Lipid Rafts:

Methods for studying membrane organization

23 - 31st May, 2010 Dresden, Germany

PROGRAM & ABSTRACT BOOK

http://cwp.embo.org/pc10-08/

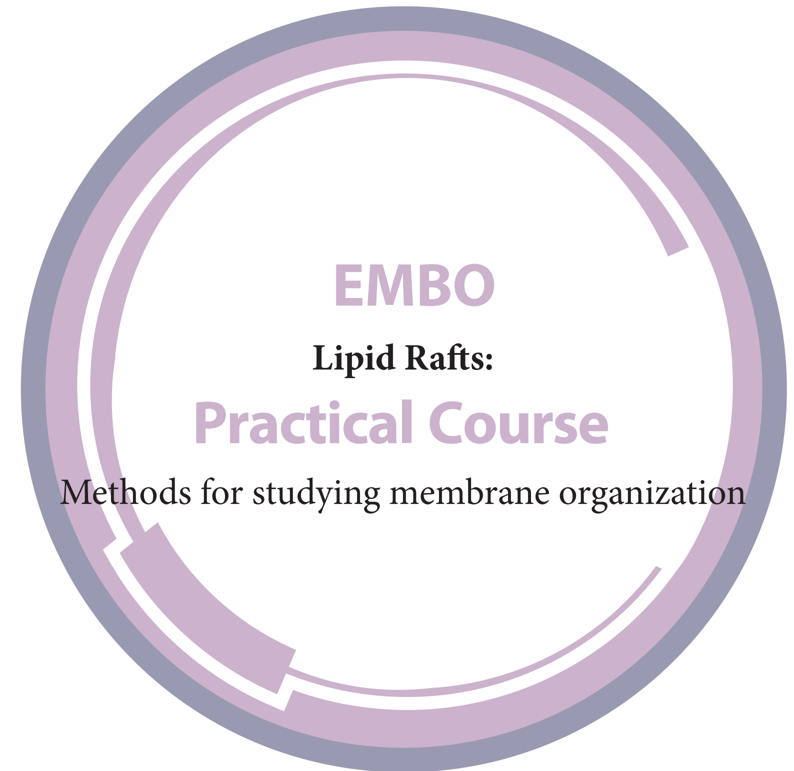
European Molecular Biology Organization

Organizers

Kai Simons • Ünal Coskun

Cover Design: Lynne Turnbull (lynne.turnbull@embo.org)
Composition: Ünal Coskun (coskun@mpi-cbg.de)
Booklet Template : Veronika Marcalíková (tatu.mamu@seznam.cz)
Cover Picture: Mikolaj Slabicki (slabicki@mpi-cbg.de)
Print & Binding: MPI-CBG Photolab

Text: MS Word
Graphics: Adobe Photoshop, Adobe Illustrator, Adobe InDesign
Font: Minion Pro
Number of prints: 50



Arrival at "Hotel am Blauen Wunder"

Loschwitzer Straße 48

01309 Dresden

Tel. +49-351-33660, Fax: +49-351-3366299

19:00 Dinner at restaurant Schillergarten

Program — Monday, 24 May

9:00	Opening lecture Kai Simons	**Small Auditorium**
9:50	Short talks: Participants	
11:20	Pinning up of posters	
12:00	Lunch at MPI-CBG canteen	
13:00	Poster Session	
14:30	Participant meeting	**Galleria 2nd floor**
16:00	Dimitrios Stamou *"Bending cell membranes"*	**Small Auditorium**
17:00	Ilpo Vattulainen: *"Modeling lipid-lipid and protein interactions"*	**Small Auditorium**

Program — Tuesday, 25 May

9:00	Christian Eggeling: *"STED microscopy in analyzing membrane dynamics"*	**Small Auditorium**
10:00	Experiments in the lab	
12:30	Lunch at MPI-CBG canteen	
13:00	Experiments in the lab	
17:00	Hai-Tao He: *"How do lipid rafts facilitate signaling at the cell surface"*	**Small Auditorium**

Program — Wednesday, 26 May

9:00	Christoph Thiele: *"Use of fluorescent lipids"*	**Small Auditorium**
10:00	Experiments in the lab	
12:30	Lunch at MPI-CBG canteen	
13:00	Experiments in the lab	
17:00	*Felix Wieland:* *"Lipid –protein interactions"*	**Small Auditorium**

Program — Thursday, 27 May

9:00	Sarah Veatch: *"Is the plasma membrane positioned close to a critical point and if yes what does this imply?*	**Small Auditorium**
10:00	Experiments in the lab	
12:30	Lunch at MPI-CBG canteen	
13:00	Experiments in the lab	
17:00	*Gerhard Schütz:* *"Single molecule methods to analyze membrane functions"*	**Small Auditorium**

Program — Friday, 28 May

9:00	Ari Helenius: *"Lipid-mediated endocytosis"*	**Small Auditorium**
10:00	Experiments in the lab	
12:30	Lunch at MPI-CBG canteen	
13:00	Experiments in the lab	
17:00	*Petra Schwille:* *"Spectroscopic imaging methods to analyze membrane functions"*	**Small Auditorium**

Program — Saturday, 29 May

9:00	Tobias Baumgart: *"Sorting of lipids and proteins by membrane domains and curvature"*	**Small Auditorium**
10:00	Experiments in the lab	
12:30	Lunch	
13:00	Experiments in the lab	

Program — Sunday, 30 May

9:45	*Boat tour to Meissen*
12:15	*Guided tour through the porcelain manufactury*
3:00	*Wine tasting at vineyard Prinz zur Lippe, Proschwitz* *http://www.schloss-proschwitz.de/*
19:00	*Concert at castle Wackerbarth* *http://www.mikhailsimonyanviolin.com/*

Program — Monday, 31 May

10:00	Finishing experiments in the lab
12:00	Summarizing meeting with participants, organizer and host lab
13:00	Lunch at MPI-CBG canteen

Departure

SPEAKERS

Tobias Baumgart

Department of Chemistry
University of Pennsylvania
231 S. 34th Street
Philadelphia, PA 19104-6323
baumgart@sas.upenn.edu

Sorting of proteins and lipids in membrane curvature and composition gradients

The sorting of lipids and proteins lies at the heart of fundamental biological phenomena such as organelle homeostasis, membrane signaling, and trafficking. Our research is directed at understanding biophysical contributions to the sorting of membrane components.
We will discuss two different, but in important situations intimately related mechanisms to sort membrane components. These are lateral segregation through preferential intermolecular interactions in mixed membranes, and molecular sorting as a consequence of membrane shape (i.e. membrane curvature).
Our experimental systems reach from self-assembled lipid model membranes, over purified cellular plasma membrane vesicles and endosomal vesicles, to membranes in whole cells. Quantitative measurements allow us to test thermodynamic and mechanical models relevant for cellular function.

Heinrich M, Tian T, Esposito C, & Baumgart T (2010) Dynamic sorting of lipids and proteins in curvature gradients: a moving phase boundary problem. Proceedings of the National Academy of Sciences of the United States of America In Print

Tian A & Baumgart T (2009) Sorting of lipids and proteins in membrane curvature gradients. Biophysical Journal 96:2 676 - 2688

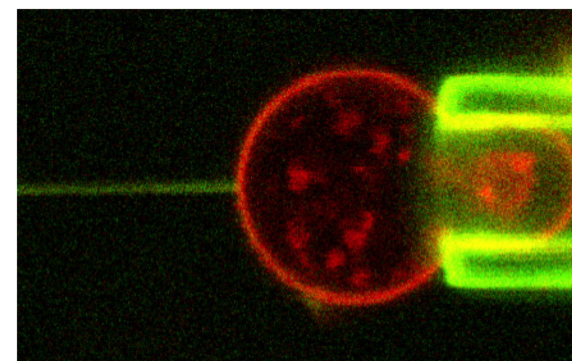
Tian AW, Capraro BR, Esposito C, & Baumgart T (2009) Bending Stiffness Depends on Curvature of Ternary Lipid Mixture Tubular Membranes. Biophysical Journal 97: 1636-1646

Capraro BR & Baumgart T (2010) Curvature sensing by the epsin N-terminal homology (ENTH) domain measured on cylindrical lipid membrane tethers. Journal of the American Chemical Society 132: 1200-1201

Johnson S, et al. (2010) Temperature dependent phase behavior and signal protein partitioning in giant plasma membrane vesicles. Biochimica Et Biophysica Acta - Biomembranes In Print

Baumgart T, et al. (2007) Large scale fluid/fluid phase separation of proteins and lipds in giant plasma membrane vesicles. PNAS 104: 3165

Baumgart T, Hess ST, & Webb WW (2003) Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. Nature 425: 821 - 824



Christian Eggeling

Max-Planck-Institute for Biophysical Chemistry
Department of NanoBiophotonics
Am Fassberg 11
37077 Goettingen, Germany
ceggeli@gwdg.de

STED microscopy in analyzing membrane dynamics

Cholesterol-assisted lipid interactions such as the integration into lipid nanodomains ('rafts') are considered to play a functional part in a whole range of membrane-associated processes, but their direct and non-invasive observation in living cells is impeded by the resolution limit of >200nm of a conventional far-field optical microscope [1]. We report the detection of single diffusing lipid molecules in nanosized areas in the plasma membrane of living cells using the superior spatial resolution of stimulated emission depletion (STED) far-field nanoscopy [2-5] or of fast single-molecule tracking [6]. Combining a (tunable) resolution of down to 30 nm with tools such as fluorescence correlation spectroscopy (FCS) or spatio-temporally following the movement of single lipids, we obtain new details of molecular membrane dynamics [4-6]. Sphingolipids or 'raft'-associated proteins are transiently (~ 10 ms) trapped on the nanoscale in cholesterol-mediated molecular complexes (Figure 1). Distinct differences show up between different lipids and molecules. For example, trapping of phosphoglycerolipids as well as cholesterol dependence of ganglioside or phosphoinositol lipid trapping is much less pronounced than for sphingomyelin lipids. STED nanoscopy and/or fast single-molecule tracking are thus exceptional tools to study the role of membrane heterogeneity in cellular functioning.

[1] E. Abbe (1873) Beitraege zur Theorie des Mikroskops und der mikroskopischen Wahrnehmung. Archiv fuer Mikroskopische Anatomie 9:413-468.

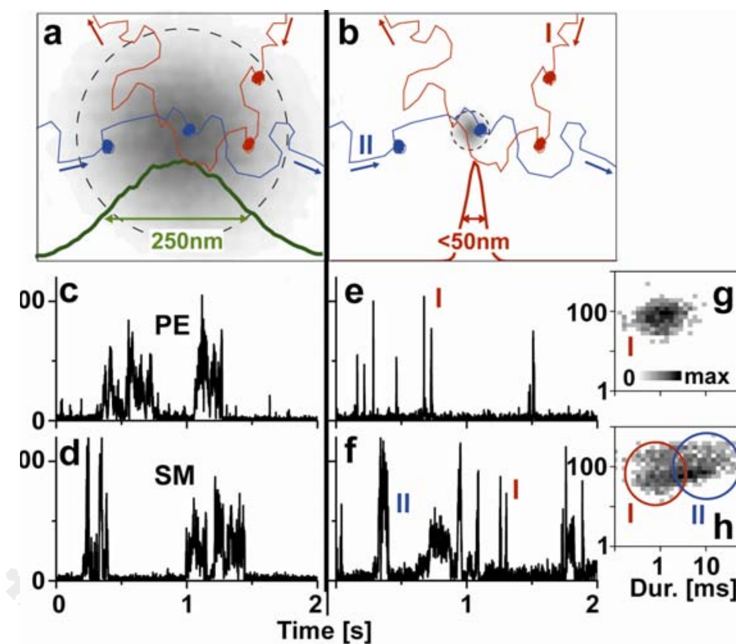
[2] S.W. Hell (2007) Far-Field Optical Nanoscopy. Science 316(5828):1153-1158.

[3] S.W. Hell, J. Wichmann (1994) Breaking the diffraction resolution limit by stimulated-emission - stimulated-emission-depletion fluorescence microscopy. Optics Letters 19(11):780-782.

[4] C. Eggeling, C. Ringemann, R. Medda, G. Schwarzmann, K. Sandhoff, S. Polyakova, V.N. Belov, B. Hein, C. von Middendorff, A. Schonle, S.W. Hell (2009) Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature 457:1159-U121

[5] C. Ringemann, B. Harke, C. von Middendorff, R. Medda, A. Honigmann, R. Wagner, M. Leutenegger, A. Schonle, S.W. Hell, C. Eggeling (2009) Exploring single-molecule dynamics with fluorescence nanoscopy. New J. Physics 11: 103054.

[6] S. Sahl, M. Leutenegger, M. Hilbert, S.W. Hell, C. Eggeling (2010) Fast molecular tracking maps nanoscale dynamics of plasma membrane lipids. PNAS 107: 6829-6834.



Hai-Tao He

Centre d'Immunologie de Marseille-Luminy
INSERM UMR631-CNRS UMR6102
Université de la Méditerranée
Case 906, 13288
Marseille France
he@ciml.univ-mrs.fr

How do lipid rafts facilitate signaling at the cell surface

Lipid rafts are sphingolipid/cholesterol-dependent dynamic assemblies proposed to be involved in various cellular processes, in particular signal transduction. Here we first discuss our recent studies on identification and characterization of raft nanodomains in the plasma membrane of live cells, using an original fluorescence correlation spectroscopy (FCS) approach. We then discuss the experimental evidences demonstrating important roles played by raft nanodomains in cell signaling, taking example of our recent work on the activation of the PI3K/Akt-mediated cell growth/survival pathway and that of the Fas-mediated cell death pathway (coll. A.-O. Hueber, ISDB, Nice), respectively.

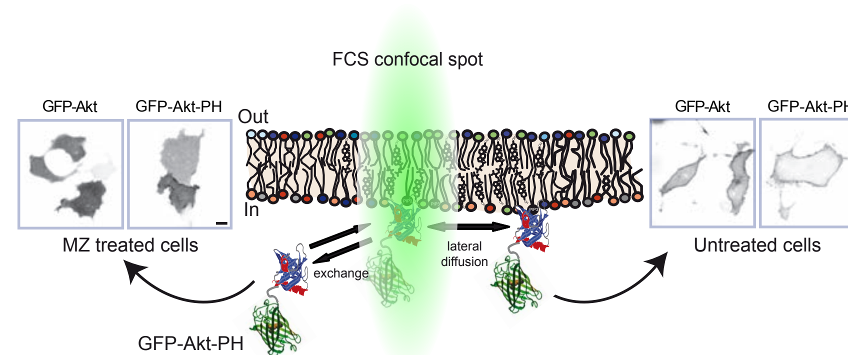
Lasserre R., Guo X.J., Conchonaud F., Hamon Y., Hawchar O., Bernard A.M., M'Homa Soudja S., Lenne P.F., Rigneault H., Olive D., Bismuth G., Nunes J.A., Payrastre B., Marguet D. and He H.T. (2008) Raft nanodomains contribute to Akt/PKB plasma membrane recruitment and activation. Nat Chem Biol. 4: 538-47

2. He H.T. and Marguet D. (2008) Lipid rafts and TCR triggering: a matter of time and space scales. EMBO Rep. 9: 525-30

3. Hérincs Z., Chakrabandhu K., Dost B., Peng L., Conchonaud F., Marguet D., He H.T. and Hueber A.-O. (2007) Palmitoylation is required for Fas to trigger cell death signalling. EMBO J. 26: 209-20

Marguet D., Lenne P.F., Rigneault H. and He H.T. (2006) Dynamic in the plasma membrane – How to combine fluidity and order. EMBO J. 25: 3446-57

Lenne P.F., Wawrezinieck L., Conchonaud F., Wurtz O., Boned A., Guo X.J., Rigneault H., He H.T. and Marguet D. (2006) Dynamic molecular confinement in the plasma membrane by microdomains and the cytoskeleton meshwork. EMBO J. 25: 3245-56



Ari Helenius

Institute of Biochemistry
ETH Zurich
8093 Zurich
ari.helenius@bc.biol.ethz.ch

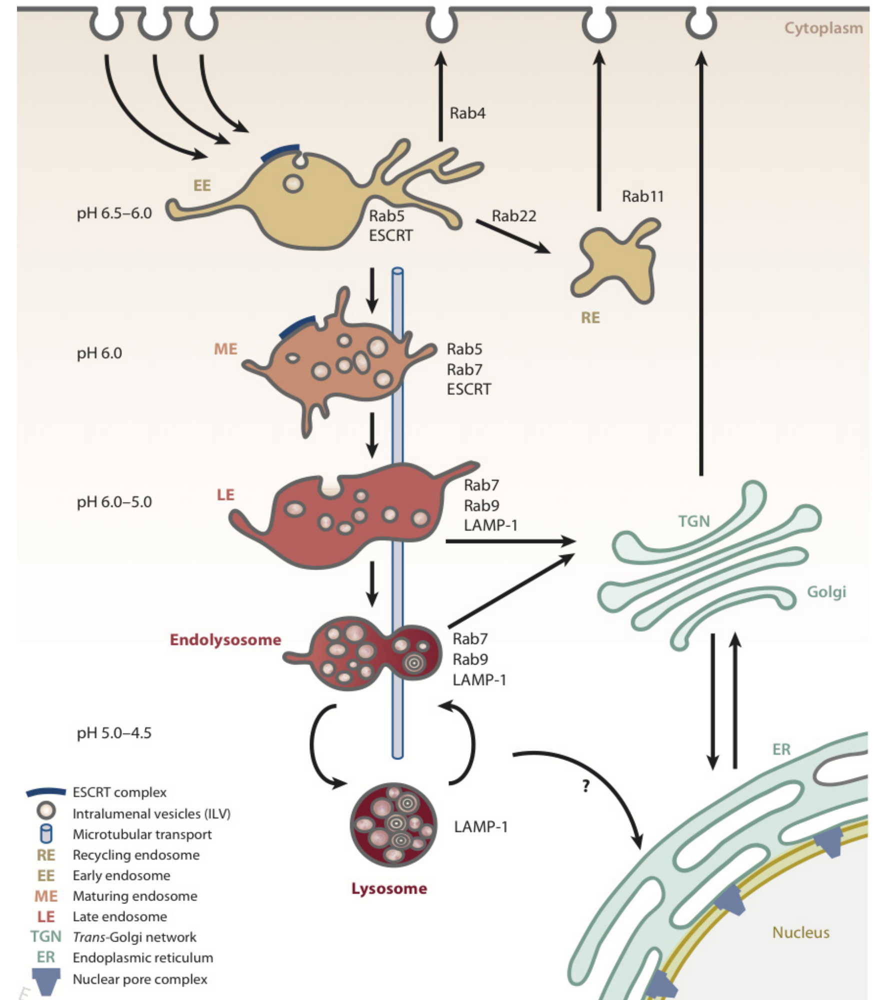
While plasma membrane lipids play a central role in the signaling, sorting, and vesicle formation during endocytosis, some lipids can actually serve directly as receptors for extra cellular ligands such as bacterial toxins and viruses. These ligands bind with high specificity to the carbohydrate moiety of gangliosides and other glycoshingolipids. Since the ligands are multimeric, binding leads to lipid clustering followed by association with lipid rafts, curvature induction, transbilayer coupling, and signaling. Endocytosis generally usually occurs by clathrin-independent mechanisms. After transport to endosomes, the ligands are usually routed to the ER. Endocytosis using lipid receptors has many interesting and unusual features, which will be discussed.

Marsh, M., and A. Helenius (2006) Virus entry: Open sesame. Cell. 124:729-740

Ewers, H., A. Smith, I. Sbalzarini, H. Lilie, P. Koumoutsakos, and A. Helenius (2005) Single particle tracking of murine polyomavirus-like particles on live cells and artificial membranes. Proc. Natl. Acad. Sci. US. 102:15110-15115

Ewers, H., Römer, W., Smith,A.E., Bacia, K., Dmitrieff,S., Chai, W, Mancini, R., Kartenbeck,J., Chambon, V., Berland, L., Oppenheim, A., Schwarzmann, G., Feizi, T., Sens, P., Schwille, P., Helenius, A. and L. Johannes (2010) GM1 structure determines SV40-induced membrane invagination and infection.Nature Cell Biology, 12(1):11-8

Sandvig K., B. and van Deurs (2005) Delivery into cells: lessons learned from plant and bacterial toxins. Gene Ther. 12(11):865-72



Gerhard Schuetz

Biophysics Institute
Johannes Kepler University Linz
Altenbergerstr. 69,
A-4040 Linz, Austria
Gerhard.Schuetz@jku.at

Single molecule methods to analyze membrane structures and dynamics

The plasma membrane structure affects interactions between membrane constituents by influencing their movements at the nanometer scale. We apply single molecule fluorescence microscopy to resolve the plasma membrane structure at a nanoscopic length-scale by employing the high precision for localizing biomolecules of down to 15nm [1]. Minimum invasive labeling via fluorescent Fab fragments is sufficient to image the lateral diffusion of individual protein molecules on a sub-millisecond time scale. We applied this technology to study the motion of single glycosylphosphatidylinositol-(GPI-) anchored proteins in the plasma membrane of living cells [2-4]. In contrast to results obtained by tracking gold-labeled membrane proteins, the single molecule fluorescence data reveal free Brownian motion of the proteins down to length scales of ~70nm, indicating no constitutive confinement zones.

In addition, we developed a technique to detect molecular cluster formation in the cellular plasma membrane of living cells [5-7]. With this methodology, individual aggregates can be selectively imaged, and the load of each cluster can be determined. We applied this technique to investigate the association of GPI-anchored proteins in living cells. Aggregates could indeed be detected and were observed to diffuse freely as stable platforms in the plasma membrane, shedding new light on the current debate concerning the existence of "lipid rafts".

Next, we are interested in measuring interactions between membrane proteins. Using single molecule Förster Resonance Energy Transfer (FRET) we are interested in measuring interactions between membrane proteins. we determined the lifetime of the trans-interaction between the T cell receptor and the peptide-loaded MHCII [8]. Finally, a general method to detect particularly weak interactions between membrane constituents using in vivo micropatterning will be presented [9].

[1] S. Wieser and G. J. Schütz (2008) Methods 46: 131-140

[2] S. Wieser, M. Axmann and G. J. Schütz (2008) Biophys J. 95: 5988-6001

[3] S. Wieser, M. Moertelmaier, E. Fuertbauer, H. Stockinger and G. J. Schütz (2007) Biophys J, 92: 3719-3728

[4] K. Drbal, M. Moertelmaier, C. Holzhauser, A. Muhammad, E. Fuertbauer, S. Howorka, M. Hinterberger, H. Stockinger and G. J. Schütz (2007) Int Immunol, 19: 675-684

[5] M. Moertelmaier, M. Brameshuber, M. Linimeier, G. J. Schütz and H. Stockinger (2005) Appl Phys Lett, 87: 263903

[6] V. Ruprecht, M. Brameshuber and G. J. Schütz (2010) Soft Matter 6: 568-581

[7] L. Zimmermann, W. Paster, J. Weghuber, P. Eckerstorfer, H. Stockinger and G. J. Schütz (2010) J Biol Chem, 285: 6063-6070

[8] J. B. Huppa, M. Axmann, M. A. Mortelmaier, B. F. Lillemeier, E. W. Newell, M. Brameshuber, L. O. Klein, G. J. Schütz and M. M. Davis (2010) Nature, 463: 963-967

[9] M. Schwarzenbacher, M. Kaltenbrunner, M. Brameshuber, C. Hesch, W. Paster, J. Weghuber, B. Heise, A. Sonnleitner, H. Stockinger and G. J. Schütz (2008) Nat Methods, 5: 1053-1060

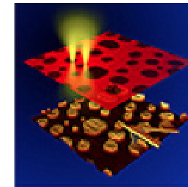
Petra Schwille

Biophysics Group
BIOTEC
TU Dresden
Tatzberg 47-51
01307 Dresden, Germany
schwille@biotec.tu-dresden.de

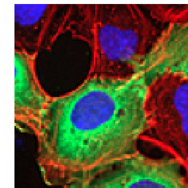
The power of minimal systems in characterizing protein-lipid interactions

Minimal systems like supported lipid bilayers and giant unilamellar vesicles (GUVs) have become very prominent in membrane protein research. GUVs in particular have been studied by membrane and lipid researchers for more than two decades. With their comfortable dimensions between single and hundreds of micrometers that are easily accessible to optical imaging and manipulation techniques, they have been proven ideal model systems to study membrane morphology and mechanical parameters, such as surface tension, elasticity, and local curvature, relevant for membrane structure and transformations. Moreover, since the advent of the raft hypothesis in cell biology, there has also been rising interest from the biological community to better understand the relevance of local lipid order for the lateral sorting and induction of functionality of membrane proteins. It is quite evident that the quantitative representation and local order of specific lipids in membranes of various organelles, in tight concert with the respective proteins inserted or attached to them, accounts to a large extent for biological functionality. However, since the exact relationships and also the structural features in live cells are often too complex or too small to be resolved quantitatively, minimal systems with reduced complexity, such as GUVs, pave the way to a more fundamental understanding of lipid-lipid and lipid-protein interactions of physiological importance.

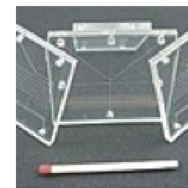
Moreover, inspired by the success of these minimal systems approaches to cell biological phenomena, many researchers nurture strong hopes that such a bottom-up approach does not stop at the membrane or at membrane-related processes, but that the GUV model system can be worked into more elaborate models of biomolecular self-organization.



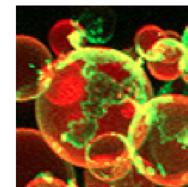
Single-Molecule Methods



Cell and Membrane Biophysics



Microfluidics



Synthetic Biology

Kai Simons

Max Planck Institute
of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden, Germany
simons@mpi-cbg.de

LIPID RAFTS- WHERE DO WE STAND NOW

The lipid raft concept introduces into membrane organization the capability of dynamic subcompartmentalization based on phase separation. Rafts form dynamic platforms with a key role in regulating membrane functions. They are dynamic assemblies of sphingolipids, cholesterol and proteins that dissociate and associate on a rapid timescale. These assemblies can be induced to coalesce to form raft clusters and these are the platforms that function in membrane trafficking, cell polarization and signalling. I will go through the recent developments in the field.

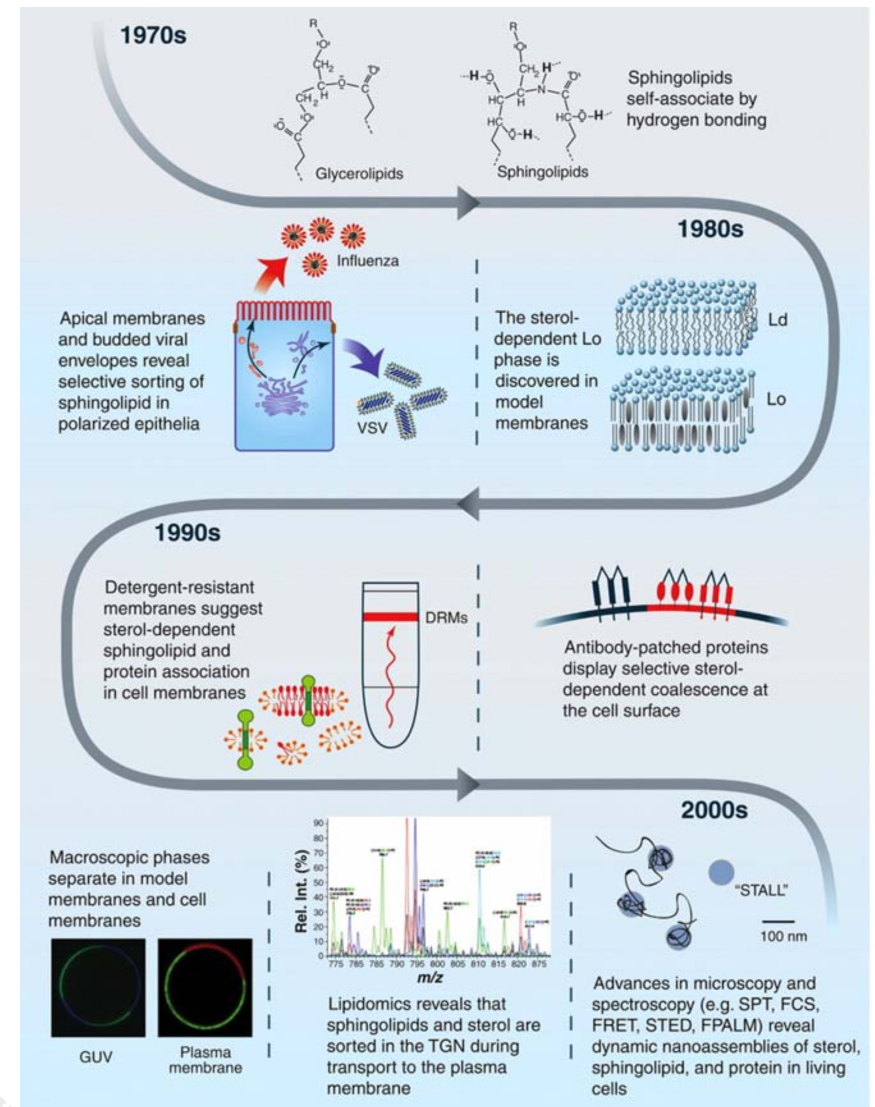
D. Lingwood and K. Simons (2010) Science 327: 46-50

Ü. Coskun and K. Simons (2010) Febs Letters 584: 1685-1693

H.J. Kaiser, D. Lingwood, I. Levental, J. Sampaio, L. Kalvodova, L. Rajendran, K. Simons (2009) PNAS. 106: 16645-50.

R. Klemm, C.S. Ejsing, M. Surma, H.J. Kaiser, M. Gerl, J.L. Sampaio, de Q. Robillard, C. Ferguson, T.J. Proszynski, A. Shevchenko, K. Simons (2009) J Cell Biol 185: 601-12

D. Lingwood, J. Ries, P. Schwille, K Simons (2008) PNAS 105: 10005-10



Dimitrios Stamou

Department of Neuroscience and
Pharmacology & Nano-Science Center
University of Copenhagen
Copenhagen, Denmark
stamou@nano.ku.dk

The Language of Shape: Membrane curvature as an allosteric modulator of protein localization and function

To date we have established exhaustive correlations between the lipid composition of membranes and its impact on membrane properties and protein function. In addition to composition the shape of cellular membranes appears to be a well-conserved phenotype in evolution. Nevertheless we largely ignore what are the consequences of membrane shape/curvature to biological functions that make it so critical for sustaining life. The lack of information on the significance of membrane shape has predominantly been due to the absence of reliable assays that allow us to perform systematic experiments as a function of membrane shape/curvature. We have recently demonstrated the possibility to construct a high throughput array of unique nanoscale membrane curvatures. The assay is based on unilamellar liposomes of different diameters (30 nm to 700 nm), and therefore curvature, that are immobilized on a surface at dilute densities allowing for imaging of single liposomes with fluorescence microscopy. One of our major scientific objectives for the coming decade will be to identify and characterize quantitatively the multiple instances during which the shape of cellular membranes influences the outcome of biological processes.

K.L Madsen, V.K. Bhatia, U. Gether and D. Stamou (2010) BAR Domains, Amphipathic Helices and Membrane-Anchored Proteins use the same mechanism to sense membrane curvature. K.L Madsen FEBS Letters, 584: p. 1848

V.K. Bhatia, N.S. Hatzakis and D. Stamou (2010) A Unifying Mechanism Accounts for Sensing of Membrane Curvature by BAR domains, Amphipathic Helices and Membrane–anchored proteins. Seminars in Cell and Developmental Biology 21: p. 381

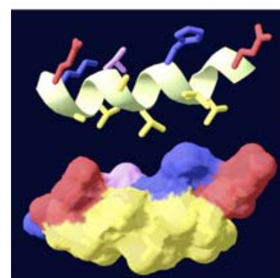
N. S. Hatzakis*, V. K. Bhatia*, J. Larsen, K. L. Madsen, P. Y. Bolinger, A. H. Kunding, J. Castillo, U. Gether, P. Hedegård and D. Stamou (2009) How Curved Membranes Recognize Amphipathic Helices and Protein Anchoring Motifs. Nature Chemical Biology, 5: p. 835

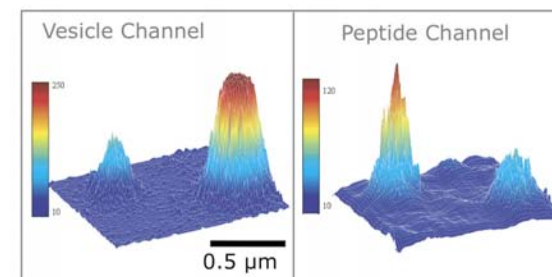
V. K. Bhatia, K. L. Madsen, P. Y. Bolinger, P. Hedegård, U. Gether, D. Stamou (2009) Amphipathic motifs in BAR domains are essential for membrane curvature sensing. EMBO Journal, 28: p. 3303

P. M. Bendix, M. S. Pedersen and D. Stamou. (2009) Quantification of nanoscale intermembrane contact areas using fluorescence resonance energy transfer. PNAS, 106: p. 12341

C. Lohr, A.H. Kunding, V. K. Bhatia and D. Stamou.(2009) Constructing size distributions of liposomes from single-object fluorescence measurements. Methods in Enzymology Liposomes. Book Series Methods in Enzymology, 465: p. 143

A. H. Kunding, M. W. Mortensen, S. M. Christensen and D. Stamou (2008) A Fluorescence–Based Technique to Construct Size Distributions from Single Object Measurements, Application to the Extrusion of Lipid Vesicles Biophysical Journal, 95: p. 1176





Christoph Thiele

LIMES Life and Medical Sciences Institute
University of Bonn
Carl-Troll-Str. 31
53115 Bonn, Germany
cthiele@uni-bonn.de

Use of fluorescent lipids

Fluorescent lipids and related functionalized compounds are important tools of biochemical and cellular lipid research. They allow analysis of metabolism, localization, transport and interactions of lipids with both high temporal and spatial resolution. But unlike proteins or nucleic acids, lipids are small molecules that react strongly on even small changes of their natural structure. The lecture will discuss in depth strategies of probe design, synthesis, and applications as well as the limitations of labeled molecules and possible label-free alternatives.

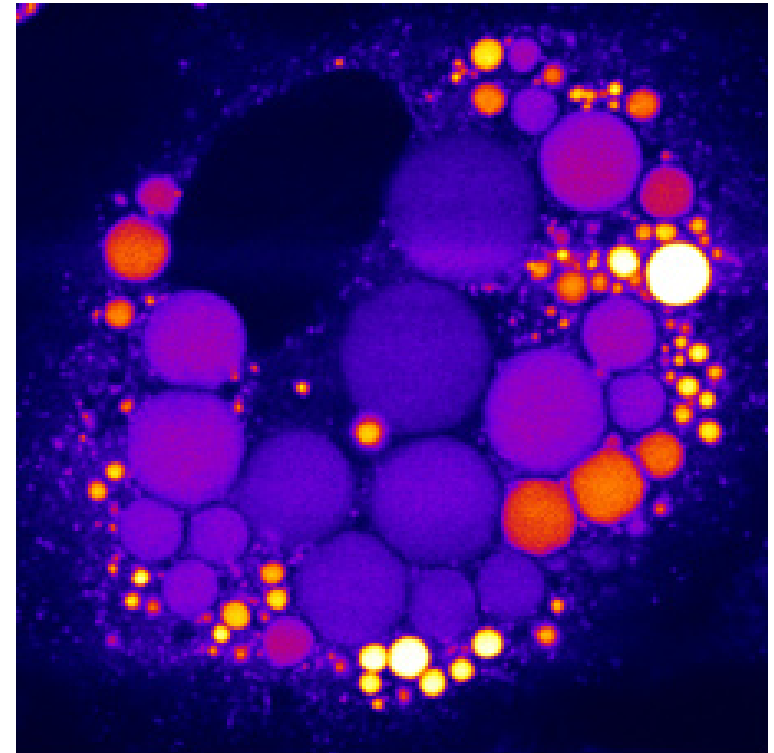
Spandl, J., White, D.J., Peychl, J. & Thiele, C. (2009) Live cell multicolor imaging of lipid droplets with a new dye, LD540. Traffic 10, 1579-1584

Kuerschner, L., Moessinger, C. & Thiele, C. (2008) Imaging of Lipid Biosynthesis: How a Neutral Lipid Enters Lipid Droplets. Traffic 9, 338-352

Haberkant, P., Schmitt, O., Contreras, F.X., Thiele, C., Hanada, K., Sprong, H., Reinhard, C., Wieland, F.T. & Brugger, B.(2008) Protein-sphingolipid interactions within cellular membranes. J Lipid Res 49, 251-262

Kuerschner, L., Ejsing, C.S., Ekroos, K., Shevchenko, A., Anderson, K.I. & Thiele, C. (2005) Polyene-lipids: A new tool to image lipids. Nat Methods 2: 39-45

Thiele, C., Hannah, M.J., Fahrenholz, F. & Huttner, W.B. (2000) Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. Nat Cell Biol 2: 42-49



Ilpo Vattulainen

Department of Physics
Tampere University of Technology
POB 692, FI-33101
Tampere, Finland
Ilpo.Vattulainen@tut.fi

Modeling lipid-lipid and lipid-protein interactions

While experiments are the cornerstone of lipid research, they are often challenged by the time and length scales associated with nano-scale phenomena. Meanwhile, as computer simulations are the method of choice for dealing with molecular processes in atomistic detail, they complement experiments and provide a great deal of added value for lipid research. Here we discuss how atomistic and coarse-grained simulation techniques can be employed to investigate a variety of phenomena in lipid systems over a multitude of scales in time and space. We first consider the basics of simulations in general, and then discuss case studies where simulations are used to study the structure and dynamics of membranes and membrane proteins, with an emphasis on systems related to lipid rafts.

P. Niemelä, M. T. Hyvönen, and I. Vattulainen (2009) Atom-scale molecular interactions in lipid raft mixtures. Biochimica et Biophysica Acta - Biomembranes 1788, 122-135

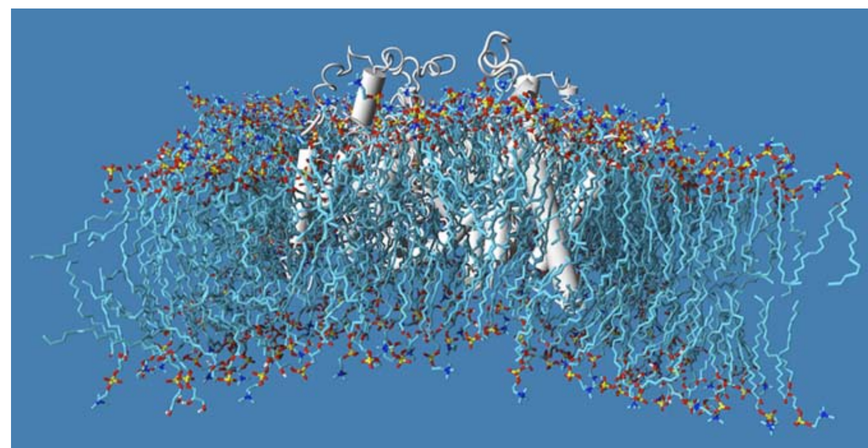
P. Bjelkmar, P. Niemela, I. Vattulainen, and E. Lindahl (2009) Conformational changes and slow dynamics through microsecond polarized molecular simulation of an integral Kv1.2 ion channel. PLoS Computational Biology 5, e1000289

T. Murtola, A. Bunker, I. Vattulainen, M. Deserno, and M. Karttunen (2009) Multiscale modeling of emergent materials: Biological and soft matter. Physical Chemistry Chemical Physics 11, 1869-1892

S.J. Marrink, A.H. de Vries, D.P. Tieleman (2009) Lipids on the move: Simulations of membrane pores, domains, stalks and curves. BBA Biomembranes 1788, 149-168

P. Niemela, S. Ollila, M. T. Hyvonen, M. Karttunen, and I. Vattulainen (2007) Assessing the Nature of Lipid Raft Membranes. PLoS Computational Biology 3, 304-312

D. P. Tieleman, S. J. Marrink, H. J. C. Berendsen (1997) A computer perspective of membranes: Molecular dynamics studies of lipid bilayers systems. Biochim. Biophys Acta 1331, 235-270

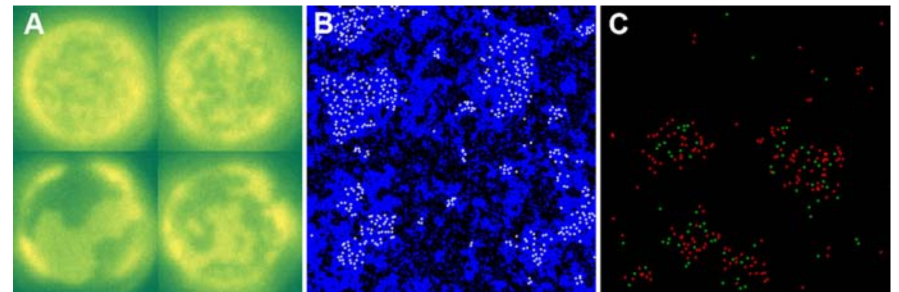


Sarah Veatch

Department of Biophysics
University of Michigan
930 N. University
Ann Arbor, MI 48109-1055
sveatch@umich.edu

Lipid rafts reach a critical point.

Multicomponent lipid bilayer membranes can contain two coexisting liquid phases, named liquid-ordered and liquid-disordered. Recently, we demonstrated that large (micron-scale) and dynamic critical fluctuations are found in simple ternary bilayer membranes prepared with critical compositions. Remarkably, robust critical behavior is also found in compositionally complex vesicles isolated directly from living cell plasma membranes. This finding strongly suggests that cells tightly regulate plasma membrane protein and lipid content to reside near a critical point and that critical fluctuations provide a physical basis of functional membrane heterogeneity in living cells at physiological temperatures. We are currently probing for critical fluctuations in intact RBL mast cells using high resolution imaging techniques (scanning electron microscopy and super-resolution fluorescence localization microscopy). In addition, we are investigating possible structural and functional consequences of plasma membrane criticality using computational approaches, and are testing these predictions experimentally using the model system of IgE mediated signalling in RBL mast cells



Micron-sized and dynamic critical fluctuations are found at the surface of a giant plasma membrane vesicle at multiple temperatures near the critical temperature. Vesicles are isolated from living RBL mast cells and are roughly 10 micrometers in diameter. (B) Monte Carlo simulation of blue 'lipids' clustering around fixed white 'proteins,' mimicking what may occur after cross-linking of cell surface receptors with antigen. (C) Reconstructed SEM micrograph demonstrating that two different proteins co-cluster on the surface of antigen stimulated RBL mast cells. Protein clusters are roughly 200nm.

Felix Wieland

Heidelberg University Biochemistry Centre
INF 328
69120 Heidelberg, Germany
Felix.Wieland@bzh.uni-heidelberg.de

Lipid-protein interactions

The complexity of biological membranes results from a network of specific lipid-lipid, protein-protein and lipid-protein interactions that are formed by a variety of proteins, lipid classes and molecular species. Whereas the mechanisms of intra-membrane protein-protein interactions as well as the interactions of soluble proteins with the polar moieties of lipids are rather well understood, remarkably less is known about the determinants that mediate specificity between membrane proteins and lipids in the hydrophobic core of biological membranes. We first discuss the potential of novel in vitro approaches, which emerged from the fields of chemical biology, biophysics and membrane biochemistry and enable the analysis of specific lipid-protein interactions, and then focus on an unprecedented example of a highly specific interaction of a membrane protein with a particular lipid molecular species. We further analyse the potential molecular mechanism of this interaction, its conservation across the membrane proteome and its impact on protein-protein and lipid-protein contacts.

D. M. Engelman (2005) Membranes are more mosaic than fluid. Nature 438: 578-580

P. S. Niemelä, M. T. Hyvönen and I. Vattulainen (2009) Atom-scale molecular interactions in lipid raft mixtures. Biochim Biophys Acta. 1788: 122–135

A. M. Ernst, F. X. Contreras, B. Brügger and F. T. Wieland (2010). Determinants of specificity at the protein-lipid interface in membranes. FEBS Lett. 3; 584(9): 1713-20

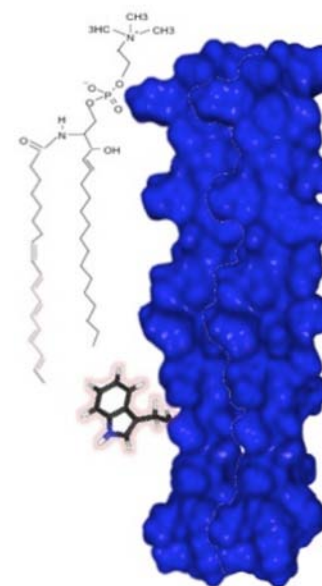
A. G. Lee (2003). Lipid-protein interactions in biological membranes: a structural perspective. Biochim. Biophys. Acta. 1612: 1-40

C. Hunte (2005) Specific protein-lipid interactions in membrane proteins. Biochem. Soc. Trans. 33(5): 938-942

L. Kuerschner, C. Moessinger and C. Thiele (2004) Polyene-lipids: a new tool to image lipids. Nat Methods. 2(1): 39-45

P. Haberkant, O. Schmitt, F. X. Contreras, C. Thiele, K. Hanada, H. Sprong, C. Reinhard, F. T. Wieland and B. Brügger (2008) Protein-sphingolipid interactions within cellular membranes. J Lipid Res. 49(1): 251-62

P. Haberkant and G. van Meer (2009) Protein-lipid interactions: paparazzi hunting for snap-shots. Biol Chem 390(8): 795-803



Participants

Zakaria Ali M. Almsherqi

Department of Physiology
National University of Singapore
Block MD9, 2 Medical Drive
SINGAPORE 117597
Singapore
phszama@nus.edu.sg

Cubic Biomembranes

Biomembranes are traditionally viewed as flat lipid-bilayer sheets, defining the cell boundaries or dividing the cell into multiple subcellular organelles with specialized functions. However, biomembranes may also fold up into 3-dimensional periodic arrangements, termed cubic membranes, which can adopt extraordinary complexity with up to 12 parallel layers of membranes. The same geometry is mathematically well described and extensively studied in synthetic liquid crystals and block copolymers systems, with a wide range of technological applications. Inner mitochondrial membranes of amoeba Chaos cells adopt cubic morphology upon starvation (Figure), which thus represents a unique experimental system to address the molecular mechanisms involved in controlling membrane morphology and associated functions.

We have studied cubic membrane formation in detail in our laboratory. Transformation of membrane morphology is accompanied by significant changes in phospholipid and fatty acid profiles of Chaos cells, and liposomes prepared from the lipids of starved Chaos cells display a high propensity to form hexagonal and cubic arrangements in vitro. These data demonstrate the importance of lipids in forming highly order membrane structures. We have further shown that isolated amoeba mitochondria containing cubic membranes efficiently incorporate and retain DNA oligonucleotides, suggesting a role of cubic membranes in intracellularmacromolecule transport, which opens potential application in gene delivery and therapy approaches.

Y Deng, Z.A. Almsherqi, M.M. Ng, SD Kohlwein (2010) Trends Cell Biol. in press

Y. Deng, Z.A. Almsherqi, G. Shui, M.R. Wenk, S.D. Kohlwein (2009) FASEB J. 23: 2866-71.

Z.A. Almsherqi, T. Landh, S.D. Kohlwein, Y. Deng. Chapter 6 (2009) Int Rev Cell Mol Biol. 274: 275-342.

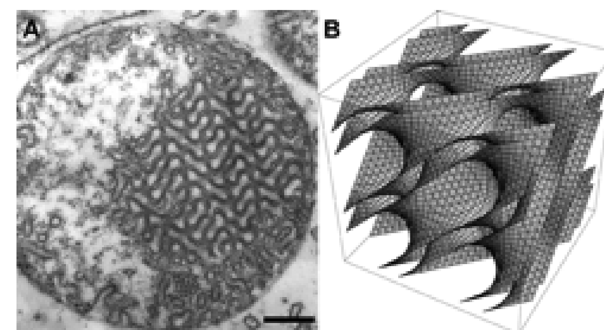
Z.A. Almsherqi, S. Hyde, M. Ramachandrana, Y. Deng (2008) J R Soc Interface. 5: 1023-9.

Z.A. Almsherqi, S.D. Kohlwein, Y. Deng (2006) J Cell Biol. 173: 839-44

Z.A. Almsherqi, C.S. McLachlan, M.B. Slocinska, F.E. Sluse, N. Kocherginsky, I. Kostetski, D.Y. Shi, S.L. Liu, P. Mossop, Y. Deng (2006) Cell Res. 16: 297-305.

O.C. Ooi, H.F. Al Habib, Z.A Almsherqi, R.M. El Oakley (2006) Cell Tissue 7: 307-17.

R.E. Oakley, Z.A. Almsherqi, S.K. Lim, S.H. Lee, K.T. Ho, A. Sutandar, C.N. Lee, Y.T. Lim. (2005) Heart Surg Forum. 8: E348-50



Andrea Balogh

Immunology Research Group
Hungarian Academy of Sciences
Pazmany Peter setany 1/C.
H-1117 Budapest, Hungary
jaworski@iimcb.gov.pl

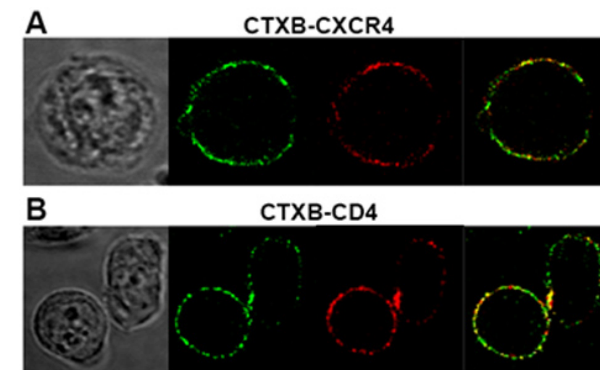
Natural cholesterol-specific autoantibodies (ACHAs), with yet unknown functions, are present in the sera of healthy individuals. We generated two new mouse IgG3 ACHAs (AC1 and AC8) reactive with clustered cholesterol, such as lipoproteins, lipid rafts of immunocytes and membranes of endoplasmic reticulum and the Golgi complex.

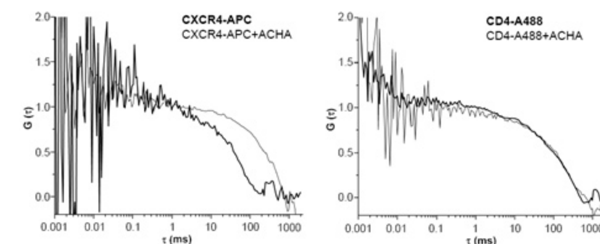
We were interested in the potential of ACHAs to modulate raft-dependent immune functions, like HIV-1 infection which takes place through these membrane microdomains. In a collaboration work we found that the antibodies significantly inhibited infection and in vitro HIV production of human macrophages and T cells. Therefore we investigated whether the new ACHAs can modulate the receptor or microdomain architecture at the surface of target cells. Namely the distribution/interaction pattern, accessibility, internalization, mobility or raft association of CD4 and chemokine receptors. These properties are all critical for membrane attachment and internalization of the virus. The new IgG3 ACHAs caused a remarkable lateral clustering of ganglioside-rich membrane rafts upon binding to the cells and remodeled the interaction pattern of CXCR4 chemokine receptors with both CD4 and lipid rafts, characterized by increased colocalization and FRET efficiency. Lateral mobility of CXCR4 co-receptors but not of CD4 or non-raft proteins (e.g. CD2) was constrained by ACHA binding. The Fab fragment of AC8 did not show any of these effects.

These data suggest a novel type of inhibition of HIV-1 infection by lipid (cholesterol)-specific mAbs, which is linked to their primary membrane remodeling effect on target cells.

Bíró A, Cervenak L, Balogh A, Lorincz A, Uray K, Horváth A, Romics L, Matkó J, Füst G, László G.(2006) Novel anti-cholesterol monoclonal immunoglobulin G antibodies as probes and potential modulators of membrane raft-dependent immune functions. J Lipid Res. Jan;48:19-29

Beck Z, Balogh A, Kis A, Izsépi E, Cervenak L, László G, Bíró A, Liliom K, Mocsár G, Vámosi G, Füst G, Matko J. (2010) New cholesterol-specific antibodies remodel HIV-1 target cells' surface and inhibit their in vitro virus production. J Lipid Res.51:286-96.





Colocalization of HIV-receptors, CXCR4 and CD4, with choleratoxin B (A.B) and lateral diffusion of these receptors in untreated and ACHA-treated macrophages (C,D)

Katharina Beck

Max Planck Institute of Immunobiology
Stübeweg 51
Freiburg, Germany
beck@immunbio.mpg.de

Role of membrane lipids in T-cell receptor (TCR) function

The composition of lipid bilayers can influence the activity of transmembrane proteins implying lipid-protein interactions. We used the TCR as a model to study these interactions. The TCR is a transmembrane-multiprotein complex existing as monovalent and multivalent TCRs on the surface of T-cells [1,2]. The multivalent receptors are much more sensitive to stimulation than the monovalent ones [1]. Since the ratio of mono- to multivalent TCRs varies, its regulation might be crucial for the sensitivity of T-cell activation. Cholesterol-extraction by MβCD disrupts multimeric TCRs into monomeric ones [1]. Hence, we wondered whether lipids interacted directly with the TCR. To this end, we synthesized beads coupled to cholesterol or the most common fatty acid chains in cell membranes. The TCR preferentially bound to palmitate- and cholesterol- compared to stearate- and arachidate-coupled beads. Furthermore, we reconstituted the TCR into PC-liposomes of different fatty acid compositions. Successful TCR integration was only achieved using PC-liposomes containing palmitoleate, suggesting that hydrophobic mismatch impairs integration.

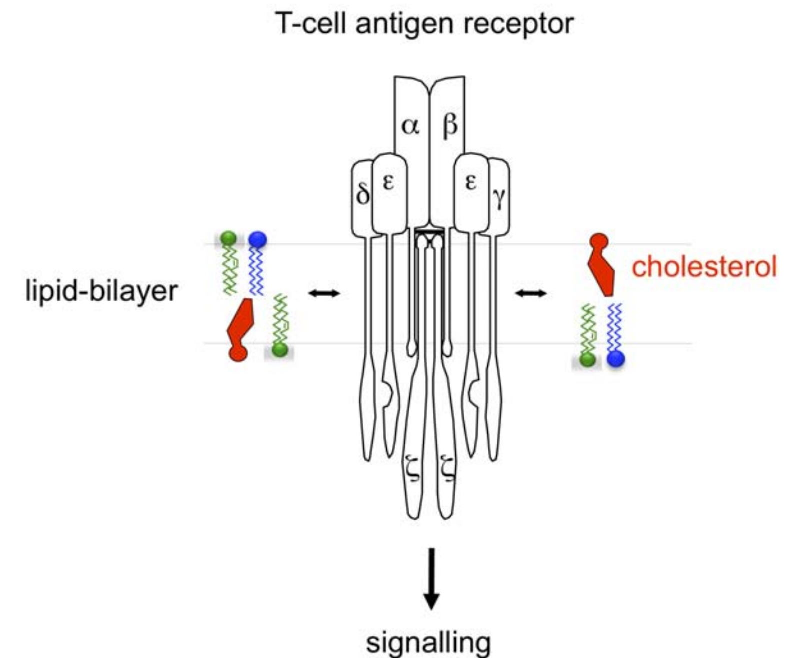
To identify cholesterol-binding sites of the TCR, we performed cross-linking experiments with radioactive photo-cholesterol in intact T-cells (3). We discovered that only two of the six TCR subunits bind cholesterol. Further, we observed that upon TCR triggering less cholesterol binds to the TCR. Altered lipid-binding to membrane proteins after activation might explain the translocation of those proteins into lipid rafts (4).

[1] Schamel et al. (2005) J Exp Med. 202: 493-503.

[2] Lillemeier et al. (2010) Nat Immunol. 11: 90-96.

[3] Thiele et al. (2000) Nat Cell Biol. 2: 42-9.

[4] Lingwood and Simons (2010) Science 327: 46-50.



Christin Bissig

University of Geneva,
Department of Biochemistry
30 quai E. Ansermet
1211 Geneva 4, Switzerland
Christin.Bissig@unige.ch

Alix-LBPA interaction in endosome biogenesis

Endosomes along the degradation pathway exhibit a multivesciular appearance and differ in their lipid compositions (1). Association of protein complexes with specific membrane lipids regulates endosomal function and biogenesis (2). Therefore it is important to investigate how protein-lipid interactions are achieved and how they are controlled.

I study the protein-lipid interaction between the late endosomal phospholipid lysobisphosphatidic acid (LBPA) (3) and its effector, the ESCRT-associated protein Alix. In vitro assays with liposomes and endosomes indicate that LBPA can drive the formation of intralumenal vesicles and that Alix negatively regulates the process (4). By combining studies of membrane transport in vivo, with assays measuring vesicle formation in vitro and biophysical strategies, I am investigating the role of Alix-LBPA interaction in the formation, dynamics and back-fusion of intralumenal vesicles.

My preliminary in vitro results indicate that the N-terminal Bro1 domain of Alix is sufficient to interact with LBPA-containing bilayers. I have identified by site-directed mutagenesis residues in the Bro1 domain that are required for this interaction, and I am using LBPA analogues and isoforms to characterize the binding determinants on the lipid. .

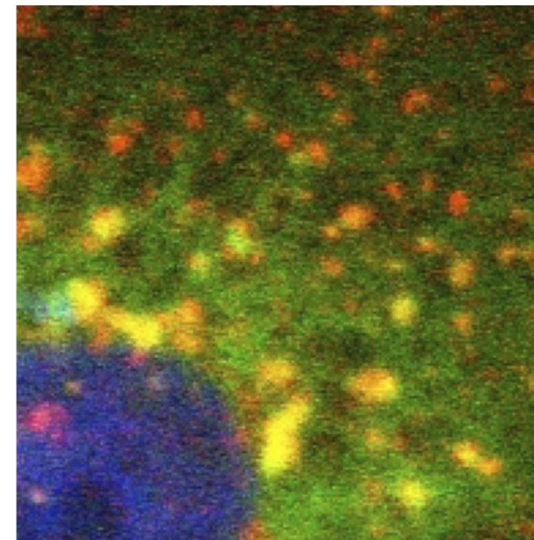
Finally my data indicate that association of the Alix Bro1 domain to LBPA-containing membrane is regulated by calcium. I wish to further investigate the molecular nature of this interaction, its regulation and its implication in membrane transport and endosome biogenesis.

J. Gruenberg, H. Stenmark (2004) Nat Rev Mol Cell Biol 5: 317

J. Gruenberg (2003) Curr Opin Cell Biol 15, 382

T. Kobayashi et al. (199) Nat Cell Biol 1: 113

H. Matsuo et al., (2004) Science 303, 531



Colocalization of AlixΔPRD-YFP with Lamp1-mCherry

Sanja Blaskovic

EPFL-SV-VDG
Bldg. AI-3147
Station 15
CH-1015
sanja.blaskovic@epfl.ch

The role of palmitoylation of Anthrax toxin receptor CMG2

The anthrax toxin receptor family comprised two members, tumor endothelial marker 8 (TEM8) and capillary morphogenesis gene 2 (CMG2). Both are type I transmembrane proteins sharing ~60% homology in their extracellular von Willebrand factor A domains and 68% identity in the first 145 residues of their cytoplasmic tails. Despite their higher degree of sequence homology, the two proteins are differentially expressed in different cell types and appear to have non-redundant function as illustrated by the fact that mutations in CMG2 lead to a sever human genetic disease, Hyaline Fibromatosis Syndrome. We have previously shown that both TEM8 and CMG2 are palmitoylated at the exact site and role of palmitoylation was however only investigated for TEM8. It was found that palmitoylation of TEM8 increase the half life of the protein at the cell surface, by sequestering it away form lipid rafts and its E3 ubiquitin ligase, thus preventing endocytosis in the absence of ligand. While it was initially assumed that palmitoylation would play a similar role for CMG2, my initial aim was to verify this assumption. To our surprise we found that CMG2 remained palmitoylated and did not undergo cycles of palmitoylation-depalmitoylation as observed for TEM8, that the palmitoylation deficient CMG2 mutant does not associate with DRMs, as opposed to the equivalent for TEM8, and that the proteins does not undergo massive ubiquitination in the absence of ligand.

These unexpected findings show that despite their high overall sequence identity, palmitoylation has very different effects on the two proteins and their ability to associate with DRMs, thus providing use with a very interesting system to understanding the respective roles of length of transmembrane domain (which differs between TEM8 and CMG2) and sequence of the transmembrane domain and palmitoylation.

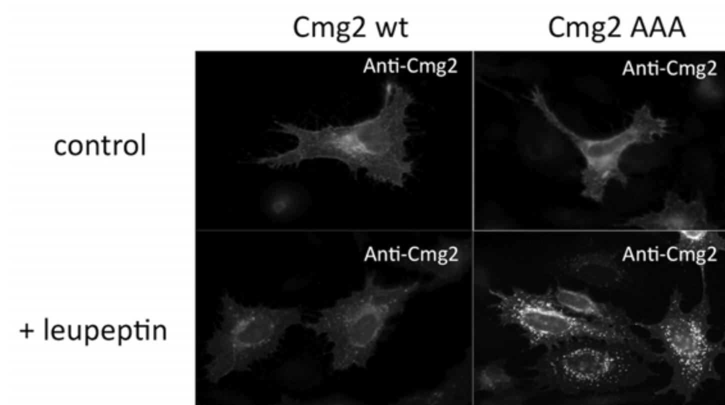
Abrami, L., Leppla, S.H. and Van der Goot F.G. (2006) Receptor palmitoylation and ubiquitination regulate anthrax toxin endocytosis. J. Cell Biol. 172:309-320.

Abrami, L., Kunz, B., Iacovache, I. and Van der Goot, F. G. (2008) Palmitoylation and ubiquitination regulate exit of the Wnt signaling protein LRP6 from the endoplasmic reticulum. PNAS. 105: 5384-9

Basu, J. (2004) Protein palmitoylation and dynamic modulation of protein function. Current Science, 87: 212-17

Shahinian S., Silvius J.R. (1995) Doubly-Lipid-Modified protein sequence motifs exhibit long lived Anchorage to lipid bilayer membrane. Biochemistry, 34:3813-3822.

Smotrys, J. E. and M. E. Linder (2004). Palmitoylation of intracellular signaling proteins: regulation and function. Annu Rev Biochem 73: 559-587.



Nir Drayman

Nir Drayman,
Hebrew University of Jerusalem
Hadassah Medical School.
Department of Hematology.
nirdra@gmail.com

SV40 mimics host ligands to promote its cell entry and infection

SV40 is a small, non-enveloped DNA virus and a member of the polyomaviridae family. While many viruses enter host cells via clathrin-dependent endocytosis, SV40 enters via lipid rafts in a cholesterol dependent endocytic process. Viral entry begins with virus binding to its cognate ganglioside receptor - GM1.

We hypothesized that in addition, SV40 binds and activates additional protein receptors in order to elicit the multiple signaling required for virus entry. We used computational approach to search for native ligands with structural homology to the viral major capsid protein VP1, assuming the virus may use mimicry for binding their cognate receptors. Candidate receptors were further investigated by biophysical, biochemical and siRNA experiments to establish their role in SV40 entry and infection processes.

We found that Gas6, a ligand of TAM (Tyro3, Axl and Mer) receptors is a structural homolog of VP1. Interestingly, the in silico study indicated that the TAM binding site does not overlap with that of GM1. While GM1 binds to the external loops of a VP1 pentamer, TAM receptors are predicted to bind at the grooves formed between adjacent pentamres. The binding of recombinant virus-like particles, VLPs, to Axl and Tyro3 was demonstrated in vitro by BIAcore experiments. The binding affinities were in the range of 1-10 nM. Blocking the receptors in CV1 cells by antibodies reduced SV40 infection (assayed by T-antigen expression), suggesting that both Axl and Tyro3 participate in SV40 cell recognition.

This finding was supported by competition experiments. Knock-down

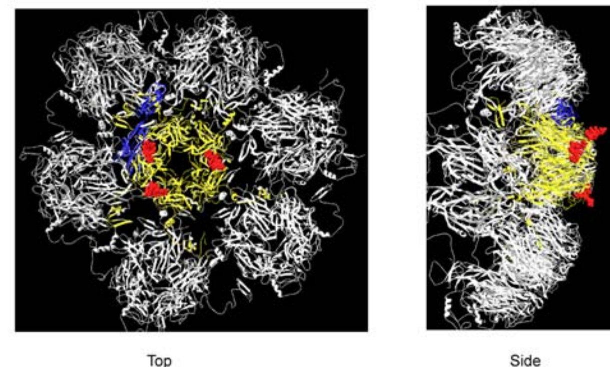
of the receptors by siRNA indicated that the receptors have a critical role in SV40 entry process.

The novel candidate receptors described here are expected to further clarify SV40 entry process. A deeper understating of SV40 entry is important for the development of safe and efficient gene therapy vectors and may have clinical implications for treating infections of pathogenic polyomaviruses.

N. Drayman, S. Kler, O. Ben-Nun-Shaul and A Oppenheim (2009) Rapid method for SV40 titration. J Virol Methods 164:145-7

V. Butin-Israeli, N. Drayman and A. Oppenheim (2010) Host-virus interactions: SV40 infection triggers balanced network that includes apoptotic, survival and stress pathways. J Virol. 84:3431-42

A. Leiba, N. Drayman, Y. Amsalem, A. Aran, G. Weiss, R. Leiba, D. Schwartz, Y. Levi, A. Goldberg and Y. Bar-Dayan (2007) Establishing a high level of knowledge regarding bioterrorist threats in emergency department physicians: methodology and the results of a national bio-preparedness project. Prehosp Disaster Med. 22:207-11; discussion 212-3



Andreas Ernst

RG Wieland/Brügger
BZH (Biochemie-Zentrum Heidelberg)
INF 328 - #226
69120 Heidelberg, Germany
andreas.ernst@bzh.uni-heidelberg.de

Together with Dr. Contreras of the Brügger/Wieland laboratory, I have studied a specific, intra-membrane protein-lipid interaction of p24, a protein which is part of the COPI-vesicle minimal machinery. We could show the p24 TMD specifically interacts with a single molecular species of sphingomyelin, SM 18. We further characterized a putative lipid-binding domain within its TMD by application of a novel in vitro FRET system, yielding a signature that we tested bioinformatically for abundance. This signature, termed "molecular-species-determining domain" revealed approx. 80 putative sphingolipid-binding proteins, which we are currently testing in vivo. Regarding the potential function of this specific protein-lipid interaction, we could recently show that this specific interaction has an impact on the oligomeric state of p24, a protein, which is monomeric in the ER and dimeric in the Golgi apparatus.

A. M. Ernst, F. X. Contreras, B. Brügger and F. T. Wieland (2010) Determinants of specificity at the protein-lipid interface in membranes. FEBS Lett. 3; 584: 1713-20

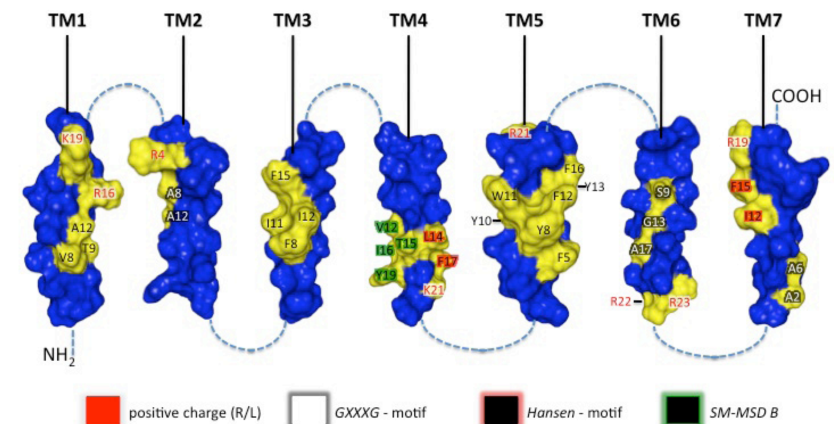
Niemelä, P.S., Hyvönen, M.T., Vattulainen I. (2006) Influence of chain length and unsaturation on sphingomyelin bilayers. Biophys. J. 90: 851–863

Niemelä, P.S., Hyvönen, M.T., Vattulainen I. (2009) Atom-scale molecular interactions in lipid raft mixtures. Biochim Biophys Acta. 1788: 122–135

Hodgkin, MN, Gardner, SD, Rose, S., Paterson, A., Martin, A. and MJ Wakelam (1997) Purification and characterization of sn-1-stearoyl-2-arachidonoylglycerol kinase from pig testes. Biochem. J. 322 : 529–34

Glukhov, E., Shulga, Y.V., Epand, R.F., Dicu, A.O., Topham, M.K., Deber, C.M. and Epand, R.M. (2007) Membrane interactions of the hydrophobic segment of diacylglycerol kinase epsilon. Biochim. Biophys. Acta. 1768: 2549-2558

Fantini, J., Garmy, N., Mahfoud, R. and Yahi, N. (2002) A common sphingolipid-binding domain in HIV-1, Alzheimer and prion protein. Exp Rev Mol Med. Cambridge University Press: ISSN 1462-3994



Eli Ezrielev

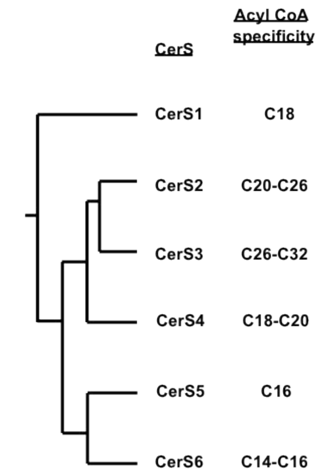
Department of Biological Chemistry
Weizmann Institute if Science
76100, Rehovot, Israel.
Eli.Ezrielev@weizmann.ac.il

Ceramide synthesis mediated by CerS proteins

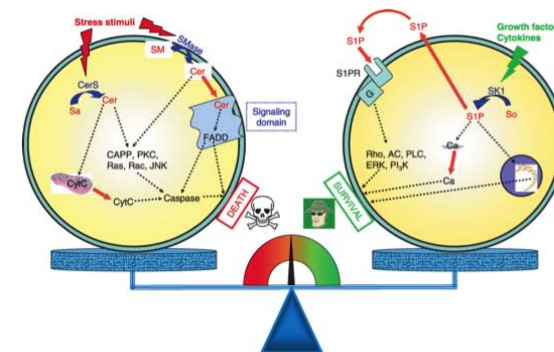
We are engaged in the study of sphingolipid biosynthesis. In particular, our interests lie with a protein family responsible for the synthesis of ceramide, an important lipid second messenger. Ceramide consists of a sphingoid long chain base to which a fatty acid is attached, the focus of our studies is the mediation if this process by the CerS enzyme family. Our studies have demonstrated that CerS proteins are highly selective towards defined fatty acid chain lengths, implying an important role in cell physiology for different ceramides containing specific fatty acids. We are currently determining which regions of the CerS proteins are involved in their activity and specificity, and are also studying the roles of each family member in ceramide-dependent signaling events.

Levy M, Futerman AH (2010) Mammalian ceramide synthases. IUBMB Life 62:347-56.

Pewzner-Jung Y, Ben-Dor S, Futerman AH (2006) When do Lasses (longevity assurance genes) become CerS (ceramide synthases)?: Insights into the regulation of ceramide synthesis. J Biol Chem. 281:25001-5



Suggested name	Adopted name	Original name	Protein domains	Number of aa residues
CerS1	LASS1	UOG1	97 311	350
CerS2	LASS2	TRH3	71 128 131 332	380
CerS3	LASS3	T3l	83 127 130 331	383
CerS4	LASS4	TRH1	84 128 131 332	394
CerS5	LASS5	TRH4	92 136 139 340	392
CerS6	LASS6	T1l	83 127 130 331	384



Artturi Kolvuniemi

Department of Physics
Tampere University of Technology
POB 692, FI-33101
Tampere , Finland
artturi.koivuniemi@wri.fi

Insights to the biogenesis of intracellular lipid droplets through coarse grained molecular dynamics simulations

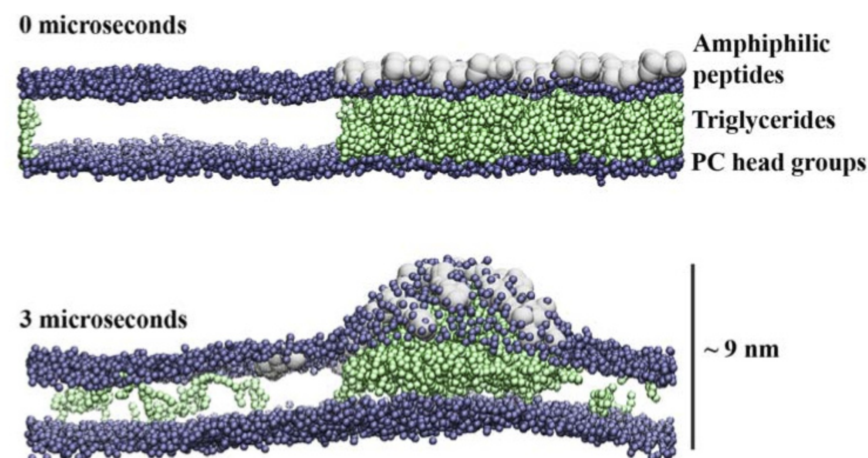
Lipid droplets are intracellular lipid storage organelles in eukaryotic cells and, lately, their role in several diseases has been recognized [1]. Lipid droplets can be divided to two parts: a surface monolayer composed of phospholipids and proteins (PAT proteins), and a hydrophobic core consisting mainly triglycerides (TGs) and cholesterol esters (CEs) [2]. Currently the properties of lipids and proteins driving the biogenesis of intracellular lipid droplets in the ER membrane are largely unknown [2]. Even the specific location for the nascent lipid droplet formation is not known. One hypothesis is that nascent lipid droplets are initially formed between the ER membrane leaflets (lens structure), after which they detach from the ER membrane to cytosol where they start to grow.

In order to shed light on the lipid droplet biogenesis we have carried out coarse grained simulations to understand the initial events of lipid droplet formation in the ER membrane. We have simulated systems composed of POPCs, TGs, CEs and perilipin-derived amphiphilic peptides. We found out that the microphase separation of TGs and CEs occurs in the middle POPC bilayer without perilipin-derived peptides. However, in the presence of peptides, the positive curvature was induced to the cytosolic side of the ER membrane that could resemble an early site for the lipid droplet detachment (see Figure).

Based on our results we suggest that perilipins that are rich of amphiphilic alpha-helixes are able to generate together with TGs a positively curved region to the cytosolic side of the ER membrane resembling a potential lipid droplet budding site. Furthermore we propose that proteins are not needed for the formation of TG-rich lenses in the ER membrane and, thus, TG phase is purely formed because of the low miscibility of PCs and TGs. Following the formation of TG lens, the curvature of TG-rich site promotes the accumulation of curvature sensing perilipins to the cytosolic side of the ER membrane promoting the budding of nascent lipid droplets.

[1] S. Martin et al. (2006) Lipid droplets: a unified view of a dynamic organelle, Nature Reviews MCB

[2] M. Digel et al. (2010) Lipid droplets lightning up, insights from light microscopy, FEBS letters



Jannik Larsen

Bionanotechnology Laboratory
Department of Neuroscience and Pharmacology
Nano-Science Center
University of Copenhagen
Universitetsparken 5, 2100 København Ø
jkl@nano.ku.dk

Raft-like membrane compositions have a significant effect on membrane curvature sensing of amphiphiles

The cell membrane has a heterogeneous lipid composition, potentially forming microdomains or membrane rafts, which are believed to be platforms involved in cellular processes like protein sorting and trafficking[1]. An alternative mechanism, potentially leading to protein sorting, has recently been proposed, suggesting that the structure and curvature of membranes can also actively regulate protein localization[2].

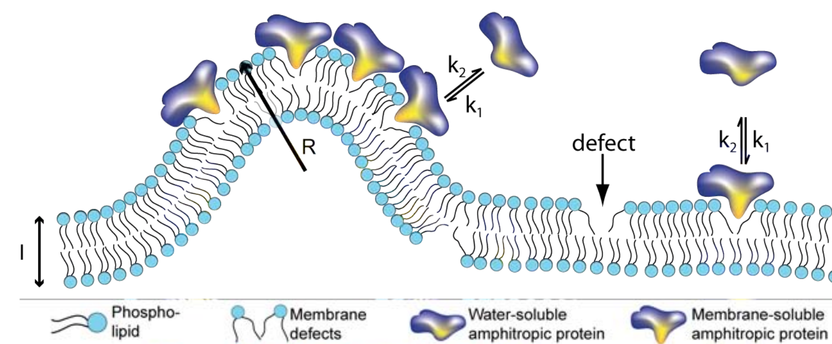
Recently, we showed that a variety of protein anchoring motifs are membrane-curvature sensors and thus up concentrate in regions of high membrane curvature[3]. Furthermore, the curvature sensing ability of the anchoring motifs persisted independently of their structural characteristics. This leads us to speculate that curvature sensing is an inherent property of curved membranes to recruit proteins anchored through hydrophobic insertion. As a consequence, the lipid composition of the bilayer could potentially be a regulator of membrane curvature sensing. We elucidated the membrane-curvature sensing properties of a model amphiphillic anchor for different lipid compositions. Employing our single vesicle membrane curvature-sensing assay, we examined lipid mixtures of DOPC, sphingomyelin and cholesterol, the last two believed to be enriched in membrane microdomains. We found that our model amphiphile-had increased membrane curvature sensing ability in all ternary lipid mixtures as compared to a reference membrane containing only DOPC.

Interestingly, the recorded increase was independent of the vesicle being in the lo or ld phase. Based on these findings we suggest that there may be an intimate, yet unrecognized, link in the way membrane microdomains and membrane curvature promote the localization and sorting of membrane-anchored proteins.

[1] D. Lingwood and K. Simons (2010) Lipid Rafts As a Membrane-Organizing Principle Science, 327: pp. 46-50

[2] V. K. Bhatia, et al., (2009) Amphipathic motifs in BAR domains are essential for membrane curvature sensing Embo J 28: 3303-3314.

[3] N. S. Hatzakis, et al. (2009) How curved membranes recruit amphipathic helices and protein anchoring motifs. Nature Chemical Biology, 5: . 835-841, 2009.



N. S. Hatzakis, V.K. Bhatia, J. Larsen et al., "How curved membranes recruit amphipathic helices and protein anchoring motifs," Nature Chemical Biology, vol. 5, pp. 835-841, 2009.

Karl Ferdinand Lechtreck

Department of Cell Biology
University of Massachusetts Medical School
Worcester
MA 01655, USA.
phone +1-508-856 4011
karl.lechtreck@umassmed.edu

Flagella, Cilia and Disease

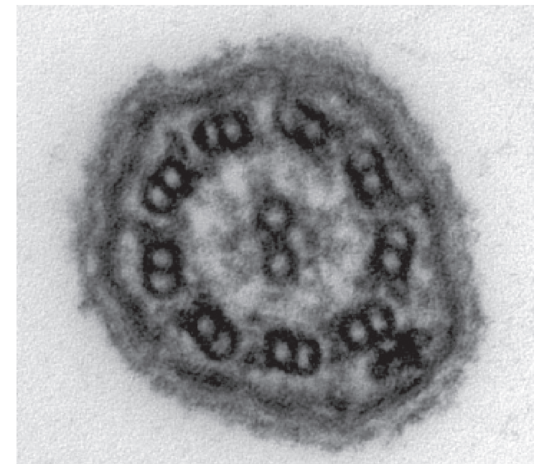
Cilia have an important role in cellular sensing and signaling, and dysfunctional cilia cause many mammalian diseases [1]. Bardet-Biedl syndrome (BBS), for example, is characterized by blindness, kidney anomalies, and obesity [2]. BBS is caused by defects in a seven-subunit protein complex, the BBSome [3]. The BBSome proteins are well conserved in the unicellular green algae Chlamydomonas reinhardtii, a preferred model for genetic, structural, and biochemical analyses of cilia/flagella. I showed that various signaling proteins accumulate with time in the flagellar membrane of C. reinhardtii bbs mutants [4]. I postulate that BBS is a degenerative disease of the cilium, particularly the ciliary membrane. How does BBSome loss results in flagellar accumulation of signaling proteins? Using TIRFM, I showed that the BBSome travels inside the flagellum on a subset of intraflagellar transport (IFT) particles. IFT is a bidirectional motility required for ciliary assembly, maintenance, and signaling. The BBSome could function as an IFT cargo adapter for the ciliary export of certain membrane-associated signaling proteins. These signaling proteins are predicted to be myristoylated and palmitoylated, and the flagellar membrane of bbs mutants differs in myristoylated protein content. In future, I want to test the possibility that the BBSome moves specialized protein/lipid domains within the cilium.

[1] J.L Badano, N. Mitsuma, P.L. Beales, and N. Katsanis (2006) The Ciliopathies: An Emerging Class of Human Genetic Disorders. Annu. Rev. Genomics. Hum. Genet. 7,125-148

[2] N.A Zaghloul, and N. Katsanis (2009) Mechanistic insights into Bardet-Biedl syndrome, a model ciliopathy. J. Clin. Invest. 119,428-437.

[3] M.V. Nachury, A.V. Loktev, Q. Zhang, C.J. Westlake, J. Peranen, A. Merdes, D.C. Slusarski, R.H. Scheller, J.F. Bazan, V.C. Sheffield, and P.K. Jackson (2007) A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. Cell. 129,1201-1213.

[4] K.F. Lechtreck, Johnson, E.C., Sakai, T., Cochran, D., Ballif, B.A., Rush, J., Pazour, G.J., Ikebe, M., Witman, G.B. (2009). The Chlamydomonas BBSome is an IFT cargo required for export of specific signaling proteins from flagella. J. Cell Biol. 187,1117-1132.



Aline Santos

Department of Biochemistry
University of Geneva
Sciences II
30 quai Ernest Ansermet
CH-1211 Geneva, Switzerland
Aline.Santos@unige.ch

CONTROL OF LIPID HOMEOSTASIS BY PROTEIN KINASES AND PHOSPHATASES.

Lipids are essential eukaryotic cellular constituents with multiple roles in membrane composition, vesicular trafficking, energy storage and signaling. Lipid metabolism homeostasis is higly relevant as its deregulation is associated with a number of diseases. Several protein kinases and phosphatases have been described to be involved in lipid metabolism regulation. The budding yeast Saccharomyces cerevisiae is a valuable model organism for studying the regulation of lipid homeostasis in eukaryotes since many of its pathways are conserved between yeast and mammals. Using lipid analysis by mass spectrometry, this work attempts to show in a semi-quantiative manner the changes in the lipid profile of 133 mutants in protein kinases and phosphatases. Yeast cells were grown to exponential phase in rich medium and different protocols were used to extract glycerophospholipids, sphingolipids and sterols. The comparative analysis of different mutants may provide insights for regulatory events that may affect lipid homeostasis. Putative integrated patterns of lipid changes may also be revealed, contributing to our understanding of how protein kinases and phosphatases regulate lipid metabolism

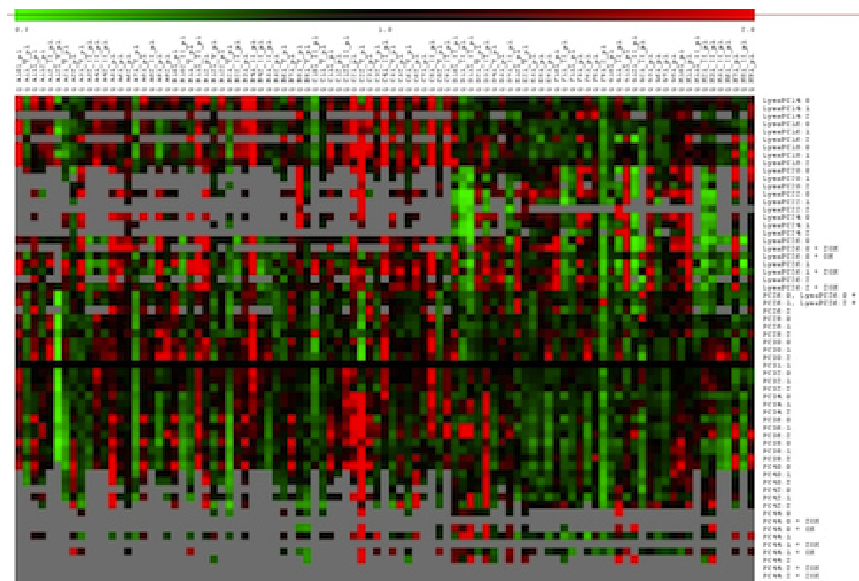
Guan XL, Souza CM, Pichler H, Dewhurts G, Schaad O, Kajiwara H, Ivanova T, Castillon GA, Picollis M, Abe F, Loewith R, Funato K, Wenk M, Riezman H. (2009) Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. Mol Biol Cell, v20, 2083-2095

Gulati S, Liu Y, Munkacsi AB, Wilcox L, Sturley SL. (2010)Sterols and sphingolipids: Dynamic duo or partners in crime? Prog Lipid Res. doi:10.1016/j.plipres.2010.03.003

Laplante M, Sabatini DM. (2009) An emerging role of mTOR in lipid biosynthesis. Curr Biol. 19: R1046-1052.

Nielsen J. (2009) Systems biology of lipid metabolism: from yeast to human. FEBS letters, 583, 3905- 3913

Usaite R, Jewett MC, Oliveira AP, Yates JR 3rd, Olsson L, Nielsen J.(2009) Reconstruction of the yeast Snf1 kinase regulatory network reveals its role as a global energy regulator. Mol Syst Biol, 5 (319) doi: 10.1038/msb.2009.67



Julia Steringer

Biochemie-Zentrum
der Universität Heidelberg (BZH)
Im Neuenheimer Feld 328
69120 Heidelberg
julia.steringer@bzh.uni-heidelberg.de

Structural and functional investigations on FGF2 membrane translocation, an unconventional secretory protein required for tumor-induced angiogenesis

Fibroblast growth factor 2 (FGF2) is a potent mitogen that is exported from cells by an ER/Golgi-independent mechanism. Unconventional secretion of FGF2 occurs by direct translocation across plasma membranes, a process that depends on the phosphoinositide PI(4,5)P2 at the inner leaflet as well as heparan sulfate proteoglycans at the outer leaflet of plasma membranes. The current project focuses on the mechanistic details of FGF2 membrane translocation with a potential role for cholesterol-dependent lipid rafts.

Using an in vitro system to study interactions between FGF2 and membranes FGF2 was shown to bind to PI(4,5)P2-containing liposomes These interactions are highly specific and depend on the presence of both cholesterol and sphingomyelin. FGF2 bound to PI(4,5)P2-containing liposomes can be recovered from detergent-resistant membranes and was found to locally disturb membrane integrity. This process is likely to be driven by PI(4,5)P2-induced oligomerization of FGF2 at the membrane interface. This observation was reinforced by cryo-electron microscopy studies demonstrating FGF2/PI(4,5)P2-dependent tethering of two opposing lipid bilayers.

Future studies aim at the in vitro reconstitution of FGF2 membrane translocation with chemically defined components with particular emphasis on a potential role of lipid rafts in this process.

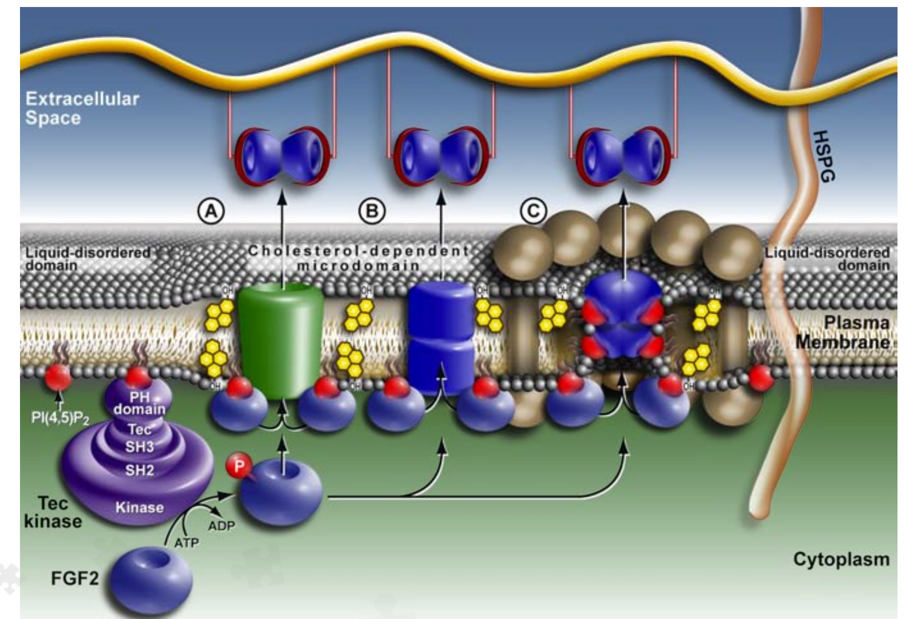
Ebert, A., Laußmann,M., Wegehingel, S., Kaderali, L., Erfle, H., Reichert, J., Lechner, J., Beer, H.D., Pepperkok, R. and Nickel, W. (2010) Traffic 11:813-826

Lucía Cespón Torrado, Koen Temmerman, Hans-Michael Müller, Matthias P. Mayer, Claudia Seelenmeyer, Rafael Backhaus, and Walter Nickel (2009) An Intrinsic Quality Control Mechanism Ensures Unconventional Secretion of Fibroblast Growth Factor 2 in a Folded Conformation. J. Cell Sci. 122:3322-3329

Nickel, W. and Rabouille, C. (2009) Nat. Rev. Mol. Cell Biol. 10:148-155

Temmerman, K. Ebert, A.D., Müller, H.M., Sinning, I., Tews, I. and Nickel W. (2008) Traffic 9:1204-1217

Christoph Zehe, André Engling, Sabine Wegehingel, Tobias Schäfer, and Walter Nickel (2006) Cell surface heparan sulfate proteoglycans are essential components of the unconventional export machinery of FGF-2. Proc. Natl. Acad Sci. U.S.A. 103:15479-15484



Goran Stepjanovic

BZH Heidelberg
Im Neuenheimer Feld 328
69120 Heidelberg, Germany
goran.stjepanovic@bzh.uni-heidelberg.de

Biochemical and biophysical characterization of membrane interactions of the Escherichia coli FtsY

In eubacteria such as Escherichia coli, co-translational targeting of membrane and secretory proteins to and across the inner membrane is mediated by the signal recognition particle (SRP). SRP binds to the N-terminal signal sequence of newly synthesized secretory and membrane proteins emerging from the ribosome. This binding leads to formation of the ribosome nascent chain (SRP/RNC) complex. The SRP/RNC complex interacts with SRP receptor (FtsY) which delivers RNC to the translocon channel located at the plasma membrane. Despite of extensive research in this area, the exact mechanisms and domains of FtsY association with the inner membrane were unclear. The association of FtsY with membrane most likely involves both lipid and proteins. Interestingly, FtsY does not contain any hydrophobic sequence that could explain its affinity for the inner membrane and putative membrane receptor has not been detected. However, biophysical studies on model phospholipid monolayer's and liposome binding studies have shown that FtsY interacts directly with E. coli phospholipids with preference for anionic phospholipids. Analysis of the structure/function differences between severely truncated active and inactive mutants of FtsY enabled us to identify an essential membrane-interacting determinant (Fig.1). Deletion experiments showed that this determinant is essential for FtsY function in vivo, thus offering, for the first time, clear evidence for the functionally important, physiologically relevant interaction of FtsY with lipids.

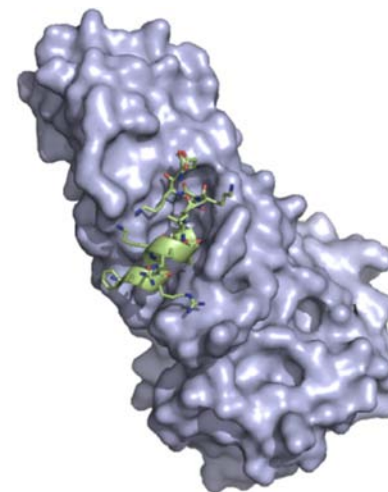
Krisko, A., G. Stjepanovic, et al. (2007) Detection of apolipoprotein B100 early conformational changes during oxidation. Biochim Biophys Acta 1768: 2923-2930

Parlitz, R., Eitan, A., Stjepanovic, G., Bahari, L., Bange, G., Bibi, E., and Sinning, I. (2007) E. coli SRP-receptor FtsY contains an essential and autonomus membrane-binding amphipathic helix, J Biol Chem 282: 32176-32184

Bahari, L., Parlitz, R., Eitan, A., Stjepanovic, G., Bochkareva, E., Sinning, I. and Bibi, E. (2007) Membrane targeting of ribosomes and their release require dinstict and separable functions of FtsY, J Biol Chem 282: 32168-32175

Bozkurt, G., G. Stjepanovic, et al. (2009) Structural insights into tail-anchored protein binding and membrane insertion by Get3. Proc Natl Acad Sci U S A 106(50): 21131-21136

Ravaud, S., G. Stjepanovic, et al. (2008) The crystal structure of the periplasmic domain of the Escherichia coli membrane protein insertase YidC contains a substrate binding cleft. J Biol Chem 283(14): 9350-9358



Surface representation of the *E. coli* FtsY (2QY9) with membrane targeting element indicated in green

Stefan Sunzenauer

Biophysics Institute,
Johannes Kepler University Linz,
Altenbergerstr.69
A-4040 Linz, Austria
Stefan.Sunzenauer@jku.at

Micropatterning for raft localization analysis of proteins in living cells

In the last years microcontact printing has been used to produce microstructured surfaces which can be differently functionalized and therefore used in a wide spread variation of applications.

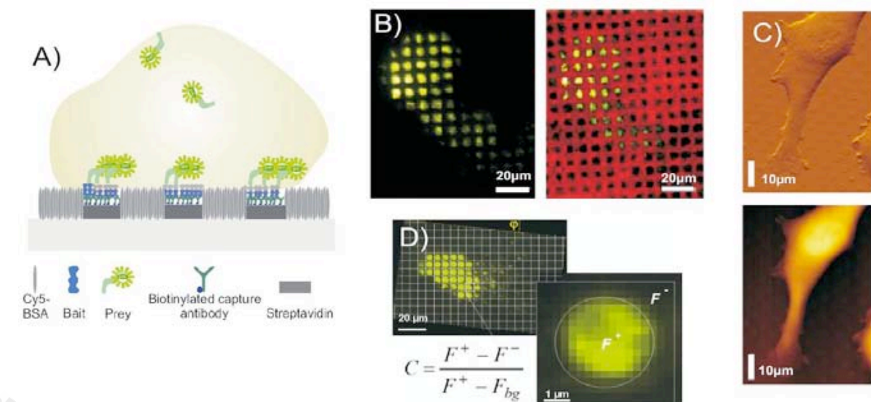
We have developed an assay for quantitative analysis of the interaction between a fluorescently marked protein (prey) and a membrane protein (bait) using microstructured surfaces covered with biotinylated ligands (antibodies) targeted against the bait. The proof-of-concept was demonstrated for the interaction between CD4, a major co-receptor in T cell signalling, and Lck, a protein tyrosine kinase essential for early T cell signalling [2].

Here we present improvements and a more precise characterization of the method as well as the applicability of the assay for the analysis of protein interactions within lipid rafts in the inner and outer leaflet of the plasma membrane. Therefore we stably expressed several fluorescently labelled raft and non-raft proteins in the human T24 cell line as prey proteins and determined the degree of interaction with the antibody targeted bait proteins CD59 (GPI-anchored protein, raft marker) and CD71 (Transferrin-receptor, non-raft marker), respectively.

We found strong interaction of CD59 with various GPI-GFP constructs, the inner-leaflet associated proteins Lck and Flottilin1 and a Pleckstrin-Homology fused to GFP sensing Phosphatidyl-Inositol (PI) in the inner-leaflet of the plasma-membrane. Importantly, we did not find interaction of CD59 with CD71-GFP and other potential non-raft proteins. If CD71 was used as the bait protein we did not find interaction with the prey proteins described for CD59.

We conclude that the micropatterning technique is a powerful method to analyse the lipid-raft mediated interaction of proteins. While the detected absence of CD71 from and the presence of CD59 in lipid rafts confirms current knowledge, it is still very unclear if a lipid-raft dependent coupling of proteins and certain, especially negatively charged, lipids across the plasma-membrane bilayer exists. Thus, our micropatterning assay will be of great interest to address this question.

Schwarzenbacher, M., et al. (2008) Nat Methods, 5(12): p. 1053-60.



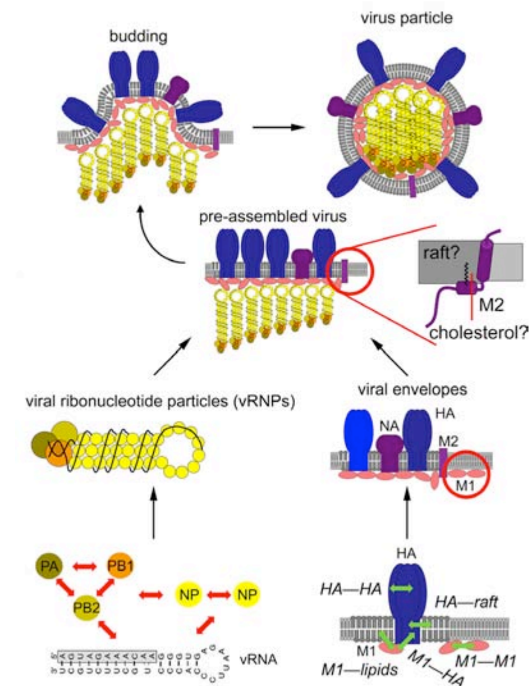
Bastian Thaa

Freie Universität Berlin
Fachbereich Veterinärmedizin
Institut für Immunologie und Molekularbiologie
Philippstraße 13, Haus 18
10115 Berlin, Germany
bthaa@gmx.de

Influenza virus particle are enveloped with a membrane derived from lipid rafts of the infected cell's plasma membrane. The spike glycoproteins hemagglutinin (HA) and neuraminidase (NA) as well as the ion channel M2 are integrated into the membrane, the matrix protein M1 lines it from beneath and thereby encapsulates the viral genome (vRNPs). During virus assembly, HA and NA are targeted to membrane rafts, while M1 shows no lipid domain preference and M2 is seemingly excluded from rafts, although the latter carries typical raft-targeting features (palmitoylation, putative cholesterol binding moiety).
The contribution of protein–lipid interactions of M1 and M2 to virus budding is not clearly known. Therefore, the intrinsic membrane association features of M1 and M2 are analysed in living transfected cells and in vitro.
M1 (as a GFP fusion protein) was equipped with a nuclear export signal to over¬come nuclear localisation. Then, membrane association could be analysed biochemi¬cally by subcellular fractionation and gradient centrifugation. It was found that a poly¬basic region, which had hitherto been believed to be the membrane interaction domain, is not solely responsible for membrane association of M1.
M2 has been suggested to play a role in the scission of the budding virus particle by association with the periphery of rafts due to cholesterol binding. To investigate this and the contribution of the supposed cholesterol-binding moiety (CRAC motif), in vitro analysis (liposome floatation assays with the puri¬fied cytoplasmic tail of M2 fused to GST) was combined with analysis of M2 labelled with GFP variants in transfected cells (association with membrane raft markers and hemagglutinin determined via FLIM-FRET) and reverse genetics (generation of recombinant virus).

The disruption of the proposed cholesterol-binding motif in M2 lowered the membrane-interactive properties of the cytoplasmic tail, but did not affect targeting of the full-length protein in cells. Furthermore, the results obtained so far point to an intrinsic, cytoskeleton-dependent interaction of M2 with HA, which has not been described so far and which adds another element to the multitude of molecular interactions in influenza virus budding.

[1] Thaa B, Hofmann KP, Veit M (2010) Review. BBA 1803, 507–519
[2] Engel S, Scolari S, Thaa B, Krebs N, Korte T, Herrmann A, Veit M (2010) Biochem J 425, 567–573
[3] Thaa B, Kabatek A, Zevenhoven-Dobbe JC, Snijder EJ, Herrmann A, Veit M (2009) J Gen Virol 90, 2704–2712
[4] Thaa B, Herrmann A, Veit M (2009) Virology 383, 150–155



Instructors

Ünal Coskun

Max Planck Institute
of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden, Germany
coskun@mpi-cbg.de

Functional and structural impact of protein-lipid crosstalk

My main interests are protein-lipid interactions and the resulting consequences at the structural und functional level. I got this strong interest as Master and PhD Student, working on the structural and functional evolution of ATP-Synthases and the eucaryotic vacuolar H+ ATP Hydrolase. As for most other membrane proteins, also here the impact of the membrane on the structure and regulation of these wonderful multi-subunit enzymes was not considered at all within the community. I then moved to Dresden, where I combine biochemical and biophysical methods to study the regulation of receptors by lipids and the consequences of lipid-protein interaction on membrane shape and identity.

Coskun, Ü., Chaban, Y.L., Lingl, A., Müller, V., Keegstra, W., Boekema, E.J. and Grüber G. (2004) Structure and subunit arrangement of the A-type ATP synthase complex from the archaeon methanococcus jannaschii visualized by electron microscopy. J. Biol.Chem., 279: 38644-38648

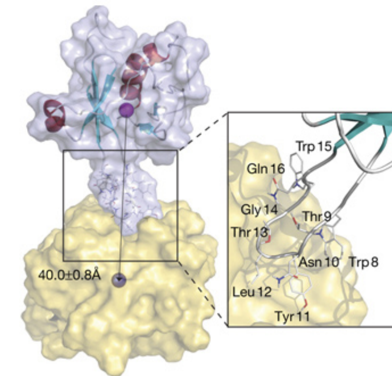
Cao, X., Coskun, Ü., Rössle, M., Buschhorn S.B., Grzybek, M., Dafforn, T.R., Lenoir, M., Overduin, M., Simons, K. (2009) Golgi protein FAPP2 tubulates membranes PNAS 106: 21121-21125

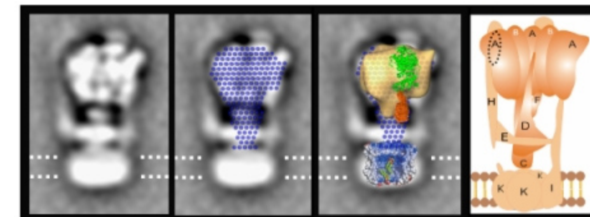
Coskun, Ü and Simons, K. (2010) Membrane rafting: from apical sorting to phase segregation FEBS Letters 584: 1685-1693

Lenoir, M., Coskun, Ü., Grzybek, M., Cao, X., Buschhorn S.B., James, J., Simons, K., Overduin, M. (2010) Structural Basis of Wedging the Golgi Membrane by FAPP Pleckstrin Homology Domains. EMBO reports 11: 279 - 284

Cherezov V., Rosenbaum D.M., Hanson M.A., Rasmussen S.G., Thian F.S., Kobilka T.S., Choi H.J., Kuhn P., Weis W.I., Kobilka B.K., Stevens R.C. (2007) High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science 318: 1258-1265

Ernst A.M., Contreras F.X., Brügger B., Wieland F. (2010) Determinants of specificity at the protein-lipid interface in membranes. FEBS letters 584: 1713-1720



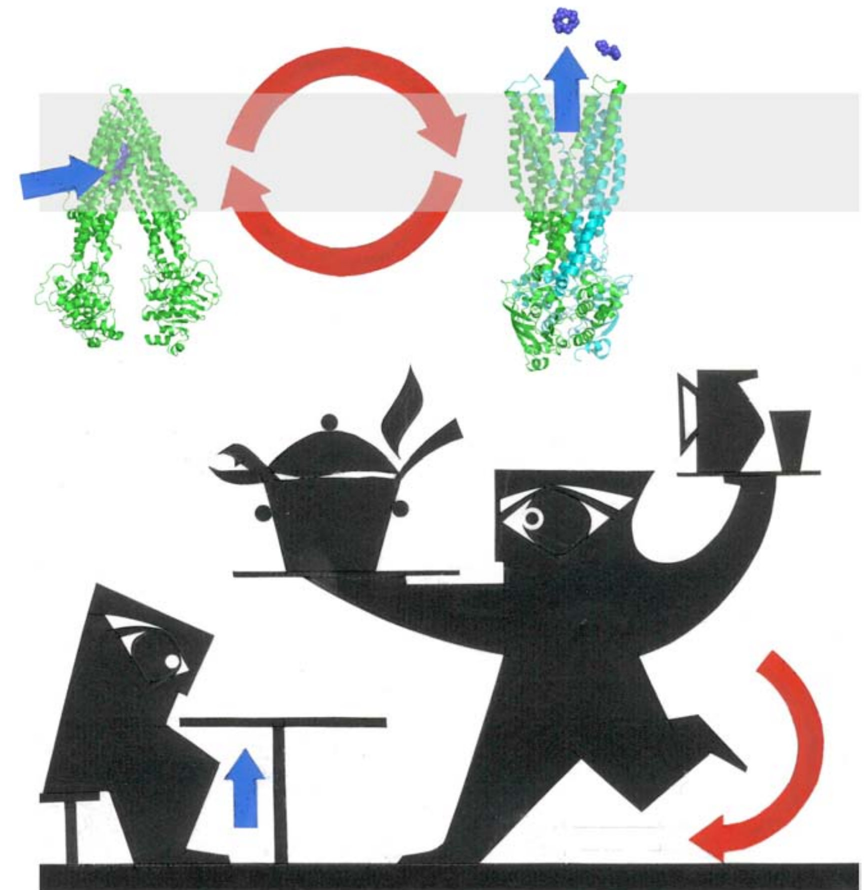


Robert Ernst

MPI-CBG
Pfotenhauerstrasse 108
Dresden , Germany
rernst@mpi-cbg.de

My Name is Robert Ernst. I studied Human Biology in Marburg and became dragged into Biochemistry during my Diploma Thesis on structure and function relationships of the peptide ABC-Transporter TAP in the groups of Robert Tampe and Lutz Schmitt (1). During my PhD thesis with Lutz Schmitt at the Goethe-University in Frankfurt and the Heinrich-Heine-University in Duesseldorf I got excited about membrane protein reconstitution, developed methods to analyze and quantify detergents. My biochemical work on the multidrug efflux pump Pdr5 from yeast resulted in a model that describes the phenomenon of substrate selection based on the dynamic behavior of the transport protein rather than on static, structural features (2, 3). Ever since those days, I wanted to work at the crossroads of proteins and lipids in a cellular context. However, I went somewhat sideways and did a PostDoc in the laboratory of Hidde Ploegh at the Whitehead Institute in Cambridge, MA. Here, I worked on the role of ubiquitintation and deubiquitination in the quality control of the ER (4) and collected valuable insights the work with mice, the identification of novel protein-protein interactions, peptide synthesis, protein circularization and so forth.
Now, I tend to say 'finally', I made it into the lipidic side of life and I am working in Kais lab on the role of specific glycolipids in membrane traffic and epithelial differentiation.

[1] R. Ernst, J. Koch, C. Horn, R. Tampe, L. Schmitt (2006) J Biol Chem 281, 27471
[2] R. Ernst et al., (2008) Proc Natl Acad Sci U S A 105, 5069
[3] R. Ernst, P. Kueppers, J. Stindt, K. Kuchler, L. Schmitt (2010) FEBS J 277, 540
[4.]R. Ernst, B. Mueller, H. L. Ploegh, C. Schlieker (2009) Mol Cell 36, 28



Mathias Gerl

Max Planck Institute
of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden, Germany
gerl@mpi-cbg.de

Lipids in Epithelial Cells

The lipid raft concept originated as a model to explain the asymmetric lipid and protein distribution in epithelial cells, whose plasma membrane (PM) is divided into two surface domains: an apical domain enriched in sphingolipids and cholesterol facing the lumen and a basolateral domain enriched in glycerophospholipids facing neighboring cells. According to the hypothesis, lipids and proteins destined for the apical or basolateral PM segregate in a domain formation process at the trans Golgi Network (TGN), one of the major sorting stations in the exocytotic pathway.

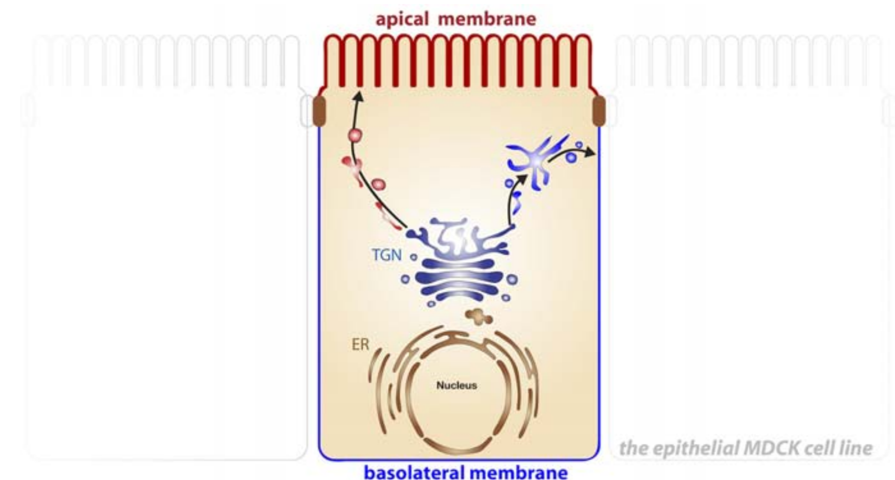
To validate some predictions of this model, I collaborated with Julio Sampaio who set up a two-step lipid extraction and mass spectrometry detection protocol that resolves glycerophospholipids, cholesterol and (glyco-) sphingolipids to the species level. We use this technique to quantify changes in global lipid composition during polarization and the concomitant assembly of the apical and basolateral membrane in MDCK cells, an epithelial cell culture model. Additionally, using novel preparation procedures including giant plasma membrane vesicle preparations together with Ilya Levental, we quantify the distinct lipid compositions of the apical PM and the basolateral PM.

Main results include the increasing concentration of the Forssman glycolipid during epithelial polarization, as well as the enrichment of cholesterol and sphingolipids in the apical PM. These results support important aspects of raft-based sorting during the genesis and maintenance of distinct lipid domains in polarized epithelial cells.

Simons and van Meer. Lipid sorting in epithelial cells. Biochemistry (1988) vol. 27 (17) pp. 6197-6202

Futerman and Riezman. The ins and outs of sphingolipid synthesis. Trends Cell Biol (2005) vol. 15 (6) pp. 312-318

Hansson et al. Two strains of the Madin-Darby canine kidney (MDCK) cell line have distinct glycosphingolipid compositions. Embo J (1986) vol. 5 (3) pp. 483-9



Michal Grzybek

Max Planck Institute of
Cell Biology and Genetics
Pfotenhauerstrasse 108
01307 Dresden, Germany
grzybek@mpi-cbg.de

The organization of biological membranes is driven by specific interactions of their components. The complexity of the cell, makes it however, extremely difficult and demanding to study these interactions in detail. By taking the components apart and assembling them back in artificial systems (lipid mono- and bilayers), we can get insight into the principles of lipid-lipid and lipid-protein associations.

At present, my research concentrates around various aspects of membrane organization, especially looking at lipid-protein interactions. First of all, we are interested in regulation of activity of Receptor Tyrosine Kinase proteins by lipids. For a direct insight, we reconstituted the EGF receptor in liposomes with defined lipid composition. The reconstituted protein is fully active as assessed by ligand binding studies and autophosphorylation measurements. We see that lateral membrane heterogeneity is needed for the proper control of EGF receptor and that GM3 strongly inhibits the activity of the receptor. This striking modulation happens only in a lipid phase separating conditions. In a cell membrane, EGF receptor associated with GM3 might be kept in an inhibited conformation. In this way the unliganded receptor is prevented from uncontrolled firing.

Another field of my interests are the interactions of various peripheral membrane proteins with membrane and my research focuses mostly on the Red Blood Cell membrane model. The shape of RBCs is defined by a combination of protein-protein and protein-lipid associations that attach the membrane skeleton to the plasma membrane. Deficiencies or defects of membrane skeleton proteins cause changes in RBC morphology, which lead to rapid clearance of affected cells from the bloodstream and result in anemia.

I am studying two aspects of these interactions: direct interaction of spectrin (major membrane skeleton protein of RBC) with lipids, which acts as additional attachment site and is suggested to play a role in maintaining erythrocyte integrity. I am also studying the role of protein palmitoylation in RBCs and its impact on membrane organization and RBC morphology

Maget-Dana R., The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membrane-lytic peptides and their interactions with lipid membranes. BBA-Biomembranes (1999) vol. 1462 (1-2) pp. 109-140

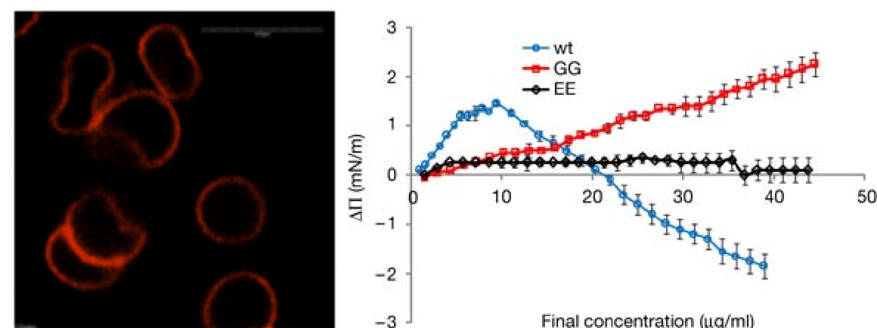
Kiessling et al. Domain coupling in asymmetric lipid bilayers. BBA - Biomembranes (2009) vol. 1788 (1) pp. 64-71

Feigenson G.W., Phase boundaries and biological membranes. Annu. Rev. Biophys. Biomol. Struct. 2007. 36:63–773 (2007)

Cherezov et al. High-resolution crystal structure of an engineered human beta2-adrenergic G protein-coupled receptor. Science (2007) vol. 318 (5854) pp. 1258-65

Ernst et al. Determinants of specificity at the protein-lipid interface in membranes. FEBS letters (2010) vol. 584 (9) pp. 1713-20

Mohandas and Gallagher. Red cell membrane: past, present, and future. Blood (2008) vol. 112 (10) pp. 3939



Herman-Josef Kaiser

Max Planck Institute of
Cell Biology and Genetics
Pfotenhauerstrasse 108
01307 Dresden, Germany
kaiser@mpi-cbg.de

Membrane order in model and biological membranes

Next to glycerophospholipids, eukaryotic membranes can contain high amounts of sterols and sphingolipids, which confer a distinct sub-compartmentalization potential. On a structural level, these lipids tend to increase the order of membranes defined as the average trans-conformation of alkyl chain-containing lipids. This molecular feature is directly related to the packing density of the molecules within a membrane.

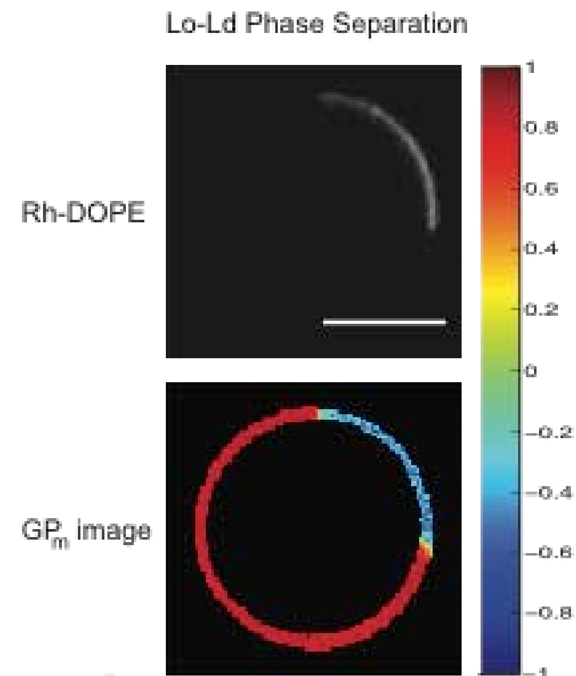
Using the fluorescent lipid probe C-laurdan we can measure this packing density (GP value) by assessing shifts in the emission spectrum. Therefore spectroscopy and fluorescence microscopy assays can reveal insights into the structure and composition of membrane domains and phases in model and biological membranes. Our main goal with these tools is to unravel how membrane composition, structure, and compartmentalization are related.

Another recent addition to our toolkit are transmembrane peptides that can be integrated at high amounts into model membranes. These proteolipid membranes mimic better the properties of natural membranes and give rise to new compartmentalization phenomena. The focus of this experimental avenue is to understand how proteins also contribute to membrane structure and organization.

Order of lipid phases in model and plasma membranes (2009) Kaiser HJ, Lingwood D, Levental I, Sampaio JL, Kalvodova L, Rajendran L, Simons K. Proc Natl Acad Sci U S A. 106(39):16645-50

Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network (2009) Klemm RW, Ejsing CS, Surma MA, Kaiser HJ, Gerl MJ, Sampaio JL, de Robillard Q, Ferguson C, Proszynski TJ, Shevchenko A, Simons K. J Cell Biol.185(4):601-12

Lipid rafts as functional heterogeneity in cell membranes (2009) Lingwood D, Kaiser HJ, Levental I, Simons K. Biochem Soc Trans. 37:955-60

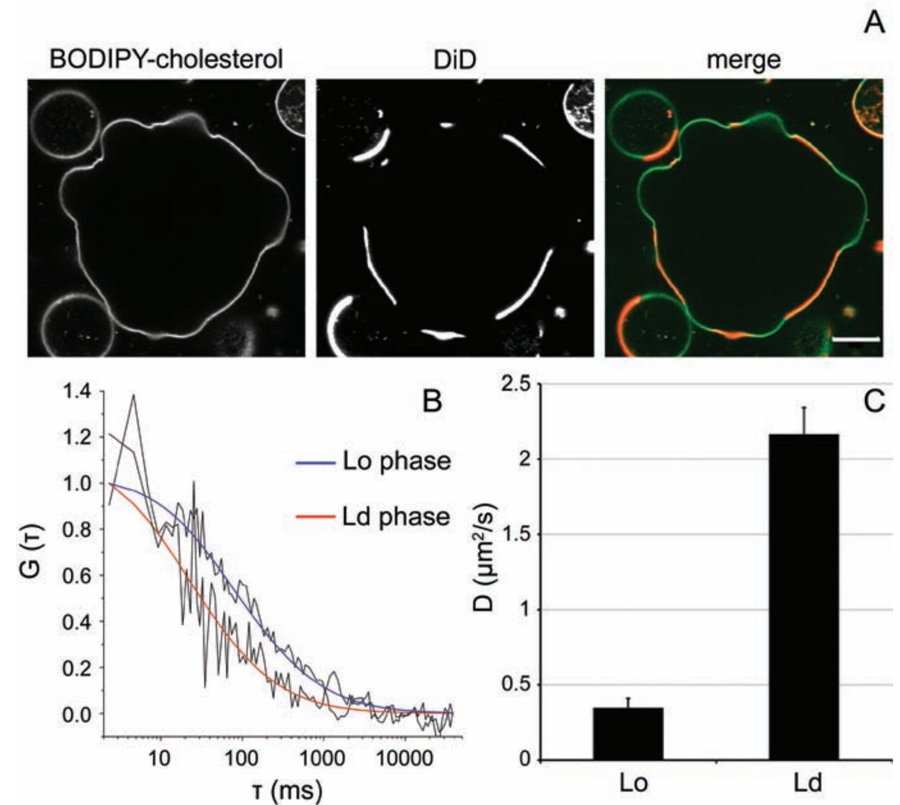


Christian Klose

Max Planck Institute
of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden, Germany
klose@mpi-cbg.de

Yeast lipids can phase separate into micrometer-scale membrane domains

The lipid raft concept proposes that biological membranes have the potential to form functional domains based on a selective interaction between sphingolipids and sterols. These domains seem to be involved in signal transduction and vesicular sorting of proteins and lipids. Although there is biochemical evidence for lipid raft-dependent protein and lipid sorting in the yeast Saccharomyces cerevisiae, direct evidence for an interaction between yeast sphingolipids and the yeast sterol ergosterol, resulting in membrane domain formation, is lacking. Here we show that model membranes formed from yeast total lipid extracts possess an inherent self-organization potential resulting in Ld-Lo phase coexistence at physiologically relevant temperature. Analyses of lipid extracts from mutants defective in sphingolipid metabolism as well as reconstitution of purified yeast lipids in model membranes of defined composition suggest that membrane domain formation depends on specific interactions between yeast sphingolipids and ergosterol. Taken together, these results provide a mechanistic explanation for lipid raft-dependent lipid and protein sorting in yeast.



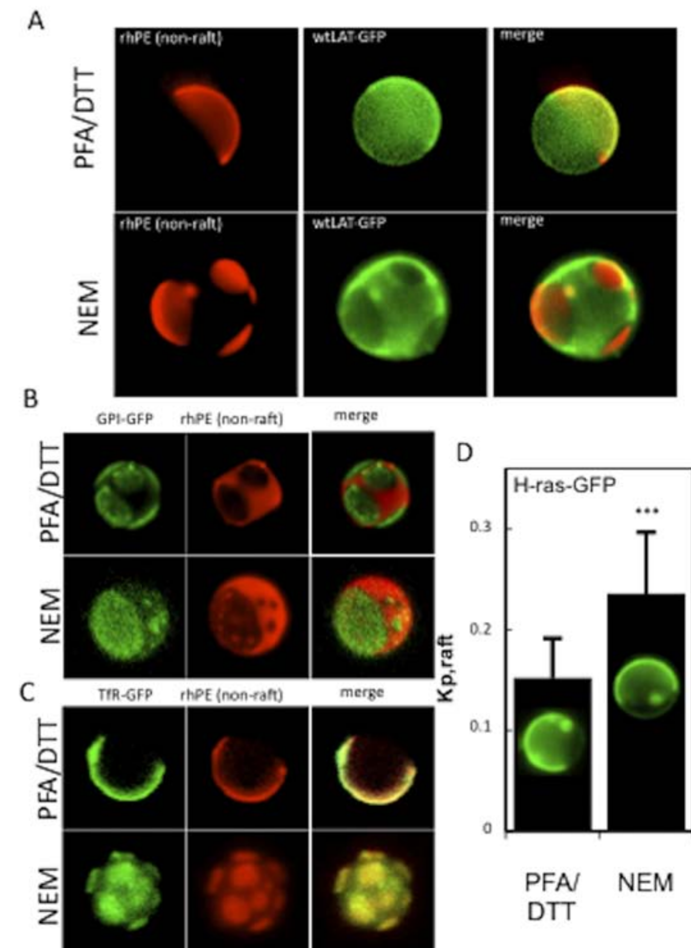
Giant unilamellar vesicles (GUVs) containing the yeast lipids IPC,PI and ergosterol (1:1:1) show phase separation into liquid-ordered and liquid-disordered domains, as judged from confocal fluorescence microscopy (A) and fluorescence correlation spectroscopy (FCS).

Ilya Levental

Max Planck Institute
of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden, Germany
levental@mpi-cbg.de

Extensive characterization of biological lipid phase behavior has confirmed the importance of lateral compositional heterogeneity in determining the function of the plasma membrane. However, the biological relevance of such studies has been disputed due to either the lack of compositional complexity or the requirement for low-temperature detergent-mediated cell lysis. The recent discovery of phase separation in cell-derived Giant Plasma Membrane Vesicles (GPMVs) enables investigation of lipid phase separation in a system with appropriate biological complexity under physiological conditions [1]. Phase abundance and miscibility transition temperature in these cell-derived vesicles is dependent on cholesterol, in quantitative agreement with simple lipid mixtures [2]. Additionally, direct quantification of partitioning of fluorescent chimeric proteins allows determination of the factors governing raft affinity of both peripheral and integral plasma membrane proteins [3].

[1] T. Baumgart et al., (2007) PNAS 104, 3165-3170
[2] I. Levental et al., (2009) Biochem J 424, 163-167 .
[3] P. Sengupta, A. Hammond, D. Holowka, B. Baird, BBA (2008) Biomem branes 1778, 20 .



Daniel Lingwood

Max Planck Institute
of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden, Germany
lingwood@mpi-cbg.de

My name is Daniel Lingwood a Canadian currently working as a post-doctoral fellow in the group of Kai Simons at the Max Planck Institute for Molecular Cell Biology and Genetics in Dresden, Germany. Originally educated in physiology and biochemistry at the University of Guelph (BSc, MSc) in Canada, I am also a trained molecular cell biologist, having also obtained my PhD from this Max Planck Institute. I employ a combination of biophysics, biochemistry and molecular biology to investigate the structural basis for functionally coherent structure in cell membranes. Specifically I focus on how cells manipulate molecular self-organizing principles to spatially and temporally coordinate bioactivity in the membrane plane.

Lingwood, D. and Simons, K. (2010) Lipid rafts as a membrane organizing principle. Science 327:46-50.

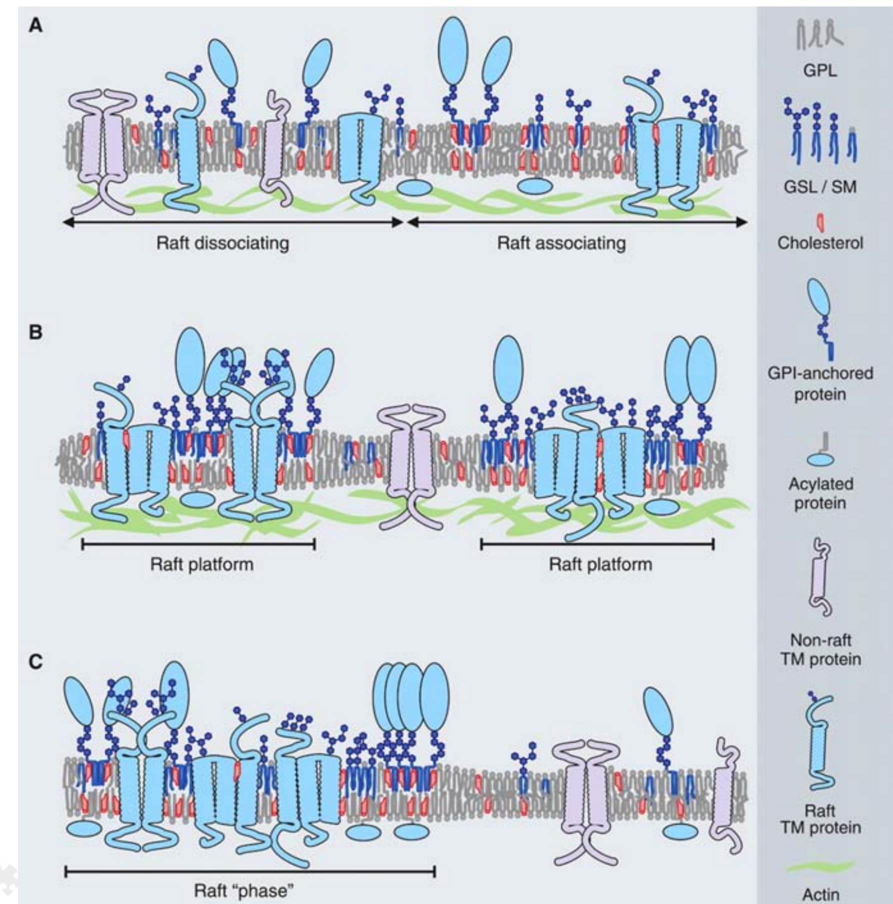
Kaiser H-J, Lingwood D, Levental I, Sampaio J, Kalvodova L, Rajendran L, Simons K. (2009) Order of lipid phases in model and plasma membranes. Proc. Natl. Acad. Sci. U.S.A. 106:16645-16650

Lingwood D, Schuck S, Ferguson C, Gerl M, Simons K. (2009) Generation of cubic membranes by controlled homotypic interaction of membrane proteins in the endoplasmic reticulum. J. Biol. Chem. 284:12041-12048

Lingwood D, Ries J, Schwille P, Simons, K. (2008)Plasma membranes are poised for activation of raft phase coalescence at physiological temperature. Proc. Natl. Acad. Sci. U.S.A. 105:10005-10010

Lingwood D, Simons K. (2007) Detergent resistance as a tool in membrane research. Nature Protocols 2:2159-2165.

Lingwood D, Harauz G, Ballantyne JS. (2005) Regulation of fish gill Na+-K+-ATPase by selective sulfatide-enriched raft partitioning during seawater adaptation. J Biol. Chem. 280:36545-36550.



Jonas Ries

ETH Zürich
Laboratory for Physical Chemistry
Wolfgang-Pauli-Str. 10, HCI F212
8093 Zürich, Switzerland
jries@ethz.ch

Scanning Fluorescence Correlation Spectroscopy

Fluorescence Correlation Spectroscopy (FCS) is a powerful technique to study concentrations, diffusion coefficients and binding parameters of biomolecules. However, its application to biological membranes is impeded by the slow diffusion and the two-dimensional geometry. Scanning Fluorescence Correlation Spectroscopy (scanning FCS) uses a moving detection volume instead of a static one. By scanning the detection volume through the membrane in a perpendicular way, this technique becomes insensitive to instabilities of the membrane. The accuracy can be increased by using two detection volumes (dual-focus scanning FCS) and binding in membranes can be studied with dual-color scanning FCS. We will apply scanning FCS to artificial and cellular phase separating membranes and compare diffusion coefficients and concentrations of several fluorescent molecules.

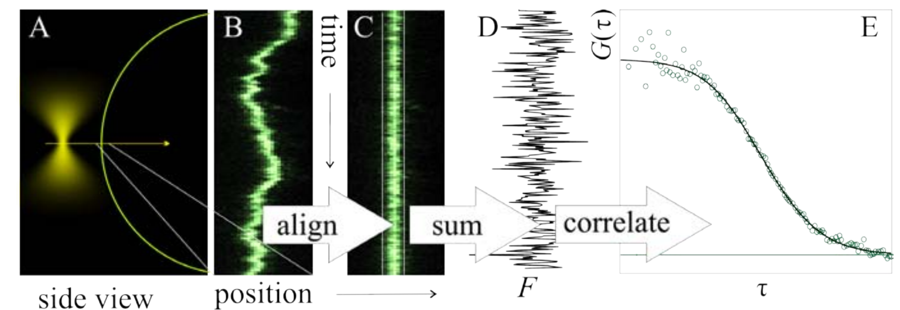
J. Ries and P. Schwille (2008) New concepts for fluorescence correlation spectroscopy on membranes. Phys Chem Chem Phys 10:3487–3497

J. Ries and P. Schwille (2006) Studying slow membrane dynamics with continuous wave scanning fluorescence correlation spectroscopy. Biophys J 91:1915–1924

D. Lingwood, J. Ries, P. Schwille, and K. Simons (2008) Plasma membranes are poised for activation of raft phase coalescence at physiological temperature. Proc Natl Acad Sci U S A 105:10005-10

J. Ries, R.S. Yu, M. Burkhardt, M. Brand, and P. Schwille (2009) Modular scanning FCS quantifies receptor-ligand interactions in living multicellular organisms. Nature Methods 6:643-645

A.J. Garcia-Saez, J. Ries, M. Orzaez, E. Perez-Paya, P. and Schwille. (2009)Membrane promotes tBID interaction with BCLXL. Nat Struct Mol Biol 16:1178-1185



Julio Sampaio

Max Planck Institute of
Cell Biology and Genetics
Pfotenhauerstrasse 108
01307 Dresden, Germany
sampaio@mpi-cbg.de

Lipid analyisis by shotgun lipidomics

All cells are delimited by membranes that protect the cell from the surrounding environment. In eukaryotic cells the same principle applies at subcellular level where membranes delimit functional cell organelles. The membrane structure, properties and function are defined in part by their lipid composition. Lipidomics is the large-scale study of pathways and networks of cellular lipids in biological systems. It involves the identification and quantitation of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites. Lipidomics has been greatly facilitated by recent advances in ionization technology and mass spectrometric capabilities which have simplified the sample processing prior to analysis, giving rise to shotgun lipidomics. Shotgun lipidomics is fast, highly sensitive, and can identify hundreds of lipids missed by other methods. Here we discuss the several generic steps of a shotgun lipidomics approach: sample preparation, lipid extraction, MS acquisition and lipid annotation.

Han, X., and Gross, R.W. (2005). Shotgun lipidomics: Electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom Rev. 24: 367-412.

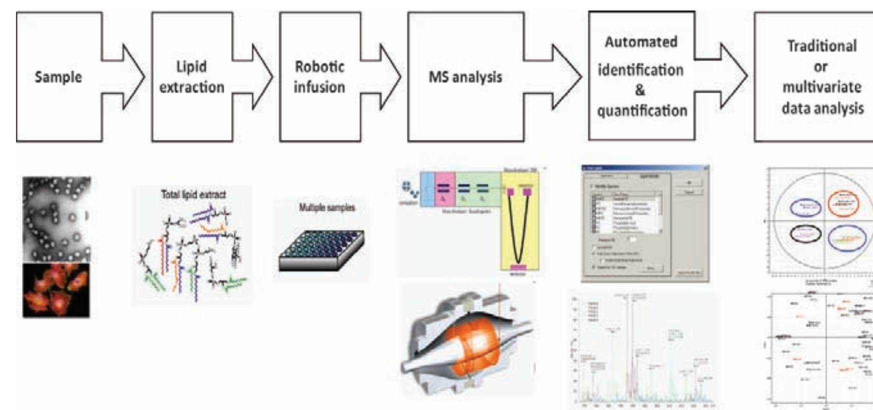
Wenk, M.R. (2005). The emerging field of lipidomics. Nat Rev Drug Discov 4: 594-610.

Wolf, C., and Quinn, P.J. (2008). Lipidomics: Practical aspects and applications. Progress in Lipid Research 47, 15-36.

Ejsing, C.S., Duchoslav, E., Sampaio, J., Simons, K., Bonner, R., Thiele, C., Ekroos, K., and Shevchenko, A. (2006). Automated identification and quantification of glycerophospholipid molecular species by multiple precursor ion scanning. Anal Chem 78, 6202-6214.

Ejsing, C.S., Sampaio, J.L., Surendranath, V., Duchoslav, E., Ekroos, K., Klemm, R.W., Simons, K., and Shevchenko, A. (2009). Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci U S A. 17;106: 2136-4

Schwudke, D., Oegema, J., Burton, L., Entchev, E., Hannich, J.T., Ejsing, C.S., Kurzchalia, T., and Shevchenko, A. (2005). Lipid Profiling by Multiple Precursor and Neutral Loss Scanning Driven by the Data-Dependent Acquisition. Analytical Chemistry 78, 585-595.



Erdinc Sezgin

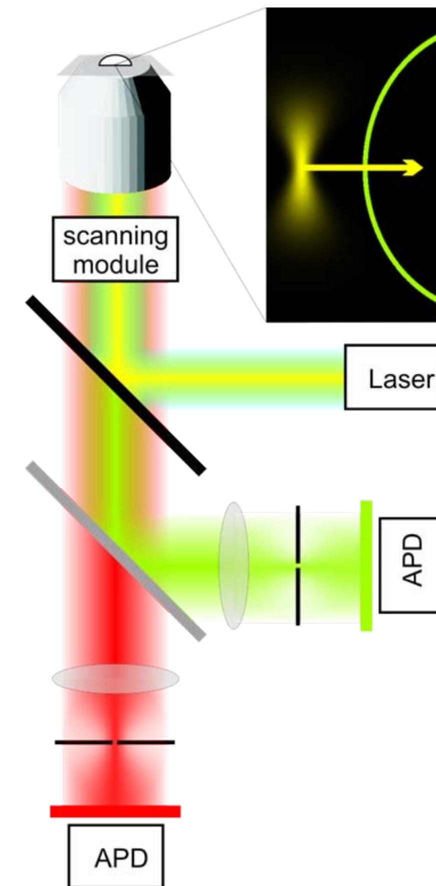
Biotechnology Center TU Dresden
Tatzberg 47-51, 01307
Dresden, Germany
erdinc.sezgin@biotec.tu-dresden.de

Lipid Rafts in Living cells by FCS

Fluorescence correlation spectroscopy (FCS) is a powerful analytical tool to get the information of concentrations, propagation, interactions and internal dynamics of molecules at nanomolar concentrations in living cells. Basically, FCS analyzes fluorescence-intensity fluctuation which is caused by the fluorescence molecules coming in and going out of a defined (in femtoliters) focal volume. To observe the interaction of two molecules, fluorescence cross-correlation spectroscopy (FCCS) is applied. It senses the co-movement of two labelled molecules through the focal volume of FCS. It has become a promising tool for living cell membrane dynamics due to its high temporal resolution and sensitivity.

S.A. Kim, Katrin G Heinze and Petra Schwille (2007) Fluorescence correlation spectroscopy in living cells. Nature Methods 4: 963-973

K. Bacia, S.A. Kim and P. Schwille (2006) Fluorescence cross-correlation spectroscopy in living cells. Nature Methods 3: 83-89



Ana Stevanovic

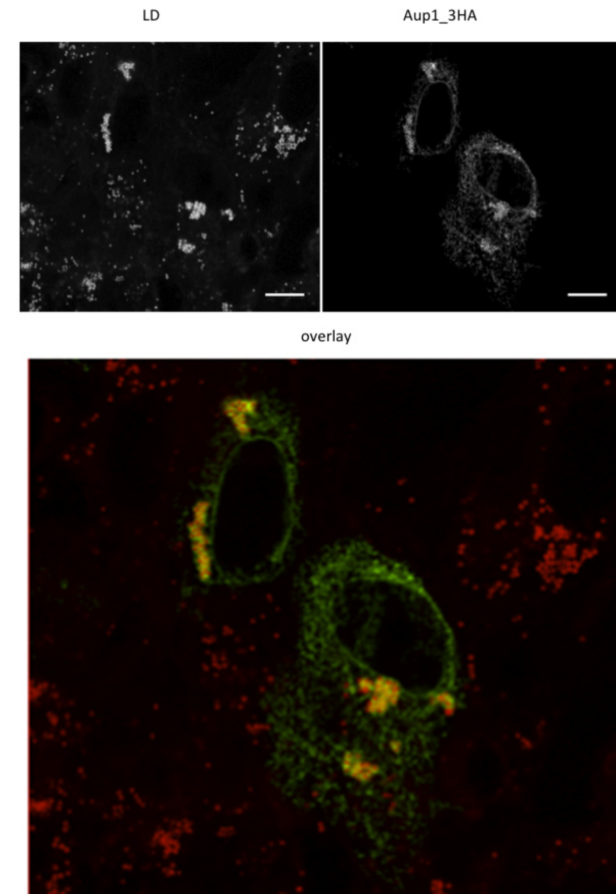
MPI-CBG
Pfotenhauerstrasse 108
Dresden , Germany &

LIMES Institute
Carl Troll Strasse 31
Bonn, Germany
astevano@uni-bonn.de, stevanov@mpi-cbg.de

Protein Targeting to Lipid Droplets

Lipid droplets are main energy storage organelles found in most eukaryotes. They are composed of a hydrophobic core surrounded by a phospholipid monolayer and associated proteins. Not much is known about the mechanism of protein targeting to LDs. We focus on understanding targeting of integral proteins to LDs. As a model protein we use Aup1 (Ancient ubiquitous protein 1) that we have previously identified and characterized as integral LD protein (1). We use mutational analysis to identify key residues within the Aup1 sequence required for its targeting to LDs. Truncation mutations have revealed that an LD targeting motif is present in the N terminal part of the protein. Point mutagenesis has identified key residues within the N terminal part of Aup1 important for its LD localization. We are currently investigating the effect of those point mutations that abolish LD localization on protein topology.
Further we looked into dynamics of LD association of Aup1 and several other LD proteins by using FRAP. These data are consistent with Aup1 being an integral membrane protein. The pathway Aup1 uses for trafficking to LDs seems to bypass Golgi, since addition of C terminal KKXX retention signal to Aup1 has no effect on its LD localization. Effect of Brefeldin A on LD targeting of Aup1 is currently being investigated.

C. Thiele and J. Spandl (2008) Cell Biology of Lipid Droplets. Current Opinion in Cell Biology 30: 378- 385



Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes