WILHELM UND ELSE HERAEUS-STIFTUNG

665. WE-Heraeus-Seminar

Physical Approaches to Membrane Asymmetry

March 25–28, 2018
at the Physikzentrum Bad Honnef/Germany

Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation which supports research and education in science, especially in physics. A major activity is the organization of seminars. By German physicists, the foundation is recognized as the most important private funding institution in their fields. Some activities of the foundation are carried out in cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft).

Aims and scope of the 665. WE-Heraeus-Seminar:

Cells and organelles establish different lipid compositions and properties in the outer and inner lipid leaflet of their membranes. The complex and energy consuming machineries needed to regulate and maintain this state imply that membrane asymmetry is a key property for proper function of the membrane and, in turn, the survival of the cell. Until recently, this apparently crucial property could hardly be addressed in model membrane studies since asymmetric models were available only to a very limited extent. As a consequence, only rather few, pioneering studies have provided insight into the specific functions of membrane asymmetry. Presently, there is encouraging progress in developing easy-to-prepare, asymmetric membrane models in the form of liposomes, free standing bilayers, and solid supported membranes. It can be expected that such models will soon become standard systems for biophysical membrane studies and allow for a boost in our knowledge of asymmetry effects on membrane proteins and membrane processes.

The seminar aims at gathering the now-forming scientific community of researchers working on membrane asymmetry and at reviewing the state of the art with respect to:

- Preparing **model membranes** with asymmetric lipid composition
- Understanding the **physical properties** of asymmetric membranes with emphasis on domain organization, lateral pressure, surface potential, order, and dynamics
- Identifying the asymmetric membrane compositions of **biological systems** at specific locations and under given conditions
- Characterizing the functions of membrane asymmetry for tuning the activity of **membrane proteins** and for governing bending, fusion, fission, and other membrane processes.

Given that asymmetric liposomes and bilayers are likely to become attractive, standard biomembrane models in the near future, the meeting will be of interest for the whole membrane biophysics community.

Scientific Organizers:

Prof. Heiko Heerklotz
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Program

Program

Sunday, March 25, 2018

16:00 – 21:00	Registration	
18:00	*BUFFET SUPPER*	
20:00 – 20:10	Sebastian Fiedler	**Welcome Note**
20:10 – 21:10	Lukas K. Tamm	**Membrane asymmetry in viral and intracellular membrane fusion**
21:10 -	*get together at the posters*	

Monday, March 26, 2018

07:30	*BREAKFAST*	
08:35 –08:40		*announcements*
08:40 – 09:20	Erwin London	**Influence of interleaflet coupling upon ordered domain formation in asymmetric vesicles**
09:20 – 10:00	Georg Pabst	**Inside asymmetric membranes: Structure and leaflet coupling**
10:00 – 10:20	Milka Doktorova	**Gramicidin increases lipid flip-flop in symmetric and asymmetric lipid vesicles**
10:20 – 10:50	*COFFEE BREAK*	
10:50 – 12:30	**Poster flashes #1-20 & poster session**	
12:30 – 12:40	**Conference Photo** (outside the lecture hall)	
12:40	*LUNCH*	

Program

Monday, March 26, 2018

14:00 – 14:40	Peter Pohl	**Registration of lipid domains from the two membrane leaflets**
14:40 – 15:20	Claudia Steinem	**ENTH-PI(4,5)P_2 interaction as a function of lateral membrane tension: Introducing membrane asymmetry**
15:20 – 16:00	Siewert-Jan Marrink	**Molecular dynamics of complex cell membranes**
16:00 – 16:30	*COFFEE BREAK*	
16:30 – 17:10	Annette Meister	**Sarcolemma tubulation induced by the peripheral membrane protein N-BAR**
17:10 – 19:00	**Poster flashes #21-43 & poster session**	
19:00	*DINNER*	
20:00- 21:00	Mark Sansom	**Molecular simulation studies of protein/lipid interactions in complex biological membranes**

Program

Tuesday, March 27, 2018

07:30	*BREAKFAST*	
08:35 – 08:40		*announcements*
08:40 – 09:20	Oscar Ces	**Generating and exploiting membrane asymmetry**
09:20 – 10:00	Sylvie Roke	**Lipid membrane asymmetry probed label-free with nonlinear light scattering and imaging: Direct probes of surface chemistry and electrostatic potential landscapes.**
10:00 – 10:20	Marie Markones Carina Drechsler	**Zeta potential quantifies charged lipids in the outer leaflet of asymmetric liposomes**
10:20 – 10:50	*COFFEE BREAK*	
10:50 – 11:30	William Dowhan	**Generating membrane protein and lipid asymmetry**
11:30 – 12:10	Joost C.M. Holthuis	**Dissecting protein-lipid crosstalk in life/death decision-making with photoactuated lipid probes**
12:10 – 12:30	Frederick Heberle	**Determining the structure of asymmetric bilayers with small-angle scattering: Insight from Molecular Dynamics simulations**
12:30	*LUNCH*	

Program

Tuesday, March 27, 2018

14:00 – 14:40	Oliver Ernst	**Phospholipid scrambling by rhodopsin**
14:40 – 15:20	Thomas Gutsmann	**Reconstitution and function of the asymmetric outer membrane of gram-negative bacteria**
15:20 – 16:00	Ana J. García-Sáez	**Membrane asymmetry in cell death**
16:00 – 16:30	*COFFEE BREAK*	
16:30 – 17:10	**Discussion: Proper choice of models for membrane research**	
17:10 – 17:30	Stefan Jorda	**About the Wilhelm and Else Heraeus Foundation**
17:30 – 19:00	**Poster session**	
19:00	*WE-HERAEUS DINNER* *(social event with cold & warm buffet with complimentary drinks)*	

Program

Wednesday, March 28, 2018

07:30	*BREAKFAST*	
08:30 – 08:40		**Presentation of poster awards**
08:40 – 09:20	Patricia Bassereau	**Shaping of membranes upon asymmetric binding of BAR-domain proteins**
09:20 – 10:00	Kirsten Bacia	**Asymmetric protein-membrane binding in intracellular vesicle formation**
10:00 – 10:20	Erik Strandberg	**Membrane pore formation of the antimicrobial peptide MSI-103 promoted by lyso-lipids**
10:20 – 10:50	*COFFEE BREAK*	
10:50 – 11:30	Sandro Keller	**Polymer-encapsulated nanodiscs for membrane biophysics**
11:30 – 12:10	Maria João Moreno	**Transient asymmetry in biomembranes due to association of small molecules. Methods for its characterization and relevance for membrane permeability**
12:10 – 12:30	Radek Šachl	**Inter-leaflet coupling of lipid driven nanodomains**
12:30 – 12:40	Heiko Heerklotz	**Closing remarks**
13:00	*LUNCH*	

End of the seminar and departure

NO DINNER for participants leaving on Thursday morning

Posters

Posters

01	Haleh Abdizadeh	**Molecular dynamics of energy-coupling factor transporters in bacterial membranes**
02	Alfred Blume	**Co-spreading of anionic phospholipids with peptides of the structure $(KX)_4K$ at the air-water interface**
03	Claudia Contini	**Patchy synthetic vesicles by polymer/polymer phase separation**
04	Bartholomäus Danielczak	**Collisional lipid transfer among DIBMA-bounded nanodiscs**
05	Manabendra Das	**Asymmetric detergents and their structural effects on human *β2* adrenergic receptor stability**
06	Jan Dedic	**Sum-frequency scattering from asymmetric liposomes**
07	Magali Deleu	**Lipid specificity of surfactin interaction with plant plasma membrane**
08	Lisa Dietel	**Fungicidal lipopeptides: Synergistic action as a mechanism of target membrane selectivity**
09	Milka Doktorova	**Interleaflet coupling in asymmetric membranes: Protocols and revelations**
10	Carina Drechsler	**Preparation of asymmetric liposomes using a phosphatidylserine decarboxylase**
11	Simon Drescher	**Synthesis and physicochemical characterization of asymmetrical glycerol diether bolalipids**
12	Barbara Eicher	**Curvature induced coupling in asymmetric lipid vesicles**

Posters

13	Hugo Filipe	**Permeation of amphiphilic drug-like molecules through symmetric and asymmetric lipid bilayers – a molecular dynamics simulation study**
14	Moritz Frewein	**Intrinsic lipid curvatures from global X-ray scattering data analysis of inverted hexagonal phases**
15	Erik Frotscher	**Structural dynamics of the bacterial membrane protein Mistic**
16	Félix M. Goñi	**Extemporaneous attempts at asymmetric vesicles**
17	Anne Grethen	**Role of Coulombic repulsion in collisional lipid transfer among SMA(2:1) nanodiscs**
18	Mona Grimmer	**Natural ER membrane interaction of COPII**
19	Heiko Heerklotz	**Consequences of membrane pressure asymmetry due to one-sided insertion of molecules**
20	Maria Hoernke	**Antimicrobial selectivity and membrane leakage mechanisms**
21	Jonas Höring	**Temperature dependence of fluorinated-surfactant micellization**
22	Yulia Ivanova	**Quantification of membrane asymmetry by continuous wave electron paramagnetic resonance spectroscopy**
23	Andreas Janshoff	**Physics of membrane fusion revealed by colloidal probe microscopy**
24	Piotr Jurkievicz	**Adsorption of Calcium ions on curved lipid bilayer**

Posters

25	Michael Kaiser	**Preparation of asymmetric liposomes - mimicking the inner plasma membrane of *Escherichia Coli***
26	Johannes Klingler	**A thermodynamic model for native nanodiscs**
27	Constanze Lamprecht	**Heat shock protein Hsp70-1A selectively inserts in dipalmitoyl phosphatidylserine domains and causes membrane blebbing**
28	Florian Mahler	**Membrane interactions of conventional and fluorinated surfactants**
29	Laura Paulowski	**A step forward in the design of liposomes: symmetric & asymmetric vesicles from lipid extracts**
30	Kristyna Pluhackova	**Interleaflet induction and registration of membrane domains under the loupe of all-atom MD simulations**
31	Ali Saitov	**Domain mirroring in asymmetric lipid membranes**
32	Jonas Schäfer	**Influence of the solid support on receptor lipid distribution in model membrane systems**
33	Jan Schatteburg	**Gramicidin A in DPhPC bilayer membranes**
34	Johannes Schnur	**Albumin-induced lysolipid depletion enhances leakage of thermoresponsive liposomes**
35	Thomas Schubert	**Novel quantitative analysis on the study of external molecules binding to different lipid-receptor species determining hierarchical orders and/or constraints**

Posters

36	Christian Schwieger	**Binding Sar1 to a lipid monolayer: Insertion and orientation studied by infrared-reflection-absorption spectroscopy**
37	Johnna St. Clair	**The effect of lipid composition upon formation of co-existing ordered and disordered lipid domains in the outer leaflet of asymmetric lipid vesicles was assayed using FRET**
38	Lena Steger	**Flipping transmembrane helices**
39	Taras Sych	**Investigation of plasma membrane reorganization during uptake processes**
40	Orly Tarun	**Label-free and charge-sensitive dynamic imaging of lipid membrane hydration**
41	Carolyn Vargas	**Formation of lipid-bilayer nanodiscs by diisobutylene/maleic acid (DIBMA) copolymer**
42	John Williamson	**How to control domains and asymmetry using flip-flop and external fields**
43	Daniel Wüstner	**Sterol asymmetry in the yeast plasma membrane**

Abstracts of Talks

(in chronological order)

Membrane Asymmetry in Viral and Intracellular Membrane Fusion

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A frequent approach to understand membrane function is to reconstitute membranes from purified components to parse out specific functions of the individual components. Although all cellular membranes are asymmetric in terms of lipid and protein topology, reconstituting asymmetry in vitro has been challenging and has frequently been ignored in reconstitution studies. This is also true for studying membrane fusion in virus entry and intracellular vesicle traffic by reconstitution approaches.

Our lab has developed methods to prepare asymmetric supported membranes. I will briefly review these methods and present results on liquid-ordered domain coupling between leaflets in such membranes. Asymmetric supported membranes have also tremendously aided studies of membrane fusion using single HIV and Ebola pseudovirus particles. Similarly, fusion of synaptic vesicles, dense core vesicles from PC12 cells, and proteoliposomes containing reconstituted SNAREs with asymmetric supported target membranes have yielded interesting new insight into the biology of these processes.

For references see: https://med.virginia.edu/tamm-lab/

Influence of Interleaflet Coupling Upon Ordered Domain Formation in Asymmetric Vesicles

Erwin London, Qing Wang and Johnna St. Clair

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Asymmetric large unilamellar vesicles were prepared by lipid exchange using alpha class cyclodextrins. Outer leaflet ordered domain (raft) formation was assessed by FRET in asymmetric vesicles composed of high-Tm saturated phosphatidylcholine (PC) or sphingomyelin (SM), low-Tm unsaturated PC and cholesterol in their outer leaflet, and unsaturated phospholipids and cholesterol in their inner leaflet. For cholesterol-containing vesicles with various saturated PC mixed with dioleoyl PC in their outer leaflet and dioleoyl PC in their inner leaflet, ordered domain stability in asymmetric vesicle outer leaflets was compared to that in symmetric vesicles with the same lipid composition as the asymmetric vesicle outer leaflet. Ordered domains in asymmetric vesicle outer leaflets were as thermally stable as in symmetric vesicles for distearoyl PC, but were progressively less stable than in symmetric vesicles as saturated PC acyl chain length was decreased from 18 to 16, 15 or 14. In the case of dimyristoyl PC (C14 acyl chains) outer leaflet ordered domain formation was suppressed in asymmetric vesicles, although easily detected in symmetric vesicles. In analogous asymmetric vesicles containing SM in place of saturated PC in their outer leaflet, the thermal stability of outer leaflet ordered domains decreased as the % SM in the outer leaflet decreased, and decreased to a greater extent than in symmetric vesicles with the same lipid composition as the outer leaflet of the asymmetric vesicles. In cholesterol-containing asymmetric vesicles in which the inner leaflet lipid was varied, while the outer leaflet contained SM and dioleoyl PC, the thermal stability of ordered domains was independent of the composition of the inner leaflet, including whether it was composed of unsaturated PC or a mixture of unsaturated phosphatidylethanolamine (PE) and phosphatidylserine (PS). In contrast, both osmotic pressure and the presence of transmembrane peptides were found to have a significant effect on ordered domain formation in the outer leaflet, especially for vesicles which contained a mixture of SM and 1-palmitoyl 2-oleoyl PC. Thus, the thermal stability of the outer leaflet ordered domains reflects high-Tm lipid amount, type, and interleaflet coupling interactions with inner leaflet lipids. Thus, depending upon lipid composition and other environmental factors, the physical state of the lipids can be dominated by outer leaflet with high-Tm lipids, in which case, as shown in prior studies, ordered domains often form in both leaflets due to interleaflet coupling, or can be dominated by inner leaflet with low-Tm lipids, in which case outer leaflet ordered domains can be destroyed by interleaflet coupling. This has implications for how domain formation may be controlled in vivo.

Inside Asymmetric Membranes: Structure and Leaflet Coupling

B. Eicher[1], D. Marquardt[2], F.A. Heberle, J. Katsaras, and G. Pabst[1]

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Biological membranes are asymmetric including the distribution of membrane lipids. Of recent, asymmetric large unilamellar lipid vesicles (aLUVs) have emerged as a new platform for studying fundamental membrane biophysics pertaining to membrane with the advantage to be amenable for broad variety of experimental techniques. In our recent studies we have focused primarily on neutron and X-ray scattering techniques, solution NMR or differential scanning calorimetry to interrogate leaflet specific structural properties of aLUVs on the sub-nanometer scale [2-4]. In particular, this allowed us to address transbilayer coupling and passive lipid flip/flop. Lipid flip/flop, for example was found to be slow in the fluid phase and practically absent in the gel phase. However, membrane defects associated e.g. with the lipid's melting transition or solid supported bilayers may cause an increase of lipid translocation [3]. Transleaflet coupling was observed only for coexisting gel and fluid phases, but not for all-fluid bilayers. In the case of DPPC/POPC aLUVs, for example, DPPC-enriched gel domains in the outer leaflet were significantly disordered by a coexisting fluid inner leaflet enriched in POPC [1]. For POPE/POPC aLUVs in turn, we found transbilayer coupling when POPE was enriched on the inner leaflet, but not for the reversed system [4]. Hence, transbilayer coupling depends strongly on lipid composition and in the case of DPPC/POPC most likely on partial hydrocarbon chain interdigitation, whereas intrinsic lipid curvature apparently dominates the coupling of leaflets in POPE/POPC aLUVs.

References

[1] F.A. Heberle, et al. Langmuir **32,** 5195 (2016).
[2] B. Eicher, et al. J. Appl. Crystallography **50**, 419 (2017).
[3] D. Marquardt, et al. Langmuir **33**, 3731 (2017)
[4] B. Eicher et al., Biophys J **114**, 146 (2018)

Gramicidin increases lipid flip-flop in symmetric and asymmetric lipid vesicles

M. Doktorova[1], F. Heberle[2], D. Marquardt[3], R. Rusinova[1], L. Sanford[1], T. Peyear[1], J. Katsaras[2], G. Feigenson[4], H. Weinstein[1] and O. Andersen[1]

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Unlike transmembrane proteins, phospholipids can reorient within the membrane by moving from one leaflet to the other. Spontaneous lipid translocation tends to be very slow, however, and cells facilitate the process with enzymes that catalyze the transmembrane lipid movement and thereby regulate the transbilayer lipid distribution. It has been proposed that non-enzymatic membrane-spanning proteins may also accelerate lipid flip-flop in a non-specific manner by introducing bilayer defects, thus easing the translocation of polar lipid head groups across the bilayer by allowing them to move along the protein-membrane interface [1-3]. We examined this possibility using deuterated phospholipids and the gramicidin channels, which have well-defined structure and function that make them ideal candidates for probing protein-membrane interactions. Making use of recently developed protocol and assays for the preparation and characterization of asymmetric lipid vesicles, we studied compositionally and isotopically asymmetric proteoliposomes containing gramicidin. Protein incorporation, conformation and function were examined with small-angle x-ray scattering, circular dichroism and a stopped-flow spectrofluorometric assay. Differential scanning calorimetry revealed the effect of the protein on the melting transition temperatures of the two bilayer leaflets, which over time merged into a single peak indicating lipid scrambling. Using proton NMR, we monitored the transbilayer lipid distribution in both symmetric POPC and asymmetric POPC/DMPC vesicles with and without the protein. Our results show that gramicidin increases lipid flip-flop in a concentration-dependent manner.

References

[1] B. De Kruijff et al, BBA **509**, 537 (1978)
[2] E. Fattal et al, Biochemistry **33**, 6721 (1994)
[3] M. Kol et al, Biochemistry **40**, 10500 (2001)

REGISTRATION OF LIPID DOMAINS FROM THE TWO MEMBRANE LEAFLETS

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Separation in biological membranes plays an important role in protein targeting and transmembrane signaling. Liquid disordered (L_d) and liquid-ordered (L_o) domains commonly span both membrane leaflets. Alternative hypotheses were put forward to explain such co-localization: (i) Lipid interdigidation at membrane midplane and (ii) minimization of elastic membrane deformations. Contrary to the expected contribution from interdigitation to interleaflet coupling, we found that the ends of both short and long chain fluorescent lipid analogues experience the same friction (Horner et al., 2013). Testing the second hypothesis was more successful: A theoretical analysis revealed that the line tension around thicker ordered domain constitutes an important driving force (Galimzyanov et al., 2015). It is complemented by thermal undulations which drive stiffer lipid domains into areas with fluctuations of lower monolayer curvature (Galimzyanov et al., 2017). These areas naturally coincide in the opposing monolayers. The required heterogeneity in splay rigidities may either originate from intrinsic lipid properties or be acquired by adsorption of peripheral molecules (Horner et al., 2009). Additional lipid mobility measurements suggest that L_d forming lipids in one leaf may experience such a large friction when opposed to L_o forming lipids in the other leaf that they may "freeze" into an L_o domain (see accompanying abstract by Saitov & Pohl). This observation is supported by molecular dynamics simulations (see abstract by Pluhackova et al.). The energetic contributions from friction, undulations, and line tension act hand in hand: (i) Since proportional to membrane area, undulations are more important for the stabilization of larger bilayer domains, while (ii) line tension is more significant for smaller bilayer domains since proportional only to domain radius.

Galimzyanov, T.R., P.I. Kuzmin, P. Pohl, and S.A. Akimov. 2017. Undulations Drive Domain Registration from the Two Membrane Leaflets. *Biophys. J.* 112:339-345.

Galimzyanov, T.R., R.J. Molotkovsky, M.E. Bozdaganyan, F.S. Cohen, P. Pohl, and S.A. Akimov. 2015. Elastic Membrane Deformations Govern Interleaflet Coupling of Lipid-Ordered Domains. *Phys. Rev. Lett.* 115:088101.

Horner, A., S.A. Akimov, and P. Pohl. 2013. Long and short lipid molecules experience the same interleaflet drag in lipid bilayers. *Phys. Rev. Lett.* 110:268101.

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ENTH-PI(4,5)P_2 interaction as a function of lateral membrane tension: Introducing membrane asymmetry

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The epsin *N*-terminal homology domain (ENTH) is a major player in clathrin-mediated endocytosis. It is known that ENTH of epsin-1 binds specifically to the receptor lipid PtdIns(4,5)P_2, resulting in tubulation or even vesiculation. Upon binding to PtdIns(4,5)P_2 an additional α-helix, referred to as helix-0 at the *N*-terminus of ENTH, is formed. To analyze the interaction of ENTH with lipid membranes, we used two different model membrane systems. In a first set of experiments, we investigated the binding of ENTH to planar lipid bilayers composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) doped with different concentrations of PtdIns(4,5)P_2 on Si/SiO_2 surfaces by means of reflectometric interference spectroscopy. It turned out that during the spreading process of large unilamellar vesicles composed of POPC and PtdIns(4,5)P_2 to form planar bilayers on the SiO_2 surface, PtdIns(4,5)P_2 appeared to distribute asymmetrically within the lipid bilayer with a lower PtdIns(4,5)P_2 concentration in the top monolayer. This notion was confirmed by comparing the obtained results on lipid bilayers with those on lipid monolayers prepared on bis(trimethylsilyl)amine-functionalized SiO_2 surfaces. However, for both membrane systems, a PtdIns(4,5)P_2 co ncentration dependent specific binding of ENTH to PtdIns(4,5)P_2 was found.

Based on these results, we addressed the question whether the specific interaction of ENTH with PtdIns(4,5)P_2 leads to tubulation and even vesiculation of artificial membranes. Thus, in a second set of experiments, we established a membrane system based on PtdIns(4,5)P_2 doped giant unilamellar vesicles (GUVs) adhered to an avidin coated glass substrate. By adjusting the Mg^{2+}-concentration in solution, we were able to control the lateral membrane tension of the adhered GUVs in a range of about one order of magnitude. Binding of ENTH to the outer leaflet of the GUVs resulted in an asymmetry in the lipid bilayer that significantly altered the GUV structure. At low membrane tension, ENTH binding induced tubular structures as observed by spinning disk confocal microscopy. At higher membrane tension, the specific interaction of ENTH with PtdIns(4,5)P_2 led to a flattening of the GUVs without the formation of tubules. This finding is attributed to an increased surface area caused by the asymmetric insertion of the ENTH helix-0 into the membrane.

References

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Molecular Dynamics of Complex Cell Membranes

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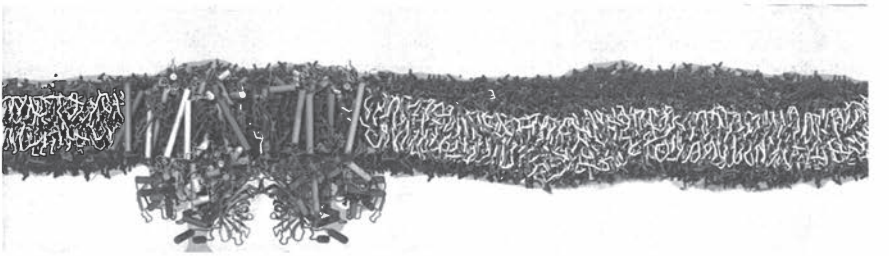
In this lecture I will describe current computer simulations of the dynamic organization of cell membranes, using the coarse-grain Martini model developed in our lab [1]. I will illustrate the power of the model by providing a few in-depth examples of large-scale simulations involving membranes with realistic composition. Applications discussed include the exchange of electron carriers in photosystems embedded in the thylakoid membrane [2], cardiolipin mediated formation of respiratory chain supercomplexes in mitochondrial membranes [3], and the lateral organization of lipids and proteins in complex plasma membrane models [4].



Snapshot from a coarse-grained MD simulation of photosystem II embedded in a realistic thylakoid membrane composed of galactolipids. The electron carrier plastoquinone that binds to or unbinds from the protein depending on its oxidation state is shown in orange.

References

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Sarcolemma tubulation induced by the peripheral membrane protein N-BAR

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The N-BAR domain of amphiphysin 2 from skeletal muscle contributes to the generation of transverse tubules by forming protein scaffolds on the sarcolemma membrane. In this study, we apply electron cryo-tomography to determine the supramolecular organization of the N-BAR domain *in situ* (1). We show that the protein assembles into a loose helical scaffold to generate long flexible membrane tubules of defined diameter. Cryo electron microscopy indicates that the natural mixture of sarcolemma lipids is essential for the formation of uniform tubules with a diameter defined by the intrinsic curvature of the N-BAR domain. The role of the individual sarcolemma lipids is discussed based on binding experiments with large unilamellar vesicles and lipid monolayers.

Langmuir monolayer experiments were performed to follow the peripheral insertion of the human N-BAR domain into artificial sarcolemma membranes of systematically varied lipid composition. We underline the role of $PI(4,5)P_2$ to protein recruitment and the limits of the monolayer technique by discussing the role of cholesterol.

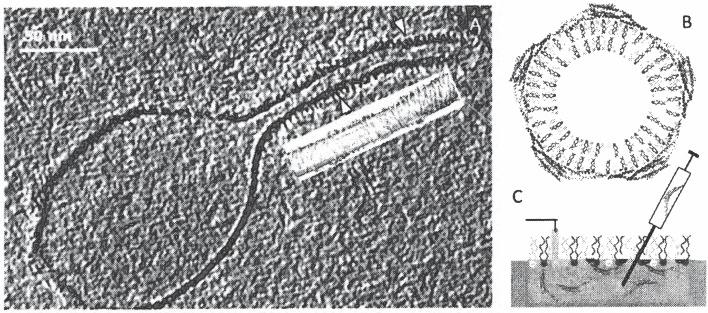

Figure 1 (A) Cryo EM image after *in vitro* tubulation of sarcolemma vesicles with the human N-BAR domain. Arrowheads point to the N-BAR scaffold. Sub-tomogram average is shown in the inset. (B) Model describing the cross section of a membrane tube decorated by N-BAR dimers. (C) Monolayer setup to study the binding of N-BAR to sarcolemma model membranes of different lipid composition.

References

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Molecular simulation studies of protein/lipid interactions in complex biological membranes

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Membrane proteins account for ca. 20% of all genes, 40% of drug targets, and are mutated in many human diseases. The past decade has witnessed an exponential rise in the number of high resolution membrane protein structures and recent developments in cryo-electron microscopy have accelerated the rate of structure determination. However, the structures do not often reveal interactions with lipid molecules present in their native membrane environments. Interactions with lipids are of crucial importance for the stability, regulation, and targeting of membrane proteins. Notably, specific lipid binding sites provide potential allosteric druggable targets, and thus these protein-lipid interactions are of immense biomedical importance. While advances in membrane lipidomics and mass spectrometry are beginning to uncover the diversity and importance of lipid/protein interactions, there is a paucity of detailed structural and biophysical/biochemical characterization of protein/lipid interactions for many families of membrane proteins. Molecular dynamics (MD) simulations provide a key tool for probing the interactions of lipids with membrane proteins. Recent advances in MD make reliable prediction and analysis of lipid interactions possible [1]. Building upon our database of membrane proteins simulated in simple lipid bilayers, MemProtMD (http://memprotmd.bioch.ox.ac.uk), we are able to use 'computational biochemistry' to probe the strength and specificity of the interactions of lipids with key membrane proteins, including transporters [2], ion channels and GPCRs [3]. More recently we have used these approaches to also explore the interactions of lipid recognition domains from peripheral membrane proteins with membrane surfaces [4]. Extending our studies to larger scale simulations allow us to address a number of aspects of the dynamic organization of lipids and proteins in models of bacterial, viral, and mammalian cell membranes [5].

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Generating and exploiting membrane asymmetry

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This talk will provide an overview of a series of microfluidic approaches we have developed for generating and characterizing membrane asymmetry. Using these technologies we have been able to develop molecular machines that are able to sense and respond to their environment through manipulation of their internal levels of membrane asymmetry.

Lipid membrane asymmetry probed label-free with nonlinear light scattering and imaging: Direct probes of surface chemistry and electrostatic potential landscapes.

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Variations between the inner and outer leaflets of cell membranes are crucial for cell functioning and signaling, drug-membrane interactions, and the formation of lipid domains. Transmembrane asymmetry can in principle be comprised of an asymmetric charge distribution, differences in hydration, specific head group/H-bonding interactions or a difference in the number of lipids per leaflet. Here, we show how nonlinear light scattering methods such as sum frequency scattering and polarimetric angle-resolved second harmonic scattering can be used to probe the molecular structure of the lipids, the membrane hydration and the interfacial electrostatic potential of liposome membranes in aqueous solutions [1-3]. We present studies of single lipid and mixed liposomes as examples. We also show how high throughput wide field second harmonic imaging [4] can be used to image the membrane hydration asymmetry with 400 nm spatial and < 200 ms temporal resolution [5]. The time lapse videos can be converted in membrane potential maps using nonlinear optical theory, from which the electrostatic energy landscape can be obtained.

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Zeta Potential Quantifies Charged Lipids in the Outer Leaflet of Asymmetric Liposomes

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Nearly all natural lipid membranes show a distinct lipid asymmetry between the outer and the inner leaflet of the lipid bilayer. Cells spend a substantial amount of energy on establishing and maintaining this asymmetry. Nevertheless, it is not fully understood how asymmetry influences membrane function, for example the behavior of embedded proteins. Here, we present two different methods to prepare asymmetric liposomes for the purpose of studying the effects of lipid asymmetry on transmembrane proteins.

To prepare liposomes with phosphatidylserine (PS) preferentially in the inner monolayer, we used a soluble form of phosphatidylserine decarboxylase (PSD), that decarboxylates PS to phosphatidylethanolamine (PE). By adding PSD to preformed liposomes, the outer PS is decarboxylated while the PS of the inner leaflet is not accessible. This results in PS asymmetric liposomes mimicking the PS asymmetry of eukaryotic plasma membranes.

As a model for bacterial membranes, we generated mixed phosphatidylglycerol (PG)-phosphatidylcholine (PC) liposomes with PG exclusively in the outer leaflet. To replace PC with PG, methyl-β-cyclodextrin (MβCD) is used as a lipid carrier. Rather than using intact donor PG liposomes, we added PG in fully MβCD-solubilized form to the PC liposomes. This allows us to accurately control the outer-leaflet content of PG. To do so, we first determined the equilibrium constants of PG/PC - MβCD complex formation by isothermal titration calorimetry. These were then used to establish defined saturation levels of cyclodextrin with PG by adjusting MβCD and PG concentrations in PG solubilization mixtures. This way, we know exactly how much PG equilibrates with the outer leaflet of the PC liposomes, which then yields the desired PG content.

In both methods, zeta potential measurements and high performance thin layer chromatography are used to quantify the degree of PS and PG asymmetries.

Generating Membrane Protein and Lipid Asymmetry

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We have focused on the role of lipid-protein interactions as a determinant of membrane protein structure [1]. Orientation of transmembrane domains (TMDs) is generally accepted to be determined at the time of initial membrane insertion by interactions between the membrane insertion machinery and topogenic signals within the protein as governed by the Positive Inside Rule. However, we established that post-translational lipid-protein interactions influence final TMD orientation at the time of and dynamically after initial folding (Charge Balance Rule). We provide evidence that the presence of net neutral lipids such as phosphatidylethanolamine (PE), which dilutes the membrane negative surface change density contributed by anionic lipids, directly reduces the effective negative charge of acidic amino acids in extramembrane domains (EMDs) thus favoring the potential of unaffected basic amino acids to orient EMDs toward the cytoplasm. We demonstrate that the free energy of insertion (ΔG) into a lipid bilayer of an acidic amino acid is more positive in the absence of PE than in the presence of PE; the ΔG for bilayer insertion of basic amino acids is unaffected by PE. In order to study the influence of membrane lipid transbilayer asymmetry on the organization of membrane proteins, methods are needed to establish stable membrane lipid asymmetry in vivo and in vitro. We have expressed in *Escherichia coli* the *mprF* gene from *Staphylococcus aureus* that encodes the synthesis of *O*-lysyl-phosphatidylglycerol (Lys-PG), a positively charged phospholipid. The full-length gene product is a bifunctional protein that synthesizes Lys-PG in the cytoplasmic leaflet of the inner membrane and flips all of the Lys-PG to the periplasmic leaflet. A truncated version of the gene product lacks flippase activity resulting in all of the Lys-PG remaining in the cytoplasmic leaflet. Small unilamellar vesicles (SUVs) containing Lys-PG only in the outer lipid leaflet can be made by methyl-ß-cyclodextrin catalyzed lipid transfer [2] between multilamellar vesicles containing Lys-PG and SUVs lacking Lys-PG. Normally the presence of polytopic membrane proteins in SUVs results in scrambling of lipids between leaflets. However, Lys-PG remains asymmetrically distributed across the bilayer of such proteoliposomes. The stable bilayer asymmetry of Lys-PG *in vivo* and *in vitro* will provide systems in which to study the importance of membrane lipid asymmetry in cellular function. Supported by NIGMS grants GM121493 and GM20487 and the John Dunn Research Foundation.

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Dissecting protein-lipid crosstalk in life/death decision-making with photoactuated lipid probes

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While sphingolipids are vital components of cellular membranes, their production relies on toxic metabolic intermediates: ceramides. Up-regulation of these putative tumour suppressor lipids is frequently linked to induction of mitochondrial apoptosis. However, the molecular principles by which ceramides commit cells to death are poorly understood. We previously identified sphingomyelin synthase-related protein SMSr as suppressor of ceramide-induced apoptosis [1-3]. Acute disruption of SMSr catalytic activity causes a dramatic rise in ER ceramides and their mislocalization to mitochondria, triggering a Bax-dependent pathway of apoptosis. Diverting CERT-mediated ceramide transport to mitochondria phenocopies SMSr inactivation [4], indicating that ER ceramides are authentic transducers of apoptosis and that their arrival in mitochondria is a crucial step in committing cells to death.

Using photo-activatable (diazirine-containing) ceramide analogues [5], we recently identified the voltage-dependent anion channels VDAC1 and VDAC2 as the principal ceramide-binding proteins in mitochondria. VDACs have previously been implicated as critical players in the cytosolic release of mitochondrial apoptogenic proteins. Combining molecular dynamics simulations with photo-affinity labeling studies, we mapped the ceramide-binding sites on VDACs and analysed ceramide binding-defective channels for their ability to support apoptosis in SMSr-deficient cells. To resolve the sequence of events underlying ceramide-induced cell death, we also applied switchable ceramide transfer proteins and photo-switchable (azobenzene-containing) ceramide analogues. As outlined in my talk, these studies yielded important new insights into how ceramides exert their tumour-suppressor activities.

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Determining the structure of asymmetric bilayers with small-angle scattering: Insight from Molecular Dynamics simulations

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Scattering techniques have contributed greatly to our understanding of how the properties of individual lipids translate to bilayer structural properties. While significant progress has been made using symmetric bilayers, much less is known about the structure and dynamics of asymmetric bilayers. Recent advances in the preparation of asymmetric model membranes now allow these questions to be addressed systematically [1], but continued progress requires the development of new scattering models that can account for the properties of individual leaflets. Because of the additional complexity of asymmetric bilayers, proposed models should be carefully scrutinized, ideally by analyzing standard samples of known structure. Standards enable validation of a proposed model, and facilitate comparison to other models. Here, we propose the use of Molecular Dynamics (MD) simulations to fill this role. Simulated bilayers meet two important criteria of standard samples: (1) the bilayer structure is known with high precision and accuracy; and (2) scattering data for different radiation types (i.e., X-ray or neutron) and sample contrasts (e.g., deuterated lipid variants) are easily calculated. Crucially, this "data collection" only requires knowledge of the time-averaged spatial distribution of matter obtained directly from the trajectory of atomic positions, and therefore does not rely on any model assumptions. Moreover, this process can be repeated with added random noise to simulate the collection and analysis of multiple replicate data sets. Statistical analyses of the resulting ensembles of fits provide valuable insight including parameter uncertainties and correlations, and the possible presence of systematic biases in the recovered structural parameters. Using this framework, we evaluate recently published models for asymmetric bilayer structure.

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Phospholipid scrambling by rhodopsin

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Rhodopsin has been intensively characterized in its role as a visual pigment and G protein-coupled receptor responsible for dim-light vision. We recently discovered that it also functions as an ATP-independent phospholipid scramblase: when reconstituted into large unilamellar vesicles, rhodopsin accelerates the normally sluggish transbilayer translocation of common phospholipids to rates in excess of 10000 phospholipids transported per rhodopsin per second. Here I summarize the work leading to this discovery and speculate on the mechanism by which rhodopsin scrambles phospholipids.

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Reconstitution and Function of the Asymmetric Outer Membrane of Gram-negative Bacteria

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The architecture of the lipid matrix of Gram-negative, Gram-positive bacteria, and mycobacteria is extremely different and specific lipid molecules can play important roles. In case of Gram-negative bacteria the outer membranes are asymmetric with respect to its lipid distribution: Whereas the inner leaflet is composed of a phospholipid mixture, the outer leaflet is built up by glycolipids. For most Gram-negative species, these glycolipids are lipopolysaccharides, for a few species, however, glycosphingolipids. We focus here on our experimental approach and results thereof to get answers on (i) the function of the asymmetric architecture, (ii) on the incorporation of porin channels into the asymmetric bilayer and their function inside the membrane [1], and (iii) the formation of transient lesion or stable pores by the interaction of antimicrobial peptides. Furthermore, we investigated the influence of the glycol-component on basic biophysical characteristics of glycolipids-containing membranes such as their electric properties [2] and the lateral organization of the glycolipids in the membrane. To this end we established and applied a number of various reconstitution systems of the outer membrane reaching from monolayers at the air-water interface, via solid supported symmetric and asymmetric bilayers, free-standing symmetric and asymmetric bilayers prepared according to Montal-Mueller technique [3] to three-dimensional aggregates such as liposomes.

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Membrane asymmetry in cell death

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All forms of regulated cell death known so far involve an execution step that affects the permeability of a cellular membrane, and very often this is accompanied of phosphatidyl serine exposure to the extracellular leaflet of the plasma membrane, which acts as an "eat me" signal for the neighboring cells. Here I will present our studies to understand the alterations in membrane organization during different forms of cell death, including the loss of plasma membrane asymmetry and its functional consequences.

Shaping of membranes upon asymmetric binding of BAR-domain proteins

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Cell plasma membranes are highly deformable and are strongly curved upon membrane trafficking or during cell motility. BAR-domain proteins with their intrinsically curved and anisotropic shape are involved in many of these processes. They spontaneously assemble on one leaflet of cell membranes and deform them, or are enriched in curved area [1]. Inspired by in vivo experiments, we have used in vitro assays based on giant liposomes (GUVs) or nanotube assays, together with theoretical models developed by our collaborators (A. Callan-Jones, V. Lorman), for understanding the mechanical action of BAR-domain proteins(N-BAR or I-BAR) on biological membranes when BAR-domain proteins bind asymmetrically to membranes. With coarse-grained simulations (with G. Voth), we could additionally understand how BAR proteins self-assemble on membranes and scaffold nanotubes [2, 3].

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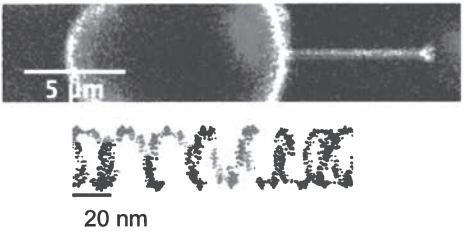

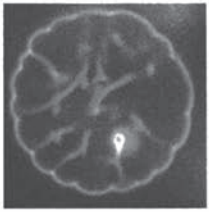

Asymmetric protein-membrane binding in intracellular vesicle formation

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The protein-mediated bending transformation of cellular membranes into buds, which ultimately separate from the donor membrane to form distinct transport compartments, constitutes an asymmetric membrane processes. Two basic topologies can be distinguished: In multivesicular body formation or some viral budding processes, protein binding on the cytosolic membrane face is involved in budding away from the cytoplasm, while in COPI, COPII and clathrin vesicle formation protein binding on the cytosolic membrane face controls budding towards the cytoplasm. We focus on the latter process. On the road towards understanding vesicle budding and fission, biophysical analysis schemes are developed that enable the quantification of protein-membrane and protein-protein interactions and shed light on the structures and dynamics of protein coat components and membranes.

COPII facilitates transport vesicle formation at the endoplasmic reticulum in the anterograde pathway. Binding and recruitment of coat proteins occurs at the cytosolic leaflet of the ER membrane. With the goal of quantifying binding affinities of coat proteins to bilayer membranes, a dual-color fluorescence cross-correlation spectroscopy (FCCS) scheme for binding analysis on liposomes has been developed [1]. Using distinct fluorescent labels on a protein and the lipid bilayer, FCCS allows determining a model-independent binding curve, as exemplified for the COPII protein Sar1. Results from applying fluorescence correlation spectroscopy (FCS) to lipid monolayers corroborate a model of asymmetric lipid displacement by amphipathic-helix (AH) mediated Sar1 binding. Although it is clear that membrane binding of the AH of GTP-activated Sar1 plays an important role in membrane bending and fission, the role of the activated protein conformation and GTP hydrolysis have not been fully disentangled. Furthermore, IRRAS measurements on monolayers suggest only a slight change of Sar1 orientation during inner coat formation [2]. COPII coat protein binding has been observed to depend on both lipid composition and the presence of cargo protein. With the goal of including more complex membranes in biophysical investigations, a new model membrane system is currently being developed.

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Membrane pore formation of the antimicrobial peptide MSI-103 promoted by lyso-lipids

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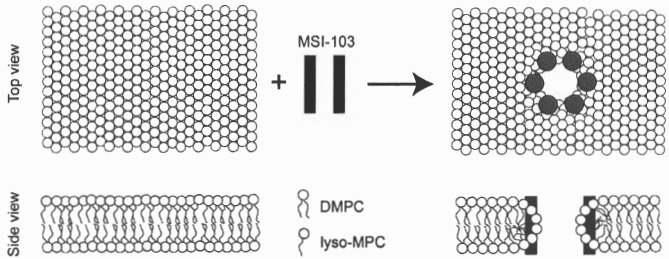
Antimicrobial peptides can kill bacteria by permeabilizing their membranes, and one proposed mechanism is by forming a membrane-spanning toroidal pore, where the peptides are lining the pore in a transmembrane orientation. It is hard to study such pores, as they are often transient and only a small proportion of the peptides are inside a pore at a given moment. We have recently shown that pore formation – and in general insertion of peptides and proteins into membranes – is promoted by lipids with a positive spontaneous curvature, like lyso-lipids [1,2]. Using KIA peptides with a repetetive amino acid sequence KIAGKIA of different length, we observed that the peptides can insert into DMPC membranes in the presence of lyso-MPC, and that the tilt angle of the peptides depend on hydrophobic matching between the peptide length and the membrane thickness, i.e., that longer peptides are more tilted than shorter peptides [3,4]. We concluded that in DMPC/lyso-MPC membranes, the peptides were able to form stable pores (see figure). In the present work, we have studied the antimicrobial peptide MSI-103 (also called KIA21) using solid-state ^{31}P-, ^{15}N- and ^{2}H-NMR in the pore state more in detail. The minimum concentration of peptide and lyso-lipid for pore formation was determined, and the effect of charges on the normal lipid or the lyso-lipid was studied. Pore formation is more favourable for this cationic peptide in the presence of anionic lyso-MPG than for neutral lyso-MPC. In the pore, the peptide orientation is such that Lys and Gly residues point into the pore. The structure of the pore did not change as a function of charge or acyl chain length of the lyso-lipids.



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Polymer-Encapsulated Nanodiscs for Membrane Biophysics

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Styrene/maleic acid (SMA) and diisobutylene/maleic (DIBMA) copolymers are attracting great interest because they solubilise membrane proteins and lipids to form polymer-encapsulated nanodiscs (Knowles et al. *J. Am. Chem. Soc.* **2009**, *131*, 7484; Orwick et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 4653; Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919). These nanodiscs retain a lipid-bilayer core that is surrounded by a polymer rim and can harbour a membrane protein or a membrane-protein complex. In contrast with other bilayer-based membrane mimics, polymer nanodiscs directly extract membrane proteins and surrounding lipids from cellular membranes, thereby retaining not only the overall bilayer architecture but also the lipid composition of the native membrane environment (Dörr et al. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 18607). Moreover, polymer nanodiscs retain vesicle-like lipid order, dynamics, and hydration to a greater extent than other membrane mimics (Vargas et al. *Nanoscale* **2015**, *7*, 20685; Cuevas Arenas et al. *Nanoscale* **2016**, *8*, 15016; Grethen et al. *Sci. Rep.* **2017**, *7*, 11517; Oluwole et al. *Langmuir* **2017**, *33*, 14378) and have even been claimed to preserve membrane asymmetry.

In this presentation, I will focus on recent findings that demonstrate fast collisional exchange of lipid molecules among polymer nanodiscs (Cuevas Arenas et al. *Sci. Rep.* **2017**, *7*, 45875; Grethen et al. *J. Membr. Biol.* **2018**, in press). This extraordinary feature means that these nanodiscs represent highly dynamic equilibrium rather than kinetically trapped membrane mimics, which has important implications for studying protein/lipid interactions in this new kind of membrane mimic. However, our finding of rapid and large-scale lipid transfer also implies that membrane asymmetry is unlikely to be retained in polymer-encapsulated nanodiscs.

Transient asymmetry in biomembranes due to association of small molecules. Methods for its characterization and relevance for membrane permeability

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The non-equilibrium distribution of drugs and biological ligands in cells and organisms leads to a preferential interaction with one of the bilayer leaflets generating a transient asymmetry in the lipid bilayer. The extent of this asymmetry depends on the rate of translocation of the solute through the membrane which is in turn affected by the properties of the asymmetric membrane. The interplay between those features is of high relevance for the bioavailability and toxicity of solutes, with fast translocation leading to a higher bioavailability and smaller toxicity.
In this presentation, current methods available to characterize the rate of translocation through lipid bilayers are reviewed, both from Molecular Dynamics simulations and experimental approaches. Strengths and limitations of the distinct methods will be discussed and some examples will be given, from literature[1-4] as well as unpublished results.

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Inter-Leaflet Coupling of Lipid Driven Nanodomains

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Presence and biophysical properties of lipid nanodomains in cellular plasma membranes have been intensively discussed in the past. We have shown recently that lipid driven nanodomains exist even in binary 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)/sphingomyelin (SM) and ternary DOPC/cholesterol (Chol)/SM lipid mixtures that should be homogeneous according to published phase-diagrams. To reveal and characterize these nanodomains we have developed and used a method b ased on the analysis of time-resolved fluorescence decays by Monte-Carlo simulations [1-2]. This method is unique and powerful in the sense that it can determine sizes and concentrations of the nanodomains down to 2 nm and also enables studying inter-leaflet coupling of these nanodomains. In this contribution, it will be shown on several examples that such lipid-driven nanodomains that have more than 20 nm in diameter are inter-leaflet coupled. Interestingly, registering of the nanodomains was revealed for a great variety of lipid compositions including bilayers containing so diverse molecules as ganglioside GM_1 or oxidized phospholipids. Therefore, it seems likely that bilayers which are symmetric in composition are also symmetric in terms of inter-leaflet organization of the nanodomains.

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Abstracts of Posters

(in alphabetical order)

Molecular dynamics of energy-coupling factor transporters in bacterial membranes

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Energy-coupling factor (ECF) transporters of the ATP-binding cassette transporter family provide prokaryotes with essential micronutrients such as water-soluble vitamins, their precursors, and transition metal ions.[1] The overall structure of ECF transporters consist of a substrate-binding S component and an ECF module. ECF transporters use the energy that is released by ATP hydrolysis to transport substrates into cells. The most remarkable feature of the structures is the orientation of the S components in the complexes. The solitary S components adopt an orientation perpendicular to the membrane plane while in the ECF complexes, the S components have 'toppled over' by almost 90 degrees and the transmembrane α-helices are approximately parallel to the membrane plane.[2,3] To obtain structural details of the ECF transporter complexes and identify the transport mechanism, we use coarse grained molecular dynamics (CG-MD) simulations. Using CG-MD simulations, we have obtained microscopic views on the specific interactions of the complex with the lipid environment. In particular, we have deciphered the S component binding and unbinding pathways in various lipid compositions and we systematically investigated how the bilayer properties affect those processes. Taken together, the findings from the CG-MD simulations have enabled us to gain an unprecedented microscopic insight into structural flexibility, organization of the lipid environment and its effect on the transport mechanism of ECF transporters.

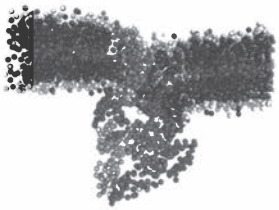

Figure 1. ECF module in a bacterial membrane. The curved structure of the membrane assists the S-component binding to the ECF module.

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Co-spreading of anionic phospholipids with peptides of the structure $(KX)_4K$ at the air-water interface

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Mixtures of anionic phospholipids (PG, PA, PS, and CL) with cationic peptides were co-spread from a common organic solvent at the air-water interface. The compression of the mixed film was combined with epifluorescence microscopy or infrared reflection adsorption spectroscopy (IRRAS) to gain information on the interactions of the peptide with the different lipids. To evaluate the influence of the amino acid X of peptides with the sequence $(KX)_4K$ on the binding, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) was mixed with different peptides with increasing hydrophobicity of the uncharged amino acid X. The monolayer isotherms of DPPG/$(KX)_4K$ mixtures show an increased area for the lift-off due to incorporation of the peptide into the liquid-expanded (*LE*) state of the lipid. The surface pressure for the transition from *LE* to liquid-condensed (*LC*) state is slightly increased for peptides with amino acids X with moderate hydrophobicity. For the most hydrophobic peptide $(KL)_4K$ two plateaus are seen at a charge ratio PG to K of 5:1 and a strongly increased transition pressure is observed for a charge ratio of 1:1. Epifluorescence microscopy images and infrared spectroscopy show that the lower plateau corresponds to the *LE-LC* phase transition of the lipid. The upper plateau is connected with a squeeze-out of the peptide into the subphase. To test the influence of the lipid headgroup structure on peptide binding $(KL)_4K$ was co-spread with different anionic phospholipids. The shift of the isotherm to larger areas for lift-off and to higher surface pressure for the *LE-LC* phase transition was observed for all tested anionic lipids. Epifluorescence microscopy reveals the formation of *LC*-domains with extended filaments indicating a decrease in line tension due to accumulation of the peptides at the *LC*-domain boundaries. This effect depends on the size of the headgroup of the anionic phospholipid.

Ref.

Hädicke A, Schwieger C, Blume A (2017) Cospreading of Anionic Phospholipids with Peptides of the Structure (KX)4K at the Air–Water Interface: Influence of Lipid Headgroup Structure and Hydrophobicity of the Peptide on Monolayer Behavior. *Langmuir* **33**, 12204-12217

Patchy synthetic vesicles by polymer/polymer phase separation

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The design of soft membrane structures with different physical-chemical properties on its surface is important for the creation of novel functional materials. In Nature, surface pattern and topologies are correlated to a precise function. For example, the phospholipids and proteins that form the virus capsid and envelop are organised in clusters and this organisation promote the cell internalisation [1,2]. Using this bio-inspired approach, it is possible to engineer polymeric vesicles that are capable to self-assemble into domains on the surface. Recently, Lo Presti and colleagues [3] show that mixing two polymersome-forming copolymers leads to the formation of patchy/hybrid vesicles characterised by different domains onto their surface. When the two different amphiphilic copolymers differ in molecular mass and properties, their separation on the polymersome surface causes curvature instabilities and therefore the emergence of topographical features [3,4]. This separation leads to the formation of asymmetric polymersomes over time. In light of these previous studies, shorter PMPC-PDPA copolymers were used to investigate the formation kinetics and morphology of the patchy polymersome. The two copolymers investigated are $PMPC_{12}$-$PDPA_{35}$ (MW 11.3 kDa) and $PMPC_{6}$-$PDPA_{17}$ (5.7 kDa) in mixture with PEO_{16}-PBO_{22} (2.2 kDa). Nine different molar ratios between PMPC-PDPA/PEO-PBO were investigated for each PMPC-PDPA copolymer staring from 10:90 to 90:10. Three different time points (i.e. 1, 7 and 14 days) were sampled and analysed using TEM.

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Collisional lipid transfer among DIBMA-bounded nanodiscs

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Amphiphilic copolymers of styrene/maleic acid (SMA) [1] or diisobutylene/maleic acid (DIBMA) [2] can solubilise membrane proteins and surrounding lipids directly from artificial and biological membranes to assemble into polymer-bounded nanodiscs. Although the latter preserve a lipid-bilayer core, they are much more dynamic than other membrane mimics. Unlike other bilayer systems, they exchange lipids not only by monomer diffusion but also by fast collisional transfer, as we demonstrated recently for nanodiscs bounded by SMA(3:1) [3]. The latter is an aromatic and rather hydrophobic copolymer and, thus, is relatively harsh toward the lipid bilayer core. By contrast, aliphatic DIBMA perturbs the bilayer core to a much lesser extent [4] but has a higher negative charge density due to its high maleic acid content.
By using time-resolved Förster resonance energy transfer (FRET) spectroscopy, we quantified the lipid-transfer kinetics among DIBMA-bounded nanodiscs (DIBMALPs). Moreover, we demonstrated the role of Coulombic repulsion and the role of nanodisc diameter in the transfer kinetics. Our experiments show that lipid transfer among DIBMALPs is relatively slow, with diffusional and collisional rate constants being several orders of magnitude slower than among SMALPs. Moreover, the kinetics of lipid transfer kinetics increases dramatically with the ionic strength of the solution. Although collisional transfer is slowed down by polyanionic DIBMA, lipids still exchange orders of magnitude faster than among MSP-bounded nanodiscs or vesicles. In summary, DIBMALPs are dynamic equilibrium rather than kinetically trapped assemblies that exchange lipids on the timescale of minutes to hours.

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Asymmetric Detergents and Their Structural Effects on Human β_2 Adrenergic Receptor Stability

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Integral membrane proteins (IMPs) comprise nearly 30% of most proteomes and facilitate material transport and signal transduction across cell membranes. Extraction and purification of IMPs using conventional detergents often creates structural denaturation or aggregation, hence there is a persistent need for new amphiphilic "assistants" that can promote solubilization and stabilization of IMPs. Herein we designed and prepared asymmetric di-maltosides with diastereomeric relationship. Depending on stereochemistry, these agents showed significantly different behavior with human β_2 adrenergic receptor (β_2AR), despite of their identical hydrophobicity.

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Sum-frequency scattering from asymmetric liposomes

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We present sum-frequency scattering as a technique to probe transmembrane asymmetry of large unilamellar liposomes in a label-free manner. Sum-frequency scattering is a non-linear optical process that probes vibrational resonances of molecules in non-centrosymmetric environments. These properties make sum-frequency inherently sensitive to transmembrane asymmetry. By using a scattering geometry we can probe the bilayer asymmetry of liposomes prepared by cyclodextrin-mediated lipid exchange. [1] The advantage of this approach is a direct detection of vibrational spectra of unlabelled lipids. Preliminary data on $POPS^{(in)}$:$DPPC^{(out)}$ mixtures shows vibrational resonances in the C-H stretch region originating from asymmetric liposomes (Fig. 1).

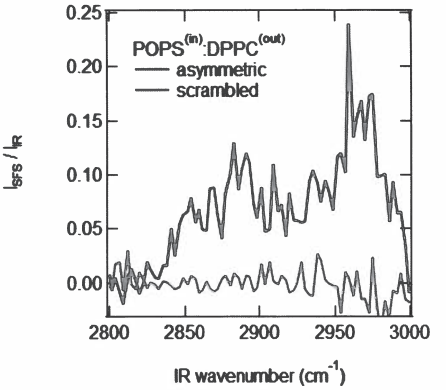

Figure 1: Sum-frequency spectra of the C-H stretch region of asymmetric and scambled liposomes.

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Lipid specificity of surfactin interaction with plant plasma membrane

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The lipid specificity of the interaction is a key factor for the detailed understanding of the penetration and / or activity of lipid-interacting molecules and the mechanisms of certain diseases. Further research in this area is expected to enhance drug discovery and the development of membrane-active molecules for many areas such as health, plant protection or microbiology.
In this poster, some "*in vitro*" and "*in silico*" complementary biophysical techniques useful to obtain information on the specificity of lipids on a molecular scale will be exposed. The approach used will be illustrated by a study carried out on a cyclic lipopeptide, surfactin, which has properties that elicit the plant's defense mechanisms [1].

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Fungicidal lipopeptides: Synergistic action as a mechanism of target membrane selectivity

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Bacterial lipopeptides of the surfactin, fengycin, and iturin families are used as biological fungicides for crop protection. Their activity involves the permeabilization of the cellular membrane of the target pathogen. Depending on the nature of the target membrane, they have been shown to synergize or antagonize with each other, with other fungicides, or with simple detergents. For a detailed, mechanistic understanding of these interactions, we have studied the permeabilizing effect of combinations of these agents against liposomes of different lipid compositions. The fluorescence lifetime based leakage assay based on time correlated single photon counting experiments was utilized to characterize in detail the efflux of calcein from these liposomes.

The types of concerted action are discussed on the basis of the specific modes of action of the individual agents. First, this opens a new avenue to improving the activity of membrane-active, antimicrobial agents for crop protection and, possibly, medical use. Second, target dependent, mutual enhancement or inhibition is discussed as a new mechanism governing target selectivity of antimicrobial agents. It is easy to kill cells in a petri dish (you recall: so does a handgun), so being able to fine tune selectivity is the key to many applications.

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Interleaflet coupling in asymmetric membranes: protocols and revelations

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In the life of cells, the many functional roles of the plasma membrane require tight regulation of properties including structure and dynamics. These properties depend on the lipid composition of the membrane, so that differences in the functions of the two membrane leaflets are best served by an asymmetric lipid distribution. In addition to exposing certain types of lipids to the extracellular or intracellular spaces, the transbilayer lipid distribution also affects the properties of the membrane itself. How these effects contribute to normal cell function remains a largely open question. To address this question we have developed both in vitro and in silico protocols for building asymmetric model membranes and investigating the dynamics, energetics, and structural consequences of interleaflet coupling. For a set of compositionally asymmetric extruded liposomes containing DPPC and different lower melting temperature lipids we have obtained ESR data on the acyl chain order and rotational diffusion of the lipids in each bilayer leaflet. Joint analysis of a large set of small-angle neutron and x-ray scattering data further reveals the structural consequences of the asymmetric distribution of DMPC in a POPC bilayer. To interpret and better understand the experimental observations, we developed a new protocol for constructing tension-free asymmetric bilayers for MD simulations, and used it to simulate the experimentally measured membranes and validate the simulation conditions. An estimate of the elastic energy of mixing in the asymmetric membranes was obtained from simulations of asymmetric and corresponding symmetric bilayers by direct comparison of the resulting elastic properties (including bending rigidity and area compressibility) of the individual bilayer leaflets.

Preparation of Asymmetric Liposomes using a Phosphatidylserine Decarboxylase

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Lipid asymmetries between the outer and inner leaflet of the lipid bilayer exist in virtually all biological membranes. Although living cells spend great effort to adjust and maintain these asymmetries, little is known about the biophysical phenomena within asymmetric membranes and their role in cellular function. One reason for this lack of insight into such a fundamental membrane property is the fact that the majority of model-membrane studies have been performed on symmetric membranes. Only rather recently, several methods have been established for preparing liposomes and other types of model membranes with asymmetric membrane compositions. Lipid exchange methods, for example using cyclodextrins, are particularly suitable to establish a minor, outside-only component. Several rounds of donor–acceptor exchange can also allow for an almost complete replacement of the outer leaflet lipid. However, obtaining minor inside-only components, as it is the case for phosphatidylserine (PS) in mammalian membranes, would require a specific removal of this component from the outer leaflet. This can be a challenging task for an unspecific lipid shuttle such as cyclodextrin, especially when complex membrane compositions are required.

Our aim is to overcome this problem by employing a targeted, enzymatic reaction to specifically prepare PS asymmetric liposomes. Those can be used for studying the effects of PS asymmetry on transmembrane proteins or utilized as model systems for eukaryotic plasma membranes in general.

For this purpose, we use a recombinant version of a water soluble phosphatidylserine decarboxylase (PSD) from *Plasmodium knowlesi* which selectively decarboxylates PS and converts it into PE.
This assay presents a straightforward and easy approach to prepare stable PS asymmetric liposomes mimicking the natural lipid asymmetry of eukaryotic plasma membranes. In addition, the use of a highly specific enzyme carries the advantage that the lipid composition of the lipid bilayer is not a limiting factor in the outcome of the experiment. This allows for the preparation of PS asymmetric vesicles in the presence of various lipid mixtures, for example also the use of cholesterol.

Synthesis and Physicochemical Characterization of Asymmetrical Glycerol Diether Bolalipids

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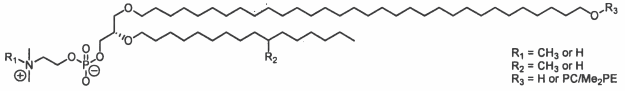
Bipolar lipids (bolalipids) are composed of two hydrophilic headgroups connected by one or two long hydrophobic alkyl spacer. This special class of phospholipids originates in membrane lipids of certain species of Archaea. These archaeal membranes can withstand harsh living conditions, such as high temperatures or low pH-values. Their outstanding stability can be explained by the chemical structure of the archaeal membrane lipids: the hydrophobic alkyl chains are connected by ether linkages in the inverse *sn*-2,3 configuration to the glycerol backbone; and these alkyl chains may contain a variable number of cyclopentane rings and/or several methyl branches in an isoprenoid substitution pattern. During the last years, our group synthesized various archaeal model lipids including symmetrical diglycerol tetraether lipids,[1,2] symmetrical single-chain bolalipids,[3,4] and asymmetrical single-chain bolalipids.[5] The general question that we aim to answer is: *How does the chemical structure of a bolalipid influences its aggregation and phase behavior?*

Here, we will present a novel type of bolalipids, namely asymmetrical glycerol diether lipids. These bolalipids contain a long, membrane-spanning C32 alkyl chain in the *sn*-3 position, a short C16 alkyl chain in the *sn*-2 position—which carries a methyl branch in the 10-position in some cases—, and a phosphocholine (PC) or a protonable phosphodimethylethanolamine (Me_2PE) headgroup in the *sn*-1 position of the glycerol moiety. Furthermore, the long C32 chain contains a small hydroxy group or a PC and a Me_2PE headgroup, respectively, at the ω-position. This leads to a set of 5 different asymmetrical bolalipids with an overall conical shape of the molecule.



We will present the synthesis of these novel asymmetrical bolalipids as well as the temperature-dependent aggregation behavior of these lipids in aqueous suspensions using DSC, FTIR spectroscopy, TEM, and X-ray scattering.

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Curvature Induced Coupling in Asymmetric Lipid Vesicles

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The effect of intrinsic lipid curvature (J0) was studied on structural properties of asymmetric vesicles composed of palmitoyl-oleoyl-phosphatidylethanolamine (POPE; J0 < 0), and palmitoyl-oleoyl-phosphatidylcholine (POPC; J0 ~0). Cryo-electron microscopy and dynamic light scattering were used to evaluate vesicle size and morphology. Furthermore, X-ray and neutron scattering combined with calorimetric measurements yielded insights into leaflet-specific lipid packing and melting processes. Strong transmembrane coupling in asymmetric vesicles with an inner leaflet composed of POPE and an outer leaflet enriched in POPC was observed below the lipid melting temperatures. This was shown by lipids melting cooperatively in both leaflets and a rearrangement of lipid packing in both layers. In contrast, no coupling was observed in vesicles with POPC inner bilayer leaflets and enriched POPE outer leaflets. In this case, the leaflets melted independently and did not affect each other's acyl chain packing. Additionally, no evidence for transbilayer coupling was found above the melting temperature of both systems regardless of the leaflet distribution of POPE. These findings are consistent with the energetically preferred location of POPE residing in the inner leaflet, where it is also located in natural membranes, most likely causing the coupling of both leaflets. The loss of this coupling in the fluid bilayers is probably the result of entropic contributions.
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Permeation of Amphiphilic Drug-like Molecules through Symmetric and Asymmetric Lipid Bilayers – A Molecular Dynamics Simulation Study

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Permeation through biomembranes is ubiquitous for drugs to reach their active sites. Membrane asymmetry of cell plasma membrane (PM) has an important role in this process. [1] Here we describe the interaction of an homologous series of NBD-labeled amphiphiles (NBD-Cn, n = 4 to 16) with lipid bilayers of different compositions, including an asymmetric bilayer. No significant effects caused by membrane asymmetry were observed. Technical details about systems setup will be discussed.

The interactions of NBD-Cn with the lipids at different membrane depths were characterized by Umbrella Sampling (US) simulations. [2] In pure POPC bilayers, the polar NBD group interacts with the lipid by H-bonds even when deeply located in the non-polar bilayer core. The presence of sphingomyelin (SpM) and/or cholesterol (Chol) leads to a decrease in those in-depth interactions, reflecting the higher bending stiffness of these membranes. Inside the POPC membrane, the NBD group tends to orient its dipole moment anti-parallel to the membrane dipole potential.

The free energy profile of NBD-Cn at different depths in the membrane was obtained from the US simulations using the WHAM method. All cases showed a free energy minimum when the NBD is in the lipid head group region. The transition state for permeation corresponds to the polar NBD group located in the non-polar center of the lipid bilayer. However, while at z = 0 a maximum is observed for pure POPC, a local minimum is observed for SpM and/or Chol enriched membranes. Quantitative matching between experimental and simulation data is hampered by the pre-exponential factor to convert free energy barriers to rate constants, smaller by orders of magnitude than that used in the absolute rate theory (TST).

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Intrinsic lipid curvatures from global X-ray scattering data analysis of inverted hexagonal phases

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Intrinsic curvature J_0 is a property of lipids, which describes the bending radius of unstressed monolayers. It is an important parameter in the calculation of interfacial energies [1], which affect lipid/protein interactions [2] or interleaflet coupling in asymmetric lipid membranes [3]. Intrinsic lipid curvatures are frequently determined using small-angle X-ray scattering (SAXS) experiments on inverted hexagonal phases (H_{II}) of dioleoyl phosphatidylethanolamine (DOPE) templates with various amounts of non-H_{II} forming host lipids (see e.g. [4]). A basic assumption of this approach is a linear addivity of the individual J_0 values. Due to diverse lipid headgroup interactions this may not be justified, however. Moreover, temperature effects or repulsive interactions between lipids rapidly decrease the scattering signal of H_{II} phases adding another limitation to this approach. In order to seek for a method to circumvent these issues we explored a full q-range SAXS data analysis method that also takes into account experimentally observed diffuse scattering contributions. Further, we included a non-linearity term for lipid mixtures based on the effective lipid headgroup size. This allows to retrieve intrinsic curvatures from weakly ordered H_{II} phases and minimizes the amount of samples required. We will discuss first results obtained upon application of our technique.

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Structural dynamics of the bacterial membrane protein Mistic

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Mistic from Bacillus subtilis is an α-helical membrane protein essential for biofilm formation [1]. It self-inserts into the membrane and has an unusual hydrophilic surface [2]. Hence, it is of great interest to investigate how such a highly polar protein resides in a hydrophobic environment and how different properties of the environment affect protein structure and dynamics. When Mistic is solubilized in detergent micelles, its stability is significantly improved in micelles that allow for more effective hydrophobic burial in the micellar core and by polar interactions with ionic detergent headgroups [3]. Here, we studied the structural dynamics of Mistic in different membrane-mimetic environments using time-resolved fluorescence spectroscopy on the lone tryptophan residue of Mistic. We combined different approaches including collisional quenching, time-resolved emission spectra, and time-resolved anisotropy. Thereby, we show that Mistic is a malleable membrane protein with its stability, conformation, and dynamics being responsive to changes in effective hydrophobic thickness and headgroup hydration of the membrane-mimetic system.

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Extemporaneous attempts at asymmetric vesicles

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Along the years, and for a variety of purposes, we have devised methods for the induction of asymmetry in lipid vesicles. These procedures include the asymmetric incorporation into liposomal membranes of: (a) glycosyl phosphatidylinositol (GPI), giving rise to a series of studies of GPI degradation by lipases, e.g. GPI-specific phospholipase D, its inhibition by cholesterol and gangliosides, (b) insulin-mimetic inositol phosphoglycan-like molecules from grass pea (*Lathyrus sativus*) seeds; (c) gangliosides, into sphingomyelin-containing vesicles, leading to the first description of lipid flip-flop motion induced by ceramides (formed *in situ* by sphingomyelinase); (d) *in situ* lipase-generated lipids, such as diacylglycerol and/or ceramide, causing vesicle-vesicle fusion through the concerted action of phospholipase C and sphingomyelinase. We have also described the electrogenic preparation of asymmetric giant unilamellar vesicles, both right-side out and inside-out, formed from erythrocyte ghost membranes in buffers containing physiological concentrations of salt. The various methods and results will be reviewed, compared and discussed.

Role of Coulombic repulsion in collisional lipid transfer among SMA(2:1) nanodiscs

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Styrene/maleic acid (SMA) copolymers are attracting great interest because they are able to solubilise membrane proteins and lipids from native or artificial membranes to form polymer-bounded nanodiscs [1]. These nanodiscs preserve a native-like lipid-bilayer core that is surrounded by a polymer shell and can harbour a membrane protein or a membrane-protein complex. SMA exists in various styrene/maleic acid molar ratios, which results in different charge densities, hydrophobicities, and thus, solubilisation properties. We have recently reported fast collisional lipid transfer among nanodiscs bounded by the relatively hydrophobic copolymer SMA(3:1) [2]. Herein, we employed time-resolved Förster resonance energy transfer to quantify the kinetics of lipid transfer among nanodiscs encapsulated by SMA(2:1), a less hydrophobic copolymer that is more efficient in terms of lipid and protein solubilization [3]. Furthermore, we assessed the role of ionic strength and, thereby, how Coulombic repulsion affects the transfer of lipid molecules among these polyanionic nanodiscs. Collisional lipid transfer is slower among SMA(2:1) nanodiscs (k_{col} = 5.9 M^{-1} s^{-1}) as compared with SMA(3:1) nanodiscs (k_{col} = 222 M^{-1} s^{-1}) but still four to five orders of magnitude faster than diffusional lipid transfer among protein-encapsulated nanodiscs or vesicles. Increasing ionic strength further accelerates lipid exchange among SMA(2:1) nanodiscs in a manner predicted by either the Davies equation, an empirical extension of the Debye–Hückel limiting law, or a modified form of the Debye–Hückel law that accounts for the finite size of nanodiscs.

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Natural ER membrane interaction of COPII

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Organelle membranes are key to cell compartmentalisation. In addition, different organelle membranes (e.g. endoplasmic reticulum (ER)- or plasma membrane) function for example as receptor platforms and signal propagators, sites of lipid synthesis or starting points for vesicle formation. As the complex environment of the cell restricts accessibility, it would be advantageous to make organelle membranes directly accessible for a wide range of biophysical investigations. One of these organelle membranes that are hard to access is the ER, which has a multitude of tasks. Among others, the ER membrane is the source of transport vesicles which deliver proteins and lipids to other organelles. Vesicle formation is mediated by the coat protein complex COPII, where the GTPase Sar1p binds GTP, supported by the ER-located membrane protein and nucleotide exchange factor Sec12. After binding of Sar1, additional COPII proteins are recruited and subsequently induce membrane curvature to finally form vesicles. Although many studies have addressed the mechanism, there are still unanswered questions as to how fission occurs. It is not fully understood which further factors regulate the process and which role membrane composition may play. To get closer to an answer, we introduce a model system that takes the complexity of the ER membrane and its content of transmembrane proteins into account while being directly accessible for biophysical investigations.

Consequences of membrane pressure asymmetry due to one-sided insertion of molecules

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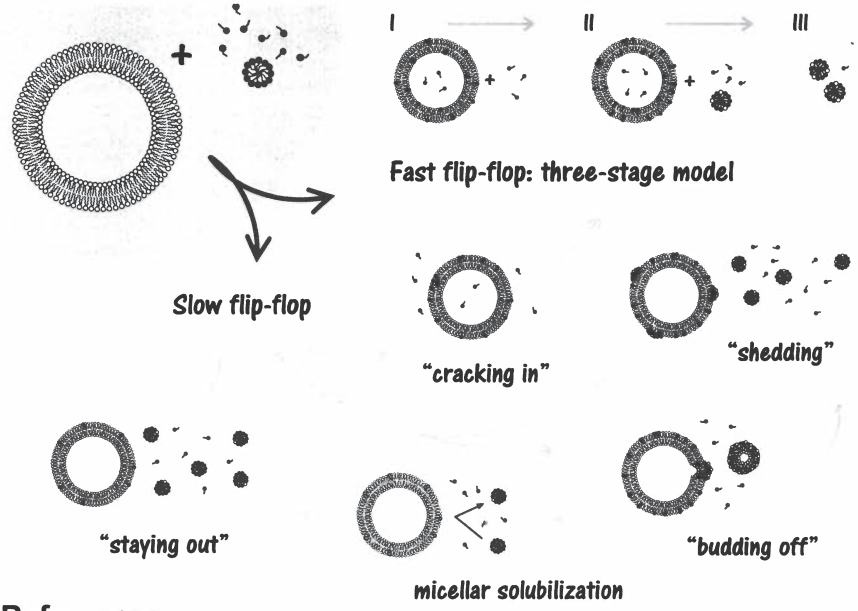
One-sided insertion of, e.g., amphiphilic peptides or surfactants increases the lateral pressure in the lipid leaflet of insertion. If the amphiphile or another membrane constituent is not able to balance the difference by partially flipping to the other lipid leaflet, a pressure asymmetry stress builds up. Depending on the nature of the additive, this stress may at a threshold value cause a transient membrane failure ("cracking in") [1], or it may reduce or stop further insertion to the overpopulated leaflet (staying out) [2,3] or promote insertion of another molecule into the underpopulated leaflet. Finally, the budding of very small vesicles can reduce stress to a certain extent.



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Antimicrobial Selectivity and Membrane Leakage Mechanisms

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Synthetic mimics of antimicrobial peptides (smAMPs) are promising alternatives to classical antibiotics because they are less prone to resistance. Like natural antimicrobial peptides (AMPs), smAMPs are thought to act on the cell membrane, but offer the advantage of better stability and easier production. Common to all antimicrobial treatment is the need for selectivity.

A series of short polymeric smAMPs with antibacterial gram-selectivity [1] is discussed exemplarily. Sophisticated analysis of vesicle leakage mechanisms [2] and kinetics reveals how the mode of action changes with the combination of smAMP design and lipid mixture.

We find that antimicrobials that seem to act by asymmetric binding via leakage upon cracking-in followed by annealing might be less selective and more haemolytic. Asymmetry stress tends to affect lipid membranes faster than other leakage mechanisms. It is mediated by high fractions of hydrophobic molecular surface in the smAMP. Surprisingly, the strength of leakage induced by asymmetry stress can increase with increasing charge in the smAMP and lipid layer. Conversely, an appropriate balance of electrostatically and hydrophobically mediated smAMP binding seems to be needed for selective antimicrobial action (possibly stabilization of local curvature). Selectivity is observed to increase by additional rare and relatively strong leakage events that seem to require assembly of smAMPS or lipids. A systematic view on membrane leakage mechanisms, antimicrobial activity and selectivity will aid future design of antimicrobials and improve membrane models for *in vitro* studies.

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Temperature dependence of fluorinated-surfactant micellization

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Membrane proteins (MPs) are crucial for plentiful physiological processes of the cell. This makes the comprehension of their structure paramount for, among others, the pharmaceutical industry, as MPs represent more than 60 % of drug targets. However, compared with soluble proteins, only a small number of high-resolution structures of MPs are available. This is rooted in their embedment in the lipid bilayer, which hampers the accessibility of MPs to biophysical investigation [1]. Therefore, it is essential to extract MPs from their native membrane. While canonical detergents, due to their solubilizing nature, are well-suited for the extraction of MPs, they are often invasive towards the structure of the MP. Therefore, new approaches have emerged to combine detergent capability for membrane extraction with a milder effect on MPs to form membrane-mimetic systems for the study of MPs [2]. Fluorinated surfactants are one of the compounds which were designed for this task. The bulky, fluorinated carbon tail of these molecules renders them both hydrophobic and lipophobic, resulting in poor miscibility with hydrocarbon chains. Therefore, fluorinated surfactants are less invasive towards extracted MPs compared with hydrocarbon-based surfactants, rendering their micelles promising membrane-mimetic systems [3]. To gain detailed insight into the molecular mechanisms underlying the mildness of fluorinated surfactants toward MPs, we characterized their micellization behavior by several calorimetric techniques. In particular, we studied the temperature-dependent properties of micellization of a homologous series of fluorinated surfactants and their hydrogenated analogs. Isothermal titration calorimetry was utilized to investigate the temperature dependence of their critical micellar concentration, as well as the Gibbs free energy and the enthalpic and entropic contributions to micellization. Furthermore, we employed differential scanning calorimetry to explore micellization of the surfactants and the associated heat capacity changes over a wide temperature range. Finally, pressure perturbation calorimetry was applied to compare volume and expansivity changes of the micelle formation of fluorinated and hydrogenated surfactants.

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Quantification of membrane asymmetry by continuous wave electron paramagnetic resonance spectroscopy

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A fundamental feature of biological membranes is the unequal distribution of different lipid species between the outer and the inner leaflets of the membrane double layer. For example, in mammalian cell membranes PC and SM lipids mainly reside in the outer leaflet, whereas PE, PS, and PI predominantly populate the inner leaflet. The underlying physical phenomena of membrane asymmetry and their functional consequences are still poorly understood. This creates high demand for well-defined model systems as well as for analytical methods that render membrane asymmetry accessible to biophysical investigation. Here, we employ the cyclodextrin–lipid exchange method to incorporate POPG into the outer leaflets of spin-labelled POPC large unilamellar vesicles (LUVs). Then, we utilize continuous wave electron paramagnetic resonance (cwEPR) spectroscopy to quantify the outer-leaflet fraction of spin-labelled POPC that was replaced by POPG. This enables us to determine the outer-leaflet content of POPG after POPG–POPC exchange and to confirm lipid asymmetry of LUVs in a single experiment. We validate the POPG–POPC asymmetry by the use of zeta potential measurements, which exclusively detect the negatively charged POPG in the outer leaflet of LUVs.

Physics of membrane fusion revealed by colloidal probe microscopy

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Membrane fusion of lipid bilayers is prevented by substantial energy barriers arising from removal of the hydration shell, formation of highly curved structures and eventually fusion pore widening. We measured the impact of normal and tensile forces on the fusion efficiency of two bilayers in the presence of SNAREs (soluble N-ethylmaleimide-sensitive-factor attachment receptor). Force-dependent lifetime of fusion intermediates were measured by means of membrane coated silica spheres serving as force probes in contact with supported lipid bilayers. Analysis of time traces obtained from force clamp experiments allowed us to assign different intermediate states during fusion. Force-dependent lifetime distributions of the various fusion states pave the way to determine the free energy barriers related to the passing of the hydration barrier, hemifusion and full fusion, the area of the transition state and the zero-force lifetime of intermediates in SNARE-mediated membrane fusion. We also investigated the tension-dependency of fusion using two different strategies. One is based on dilatable supported bilayers and the other one employing sessile giant liposomes. In both approaches fusion efficiency increases considerably with lateral tension and we identified a threshold tension at which the number of fusion events is increased substantially.

Adsorption of calcium ions on curved lipid bilayer

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While calcium interactions with lipid membranes have been studied for decades, its adsorption at curved membranes is still little understood [1]. In the presented work we combine fluorescence spectroscopy with computer simulations to get a molecular details of the association of calcium ions with model POPC membranes. We found a qualitative difference in the effects of calcium adsorption in the regions of lipid carbonyls and phosphates for differently curved lipid bilayers. At the positively-curved leaflet of small liposomes larger changes are detected at the level of lipid phosphates, while in large vesicles the effect at lipid carbonyls dominates. These findings satisfactorily match the molecular mechanism of calcium adsorption observed in molecular dynamics simulations. Calculations of the mean force potential show that the Ca^{2+} binding to the positively curved side of the bilayer is significantly stronger compared with that to a flat membrane [2]. Our findings can be of great importance for neuronal signaling and fusion of synaptic vesicles, which cannot be faithfully modeled with flat model membranes.

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Preparation of Asymmetric Liposomes - Mimicking the Inner Plasma Membrane of *Escherichia Coli*

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The lipid composition of inner and outer leaflet of naturally occurring plasma membranes usually differs distinctly. This asymmetry is actively maintained, as it is crucial for the function and recognition of the cell. In bacterial membranes for example, PI and PE are preferentially located in the inner leaflet, while PG tends to be in the outer leaflet. Cardiolipin is distributed equally across both leaflets. [1]

With the rise in antibiotic-resistance, understanding the properties of bacterial membranes has garnered special interest: It is key in developing new anti-bacterial agents, targeting the plasma membrane. However, current liposome preparations - often used as membrane mimetic systems - lack asymmetry and are therefore not ideally suited for the task.

We employed a novel method [2] for the preparation of asymmetric liposomes, mimicking the inner plasma membrane of *Escherichia coli*: Using Methyl-β-Cyclodextrin, DPPG was incorporated into the outer leaflet of liposomes composed of POPE and POPC. Asymmetry was confirmed by zeta potential and HPTLC. Stability of the asymmetric nature of the liposomes over a period of several weeks was confirmed by zeta potential.

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A thermodynamic model for native nanodiscs

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Nanodiscs formed by phospholipids and amphiphilic copolymers play an increasing role as membrane mimics for membrane-protein research (1; 2). Following a simplistic concept, such native nanodiscs are described to be a dozen nanometers in diameter and to harbor a lipid-bilayer core surrounded by a thin polymer rim. Reality, however, is more complex, as the size and composition, as well as the bilayer stability of native nanodiscs vary with the types and the concentrations of both lipid and polymer components (2; 3)

Here, we present a quantitative approach that relates nanodisc size and the characteristics of its lipid-bilayer core and polymer rim to the concentrations of lipid and polymer species. This model combines the pseudophase concept frequently applied to canonical lipid/detergent systems (3), with geometrical considerations and Flory–Huggins solution theory. Together with experimental data from various scattering techniques, calorimetry, and ^{31}P NMR, a detailed morphological description of nanodiscs is obtained.

Importantly, we were able to quantify the perturbation exerted by different types and concentrations of polymers on the bilayer structure in nanodiscs, thus allowing for optimization of these parameters to improve the "nativeness" of nanodiscs. These findings help gaining a deeper understanding of nanodisc structures and the underlying thermodynamics to evaluate their potential and limitations as membrane mimics for studying membrane proteins and lipids.

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Heat shock protein Hsp70-1A selectively inserts in dipalmitoyl phosphatidylserine domains and causes membrane blebbing

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Hsp70-1A - the major stress-inducible member of the HSP70 chaperone family – is being implicated in cancer diseases with the development of resistances to standard therapies [1]. In normal cells the protein is purely cytosolic, but in a growing number of tumor cells a significant fraction can be identified on to the cell surface. The anchoring mechanism is still under debate, as conventional signaling sequences for translocation from the cytosol to exoplasmic leaflet of the plasma membrane and common membrane binding domains are lacking. Recent reports propose a lipid mediated anchoring mechanism based on a specific interaction with charged, saturated lipids such as dipalmitoyl phosphatidylserine (DPPS) [2, 3]. Here we prepared planar supported lipid bilayers (SLBs) to visualize the association of Hsp70-1A on the single molecule level by atomic force microscopy (AFM). We compared the binding of the protein to bilayers with 20% DPPS lipid content both in the absence and presence of cholesterol. Hsp70-1A inserted exclusively into DPPS domains and assembled in clusters that reached a critical density upon incubation with 0.5 µg/ml (7nM) of the protein. Higher concentrations introduced membrane defects that originated from cluster centers. In the presence of cholesterol the critical concentration caused the formation of membrane-blebs, which burst at higher concentrations. Our data thus support a previously proposed non-classical pathway for the export of Hsp70-1A by tumor cells and link the DPPS facilitated plasma membrane localization of Hsp70-1A to its involvement in resistance to radiotherapy.

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Membrane interactions of conventional and fluorinated surfactants

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Membrane proteins (MPs) play a vital role in all kind of cells and processes. Therefore, MPs are promising targets for drug development. However, in general, little is known about their structures and mechanisms. This is because they are embedded in lipid bilayers and especially spectroscopic techniques are hindered by the large, formed aggregates. One way to overcome this problem is to extract MPs from their membrane with amphiphilic molecules, such as copolymers or detergents [1]. Detergents are able to solubilize lipid membranes and form mixed lipid–detergent micelles which can accommodate MPs. Additionally to conventional hydrocarbon–based surfactants, fluorinated surfactants have emerged, which contain partially or fully fluorinated carbon chains. Fluorocarbon groups show a bigger cross–section than hydrocarbon groups and additionally, as fluorocarbon and hydrocarbon chains are poorly miscible, fluorinated detergents interact less avidly with lipid bilayers [2]. Hence, fluorinated detergents show less distortion of membrane order and interactions of MPs. This makes them a promising tool to sustain the MPs structure in solution. Here, we present the physicochemical characterization of membrane interactions and biological application of a homologous series of hydrogenated and fluorinated surfactants. We investigated possible surfactant-induced membrane disordering using fluorescence anisotropy decay measurements. With DLS we were also able to show that both series, hydrogenated and fluorinated surfactants, are able to solubilize POPC vesicles, and thus show detergent–like behavior. For the hydrogenated surfactants we could construct pseudophasediagrams to access the thermodynamics of lipid membrane solubilization. Further, we were able to solubilize MPs from native *E. coli* membranes with all tested detergents and quantify the extracted amount via SDS-PAGE.

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A Step Forward in the Design of Liposomes: Symmetric & Asymmetric Vesicles from Lipid Extracts

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Nature's complexity in the design of cellular boundaries represent a challenge in monitoring processes that are located on or associated with the membrane. Liposomes are the method of choice when it comes to studying membrane-peptide or -protein interactions due to the good control regarding composition and the reduced number of component variables. One major drawback in their design is the symmetric architecture and the related lack of controlling different lipid compositions between inner (IL) and outer leaflet (OL). Since biological membranes are highly asymmetric, we compare different techniques for engineering lipid vesicles and point out challenges, opportunities and potential limitations of these design concepts: Symmetric vesicles are prepared by electroformation whereas asymmetric vesicles are generated by a double-emulsion phase. The different fashioned vesicles are either reconstituted from pure DOPC for obtaining vesicles undergoing no phase separation, multi-component mixtures such as DOPC/SM/Chol as host cell mimicry and model for lipid rafts as well as POPE/POPG as inner membrane model of Gram-negative bacteria. An extension from these synthetic lipid species to natural lipid extracts, e.g. *S. cerevisiae*, *E. coli*, and bovine liver and heart build a bridge between the pure model and native cellular systems. Asymmetric vesicles provide a perfect tool in modelling the bacterial outer leaflet by using lipopolysaccharides (LPS) as OL-mimicry and a phospholipid mixture composed of PE/PG/CL for the IL. Both vesicle design concepts are compared regarding size distribution, reconstitution potential in terms of the applied lipid spectrum, introduction of fluorophores, the ability to undergo phase separation and the dominating domain-sizes as investigated by fluorescence and confocal microscopy. Additionally, asymmetric vesicles are used for the determination of lipid flip-flop with respect to the OL-composition. To which extent lipid specificity and interleaflet coupling play a role in phase separation and lipid flip-flop should be elucidated.

Interleaflet Induction and Registration of Membrane Domains under the Loupe of All-Atom MD Simulations

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Biological membranes have been recognized to be heterogeneous in space and time. Phase separated nanodomains are believed to be functionally relevant. However, the forces and mechanisms behind domain formation and interleaflet registration remained elusive so far. Here, we induced different phases (gel or liquid crystalline) in a single component lipid bilayer by coupling parts of the lipids to baths of different temperatures. This methodology allowed us to study both structure and dynamics of domain interleaflet coupling at all-atom resolution on computationally affordable time-scales. Our results support the theoretical prediction that the line tension between the thicker gel and thinner liquid disordered regions is minimal when the spatial positions of the domains in the individual leaflets differ by a few nanometers [1] a result recently also confirmed in atomistic MD simulations of the main phase transition in phospholipid bilayers [2]. Moreover, shorter lipids at the boundary between the gel and liquid crystalline regions decrease the line tension and cause mismatch-free interleaflet domain registration. We also witnessed how a gel phase region in one leaflet induced a fluid-to-gel phase transition in the other leaflet. Our *in silico* observation is in line with *in vitro* experiments (see abstract by Saitov & Pohl).

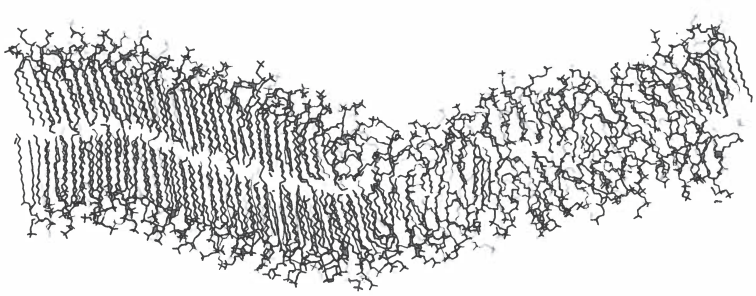

Figure 1. Spatial shift of ordered domains in the two leaflets of a DPPC bilayer. Individual lipids are colored according to their root mean squere fluctuations.

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Domain mirroring in asymmetric lipid membranes

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Biological membranes contain ordered lipid domains (L_o) that are thicker and more densely packed than surrounding disordered membrane domains (L_d). The L_o domains of the two membrane leafs localize in matching positions. In compositionally symmetric membranes, this registration is driven by (i) the line tension between the L_o and L_d regions that is minimal when there is a mismatch of a few nanometer in the alignment of the L_o domains from the two leaflets (Galimzyanov et al., 2015; Galimzyanov et al., 2016) and (ii) membrane undulations, which require stiffer regions from the two leafs with higher splay rigidity to colocalize into areas of lower curvature (Galimzyanov et al., 2017; Horner et al., 2009). For these two mechanisms to work in asymmetric biological membranes, the domains in the outer leaflet have first to induce domain formation in the inner leaflet, since otherwise the latter would be disordered throughout. Here we measured the diffusion constants of fluorescently labeled lipids in the L_o and L_d domains (Horner et al., 2013) of asymmetric planar lipid bilayers by fluorescence correlation spectroscopy. The enslavement of lipids from the inner leaflet into newly triggered L_o domains was accompanied by increasing lipid mobility in the L_o domains of the outer leaflet. In contrast, average lipid mobility in the remaining region of both leaflets decreased - it was no longer homogeneous but contained small, optically-non-discernable domains, which were characterized by an intermediate diffusion coefficient. The observation suggests that friction between opposing L_o and L_d domains (i) slows down both the rotational and the translational motion of lipids in central regions of the L_d domain, thereby forcing them into an ordered state, while (ii) increasing lipid mobility in the L_o domain. This causes fragmentation of the L_o domain in the outer leaflet at its edges. In turn, these fragments induced the formation of new matching L_o domains in the inner leaflet that were equally small in size.

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Influence of the solid support on receptor lipid distribution in model membrane systems

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Phosphatidylinositol phosphates (PIPs) function as receptor lipids for a number of proteins. PIPs differ in their degree of phosphorylation resulting in different net charges. To analyse the interaction of PIP-binding proteins with different PIPs solid supported lipid bilayers are a state of the art system. They are frequently used due to their simple preparation and they allow a controlled bilayer composition. Solid supported membranes can easily be combined with numbers of techniques like atomic force microscopy (AFM), fluorescence microscopy and reflectometric interference spectroscopy (RIfS).

We are focussing the influence of the support on the lipid distribution in the membrane leaflets. Therefore, we utilize two different supported lipid membrane systems. The systems are either lipid bilayers or lipid monolayers which are obtained by spreading vesicles on hydrophilic or hydrophobic silicon dioxide substrates, respectively. Hydrophobic properties are obtained by treating the substrates with Bis(trimethylsilyl)amine (HMDS) to facilitated hybrid membrane formation. To determine the effect of the solid support we investigated the adsorption behaviour of different types of PIP-binding proteins (Ezrin, Collybistin 2 and the epsin *N*-terminal homology domain) on membranes with varying receptor lipid content and net charge.

The comparison of both systems at identical PIP concentrations showed a decreased in protein surface coverage on bilayers determined by AFM. Furthermore, adsorption of all proteins tested to the hybrid membrane system resulted in higher signal intensities in RIfS. These results suggest a reduced amount of receptor lipid accessible in the supported lipid bilayer and can be explained by electrostatic interactions of the charged PIPs with the hydrophilic substrate surface resulting in an inhomogeneous PIP distribution between upper and lower leaflet.

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Gramicidin A in DPhPC bilayer membranes

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Ion channels have long been a hot topic in membrane biology: They are responsible for signal transduction, communication between cells, maintenance of cellular equilibria, and many more [2]. This means their investigation is essential to obtain a deeper understanding of cellular processes. The gramicidin A channels used here are not only part of multiple antibiotics mixtures [1] but are also used as model channels because of their favorable properties such as their stability [1] and straightforward reconstitution. Moreover, the fact that they have been investigated in great detail makes them easy to work with even for beginners. This work focusses on setting up an experimental protocol that enables students to gain easy access to the fundamental aspects of ion-channel dynamics.

In our basic setup protocol, we painted black lipid membranes using 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC)/nonane or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)/nonane solutions. DPhPC membranes proved to be much more stable than POPC membranes, enabling measurements over multiple hours without membrane rupture. Gramicidin A was then reconstituted into the membranes, thus allowing the measurement of ion flow by keeping track of the current, resulting in multiple *I/V* curves. Furthermore, single-channel measurements were performed and analyzed to calculate the average open time of the channels at different voltages. Finally, the effect of capsaicin on the channel open time was examined and studied as a function of additive concentration.

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Albumin-Induced Lysolipid Depletion Enhances Leakage of Thermoresponsive Liposomes

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Thermoresponsive Liposomes (TSLs) are lipid-based drug carriers that allow the controlled release of their content when triggered by mild hyperthermia. While TSLs have been developed since the 1970s [1], only a few formulations have reached the stage of clinical trials. While numerous efforts were made to improve formulations of TSLs, there is only little systematic data on how plasma proteins interact with TSLs and thus improve or interfere with a controlled release of their payload.

A recent development in the formulation of TSLs is the incorporation of lysolipids as release boosters [2]. We investigated the interaction of human albumin (HSA) on lysolipid-containing TSLs (LTSLs) by performing microcalorimetric uptake assays, as well as time-resolved fluorescence-based release experiments.

The uptake assays revealed that the membrane partition coefficient of HSA increases above the transition temperature (T_m) of the LTSLs and correlates positively with the lysolipid content in the liposomes. In the presence of HSA LTSLs also showed an increased release rate above T_m.

This could be explained by HSA depleting the lysolipids in the outer leaflet and hence an increase in bending stress in the membrane of the LTSLs. As lysolipids do hardly flip [3], this prolonged bending stress might increase duration and size of defects, allowing more of the TSLs' content to be released.

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Novel quantitative analysis on the study of external molecules binding to different lipid-receptor species determining hierarchical orders and/or constraints

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In synthetic biology, the power of artificial membrane systems such as giant unilamellar vesicles and supported lipid bilayers has been shown in a variety of studies especially concerning biological processes occurring at or through membranes. They helped in the understanding and postulation of the emerging concept in lipid-driven endocytosis summarizing that the lipid-receptor and protein must display a distinct geometrical structure and binding valency in order to trigger the uptake event. The molecular mechanism is based on the local alteration of the membrane architecture facilitating shape deformations until complex bending into tubular invaginations takes place. Thereby, the determining factors are directly linked to lipid-protein interactions however so far only rare information is given about lipid diversity and matrix environment affecting protein binding. The presented study facilitates for the first time a quantitative approach on the examination of lipid-protein interactions in GUVs. The assay is developed for the calculation of numerous replicates enhancing a statistical evaluation on single lipid-protein events. The design is sensitive enough to calculate binding efficiencies to single lipid-species and their differences based on a fluorescent read-out. This tool not only facilitates the study on none phase-separated GUVs furthermore we are able to process also phase-separated GUVs providing a thorough and robust statistical analysis on the binding efficiencies of proteins to single lipid-species incorporated in a manually controlled membrane composition.

Binding Sar1 to a Lipid Monolayer: Insertion and Orientation studied by Infrared-Reflection-Absorption Spectroscopy

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The small eukaryotic GTPase Sar1 functions in phospholipid bilayer remodeling and vesicle formation as part of the multimeric coat protein complex (COPII) [1,2]. The membrane interaction of Sar1 is strongly dependent on its N-terminal 23 amino acids. By monolayer adsorption experiments and infrared reflection-absorption spectroscopy (IRRAS) we elucidate the role of lipids in inducing the amphiphathicity of this N-terminal stretch, which inserts into the monolayer as an amphipathic helix (AH) [3]. The AH inserting angle is determined and is consistent with the spatial distribution of hydrophobic and hydrophilic amino acid monomers. Using an advanced method of IRRAS data evaluation, the orientation of Sar1 with respect to the lipid layer prior to the recruitment of further COPII proteins is determined [3]. It turns out that the orientation of the protein is not only controlled by the anchorage through the AH, indicating that other interactions between the protein and the membrane exist. Only a slight reorientation of the membrane-bound Sar1 is needed to allow coat assembly. The time-course of the IRRAS analysis corroborates a role of slow GTP hydrolysis in Sar1 desorption from the membrane.

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The effect of lipid composition upon formation of co-existing ordered and disordered lipid domains in the outer leaflet of asymmetric lipid vesicles was assayed using FRET.

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Spontaneous domain formation was observed under many conditions in cholesterol-containing asymmetric vesicles with sphingomyelin (SM) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in their outer leaflet, and any of a variety of unsaturated lipids in their inner leaflet, including unsaturated phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) with dioleoyl or palmitoyl oleoyl acyl chains. However, domain formation was more sensitive to solution conditions in the outer leaflet of cholesterol-containing asymmetric vesicles composed of SM and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in their outer leaflet. A lack of ordered domain formation was observed when the vesicles were under an osmotic pressure gradient, and this was again independent of the nature of the unsaturated lipids in the inner leaflet. Domains were observed in these lipid compositions when the vesicles were not under an osmotic pressure gradient. Domains were also observed when under an osmotic pressure gradient if a transmembrane helix-forming peptide was incorporated in the asymmetric vesicles. Because outer leaflets composed of SM and POPC come closest in structure to what is present in mammalian cell plasma membrane outer leaflets we conclude that the lipids of the plasma membrane are likely to exist at a borderline between being able to spontaneously form ordered domains and not being able to spontaneously form such domains. Even small perturbations may be crucial in driving ordered domain formation in the plasma membrane. Also, because changing the inner leaflet lipids did not greatly affect domain formation, it appears that coupling may not be very dependent upon the structure of the inner leaflet lipids

Flipping Transmembrane Helices

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It is commonly perceived that hydrophobic α-helical transmembrane segments are stable inserted inside the lipid bilayer. Nevertheless, for some membrane proteins it has recently been postulated that they possess transmembrane helices (TMHs), which can change their orientation from a membrane-inserted to a surface-oriented state. To find first experimental proof of this unconventional behavior, we investigated the membrane alignment of two postulated flipping TMHs using a combination of synchrotron radiation circular dichroism (SRCD) and solid-state nuclear magnetic resonance (ssNMR) spectroscopy.

The twin arginine translocase, transports fully folded proteins across cellular membranes driven only by the proton motive force. One of its components, the small membrane protein TatA, possesses an unusually short TMH[1]. We postulate that this TMH plays an important role during translocation. Therefore we analyzed the influence of hydrophobic mismatch and lipid shape on its membrane alignment. In lipid bilayers with a thickness comparable to the length of the TMH, we found that the TatA TMH is inserted essentially upright in the membrane, whereas positive hydrophobic mismatch increased the tilt angle of the TMH, and a strong negative hydrophobic mismatch led mostly to protein aggregation. Remarkably, when reconstituted in lipid bilayers with a high negative spontaneous curvature, the TMH flipped onto the membrane surface, resulting in a stable, flat surface-aligned orientation of the entire TatA protein.

Additionally we investigated the Pinholin $S^{21}68$ which regulates the host cell lysis during the lytic cycle of the bacteriophage 21 and which is supposed to be activated by a flipping of the N-terminal TMH onto the lipid bilayer surface. $S^{21}68$ consists of two TMHs and we investigated its membrane alignment in different lipid systems. We could show for the first time that the N-terminal TMH indeed possesses a high tendency to flip onto the bilayer surface and only in very thick membranes the protein inserts both TMHs into the bilayer.

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Investigation of plasma membrane reorganization during uptake processes

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To be taken up by the host cell, pathogens (viruses, bacteria) and pathogenic products such as toxins must modify the structure of the cell membrane by tuning the membrane composition and/or changing the lipid membrane order and curvature via binding to specific receptors. For these processes, the role of glycosphingolipids as receptors for many human pathogens is of key importance. The glycosphingolipid (globotriaosylceramide (Gb3) is a plasma membrane receptor of Shiga toxin from *Shigella dysenteriae* [1] and the lectin LecA from *Pseudomonas aeruginosa* [2]. The B subunit of Shiga toxin (StxB) and LecA [2] bind specifically to the Gb3 trisaccharide triggering their uptake. Sharing the same receptor, both proteins lead to different events in downstream signaling [3], which can originate from the differences in binding and domain formation already at the plasma membrane. This can be resolved by studying membrane dynamics induced by Gb3-lectin interaction. As the plasma membrane contains a number of other components that can participate in the uptake processes, we elucidated the role of the Gb3 receptor by using artificially prepared membrane systems – giant unilamellar vesicles (GUVs) and supported lipid bilayers (SLBs) containing 5 molar percent of Gb3. Such synthetic membranes were further examined by fluorescence microscopy to visualize protein-induced membrane reorganization. Moreover, to investigate the protein-induced receptor clustering and reveal changes in lipid membrane order, we used environmental sensitive membrane probes in association with fluorescence lifetime imaging microscopy. Our results demonstrate that StxB and LecA cluster Gb3 receptor molecules and reorganize synthetic membranes in a different manner, which may explain also differences in downstream signaling.

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Label-free and charge-sensitive dynamic imaging of lipid membrane hydration

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Biological membranes are highly dynamic and complex lipid bilayers, responsible for compartmentalization, signaling, transport and flow of charge in living cells. Membranes self-assemble in aqueous solutions and without the aqueous environment, membranes cannot exist. It is thus surprising to note that membrane imaging typically neglects water. Indeed, membrane-imaging methods are either geared to measure the fate of membrane-inserted probes and their relation with the membrane, or to probe the resonant response, refractive index contrast or height difference of the hydrophobic core of the membrane. Here, we use second harmonic imaging to follow membrane hydration of freestanding lipid bilayers on millisecond time scales. We show that the non-resonant second harmonic response of water that is aligned by charge-dipole interactions with charged lipids can be used as a label-free probe of membrane structure and dynamics. We report on th e spatial and temporal mapping of membrane structure, membrane dynamics and surface potentials. Probing in real time and space the hydrating water and associated membrane potentials can undoubtedly bring molecular level understanding into diverse membrane processes that involve hydration, such as membrane (inter-leaflet) structuring, specific ion effects, charge dependent protein activity, ion pumps/pore structure and activity, surface acid/base reactions and dynamics of action potentials.

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Formation of lipid-bilayer nanodiscs by diisobutylene/maleic acid (DIBMA) copolymer

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Diisobutylene/maleic acid (DIBMA) copolymer solubilizes membrane proteins and their surrounding lipids into bilayer nanodiscs with mild impact on lipid acyl-chain order and thermotropic phase behavior. Here, we examined th e self-association behavior of DIBMA and its membrane-solubilization properties against lipids of different acyl chain length and saturation by fluorescence spectroscopy, small-angle X-ray scattering, size-exclusion chromatography, dynamic light scattering, and ^{31}P NMR spectroscopy. We found that, although DIBMA is less hydrophobic than commonly used styrene/maleic acid (SMA) 2:1 and 3:1 copolymers, it efficiently formed lipid-bilayer nanodiscs that decreased in size with increasing polymer/lipid ratio while maintaining the overall thickness of the membrane. DIBMA fractions of different molar masses were similarly efficient in solubilizing a saturated lipid. The solubilization efficiency of DIBMA was enhanced by Coulombic screening at elevated ionic strength or reduced charge density on the polymer at low pH. Increasing the acyl chain length or the degree of unsaturation incurred an additional free-energy penalty for transferring phospholipids from vesicular bilayers into nanodiscs. Taken together, these findings serve as guideposts for the application of DIBMA in membrane-protein research.

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How to control domains and asymmetry using flip-flop and external fields

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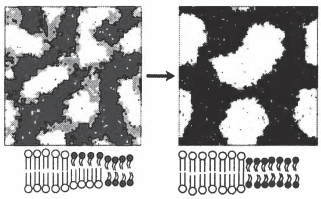
Understanding domains in asymmetric membranes is made easier by the use of phase diagrams that are properly aware of the distinct, yet coupled, leaflets. With this approach, we and others have successfully modelled a number of static and kinetic features in both symmetric and asymmetric membranes [1-4].

However, in biological and model systems, the compositional asymmetry is not a fixed parameter. Lipids are exchanged between leaflets thanks to flip-flop, via active enzymes, scramblase proteins, defects, and transient pores. We introduce a theoretical method to predict how passive inter-leaflet flip-flop alters the types of domain formation a bilayer can exhibit. We predict observable transitions in which qualitative changes in domain patterning accompany the dynamical transport of lipid species between leaflets.

Our approach further allows to include a symmetry-breaking external field, such as a substrate or electric field. We use our findings to explain available observations in the experimental literature. Most excitingly, our work indicates a way to control domain formation and asymmetry in concert, via the systematic manipulation of flip-flop rates and applied external fields.



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Sterol asymmetry in the yeast plasma membrane

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The transbilayer distribution of sterols, such as cholesterol in mammalian cells and ergosterol in yeast, in the plasma membrane (PM) is highly debated [1, 2]. Exploiting yeast cells in which ergosterol is entirely replaced with closely related fluorescent dehydroergosterol (DHE), we show using spectrophotometry and fluorescence microscopy combined with site-specific quenchers that PM sterols reside primarily (~80%) in the inner leaflet of the yeast PM. Maintenance of this pronounced asymmetry does not require metabolic energy; however, asymmetry is weakened upon sphingolipid depletion and loss of PM phospholipid asymmetry. We deduce that sterols comprise up to ~45% of all inner leaflet lipids in the PM, a result that necessitates revision of current models of the architecture of the PM lipid bilayer.

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