions was raised by our observation that N-stearoyl-sphingomyelin is enriched in COPI vesicles, while the bulk of sphingomyelins is segregated. To understand the molecular basis of this enrichment, we employed functionalized lipids that can be used to identify interacting proteins within cells. Using this approach, we identified p24, one of the key



**Fig. 1: Analysis of protein-lipid interactions within membranes.** To elucidate if and how lipids interact with proteins, we employ cellular, in vitro and in silico approaches.

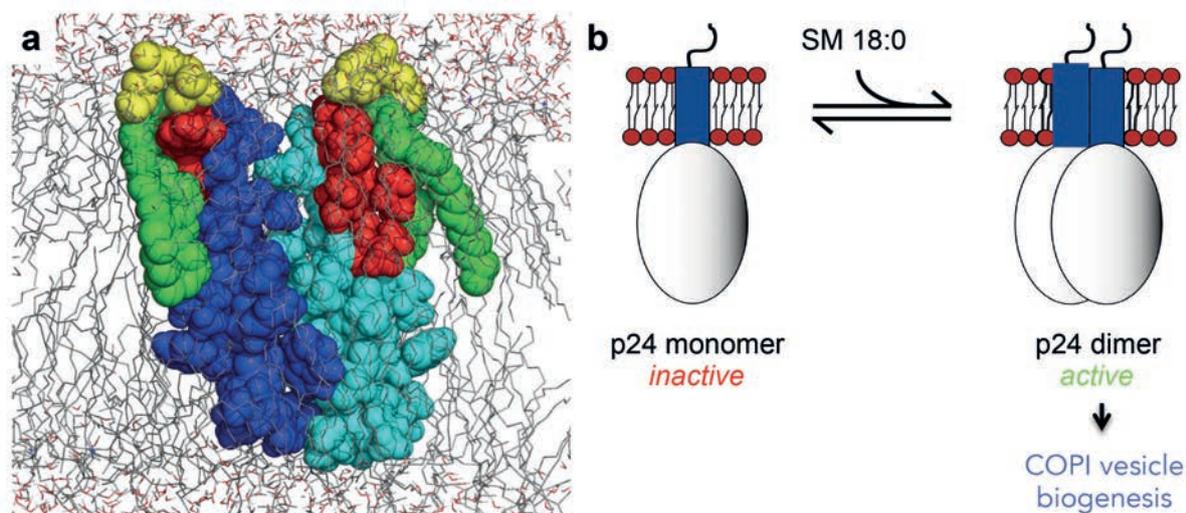
players in COPI vesicle biogenesis, to specifically interact with a sphingolipid. Combining different cellular and FRET-based in vitro reconstitution assays, we identified N-stearoyl-sphingomyelin as the preferred lipid interacting with p24. As a consequence of this specific protein-lipid interaction the formation of active p24 dimers is facilitated (Fig. 2).

## Research Highlights

### Protein-lipid interactions

Based on our initial studies on the presence of a sphingolipid-binding motif in single-spanning transmembrane proteins, we extended the analy-

ses towards multi-spanning transmembrane proteins. Together with Gunnar von Heijne a motif-probability algorithm was established for large datasets to evaluate customized amino acid motifs in transmembrane helices. With this approach we identified and validated a number of proteins that contain the putative sphingolipid-binding motif in single- and multi-spanning transmembrane proteins. Among the candidate proteins, G-protein coupled receptors (GPCRs) were significantly enriched, suggesting a role of sphingolipids in modulating GPCR function. For one member of this group, a metabotropic glutamate receptor, in collaboration with Irmi Sinning we had shown a coupling of activity with localisation to cholesterol-enriched membranes. Based on these previous studies, we have now identified variants compromised in lipid binding. These variants are now investigated to understand structural and functional consequences of compromised lipid binding. In case of EBOLA virus, we could unravel a role for a specific interaction of the viral membrane protein GP2 with cholesterol in conferring membrane activity, an important function both in viral entry and exit that might point towards novel therapeutic strategies for EBOLA infections. To allow for an unbiased identification of sphingolipid-interacting proteins we synthesised a novel



**Fig. 2: A specific interaction of the membrane protein p24 with a single sphingomyelin species facilitates formation of p24 dimers.** a, A molecular dynamics simulation of a homodimer interacting with two molecules of N-stearoyl-sphingomyelin (Erik Lindahl, Stockholm). b, Interaction of N-stearoyl-sphingomyelin with p24 modulates its function by facilitating formation of dimers. Dimeric p24 serves as platform for the recruitment of cytosolic COPI coat components to the membrane, a first step in the formation of COPI vesicles.

sphingolipid tool, a photoactivatable and clickable sphingosine (with Per Haberkant and Carsten Schultz, EMBL). This bifunctional sphingosine is metabolised in cells to bifunctional sphingolipids but its degradation via sphingosine-1-phosphate also generates bifunctional glycerolipids. To achieve specific labelling of only sphingolipids we employed a CRISP/Cas9 approach to knock-out the enzyme sphingosine-1-phosphate lyase in different cell lines, including fibroblast, T cells and lung epithelial cells. As additional tools, we also established the cellular synthesis of a clickable and photoactivatable phosphatidylcholine. Together with bifunctional cholesterol, we can now target the major membrane lipids.

One important and experimentally challenging tool is the mass spectrometric analysis of protein-lipid crosslink products. Here we have succeeded in establishing a method to identify crosslinked molecules via MALDI-TOF. In addition, we have established a protocol for mass spectrometry-reporter based identification of clicked lipid species and of crosslinked protein-lipid adducts by nano-electrospray ionisation mass spectrometry.

To extend the experimental approach for the identification of protein-sphingolipid interactions to those specific for a given sphingolipid species, we have generated by CRIPR/Cas9 a set of ceramide synthase knockout cell lines. Lipidomics analysis of these cells showed that we successfully manipulated the sphingolipid species profiles of these cells. A SILAC proteomics approach in combination with labelling of cells with bifunc-

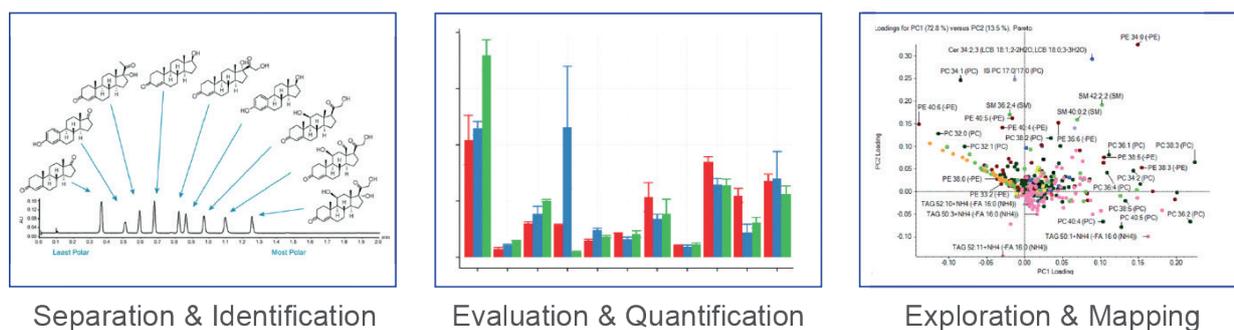
tional sphingolipids identified a set of proteins interacting with very-long chain sphingolipids. We now focus on validation and functional characterization of a subset of these proteins.

## Lipidomics

The second focus of our group is lipidomics, with the aim to define lipid classes including their molecular species at a quantitative level. Lipidomics has experienced rapid progress in recent years, mainly because of continuous technical advances, now providing quantitative lipid analysis with an unprecedented level of sensitivity and precision. The growing category 'lipid' includes a broad diversity of chemical structures with a wide range of physical-chemical properties. Reflecting this diversity, we apply different methods and strategies to quantify lipids (see also the report on the Lipidomics platform). In order to facilitate and accelerate data evaluation we have developed an R-based application for data analysis, including visualisation, exploration and statistical analyses of big data sets (Fig. 3).

With support of the cluster of excellence CellNetworks Heidelberg we have expanded the lipidomics platform, now also including ion mobility as well as supercritical fluid chromatography.

In the last three years, we have extended our analysis towards minor species, including lipid second messengers such as sphingosine and sphingosine-1-phosphate and complex glycosphingolipids such as gangliosides. As



**Fig. 3: A lipidomics workflow from mass spectrometry to data evaluation.** Following extractions with organic solvents to recover lipids from biological samples, mass spectrometric analysis is performed. Data sets are then analysed, explored and subjected to statistical interpretations.

for the separation of structural isomers, we do have promising preliminary data using a novel differential ion mobility setup. We are currently exploring this approach for the analysis of phosphoinositide isomers.

Besides our own work and collaborations on Heidelberg Campus, we continued to perform analyses for external national and international collaborators.

In one of these collaborations we investigated alterations in the cardiolipin homeostasis in Barth Syndrome, a cardiomyopathy caused by loss of tafazzin (Peter Rehling, Göttingen). Tafazzin is a mitochondrial acyltransferase, which is required for remodelling-based maturation of cardiolipin species. In a mouse model severe structural changes of respiratory chain supercomplexes at a pre-onset stage of the disease were found. In addition, we could show that the enzymatic block in cardiolipin maturation in mice led to a shift in cardiolipin species distributions and an increase of the cardiolipin precursor monolysocardiolipin, with both effects being strictly tissue-dependent. However, the molecular mechanisms linking defects in respiratory chain complexes to specific cardiolipin species are not yet understood and subject of on-going analyses.

With work for the group of Yves Rouille (Pasteur Institute, Lille) we expanded our analyses of viral lipid envelopes to bovine viral diarrhoea virus (flaviviridae family) that is characterised by a lipid composition, which is significantly distinct from the site of virus budding, the endoplasmic reticulum. These data suggest extensive lipid sorting prior to/during budding of progeny viral particles.

## Selected Publications 2014 - 2016

Luján P, Varsano G, Rubio T, Hennrich ML, Sachsenheimer T, Gálvez-Santisteban M, Martín-Belmonte F, Gavin AC, Brügger B, Köhn M. PRL-3 disrupts epithelial architecture by altering the post-mitotic midbody position. *J Cell Sci*. 2016;129(21):4130-4142.

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Gerl MJ, Sachsenheimer T, Grzybek M, Coskun U, Wieland FT, Brügger B. Analysis of transmembrane domains and lipid modified peptides with matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. *Anal Chem*. 2014;86(8):3722-6.

Brügger B. Lipidomics: Analysis of the lipid composition of cells and subcellular organelles by electrospray ionization mass spectrometry. *Annu Rev Biochem*. 2014 Jun 2;83:79-98.

## Awards and Honors

2015 Walter A. Shaw  
Young Investigator Award  
in Lipid Research

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