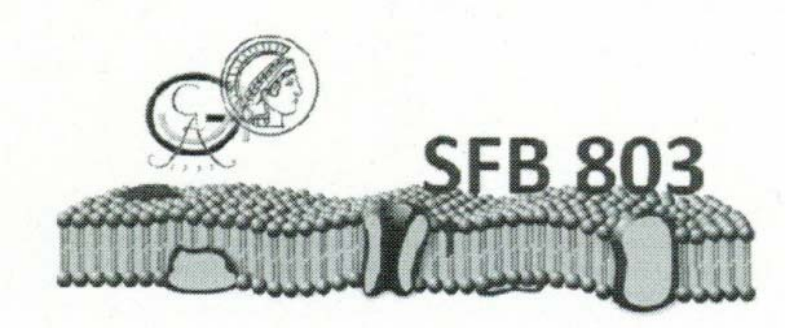
SFB 803 Symposium

Functionality controlled by organization in and between membranes



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University of Göttingen

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Program

Monday, September 6th 2010

09:30 – 09:40	**Claudia Steinem** Welcome
09:40 – 10:20	**Alfred Blume** (Halle) The interaction of antimicrobial peptides with lipid monolayers and bilayers
10:20 – 10:40	**Ulf Diederichsen** Interaction of peptide/PNA hybrids in and between membranes
10:40 – 11:00	**Marina Bennati** Towards determination of orientations in transmembrane peptides by pulse EPR spectroscopy
11:00 – 11:30	**Coffee Break**
11:30 – 12:10	**Petra Schwille** (Dresden) The power of in vitro systems in characterizing protein-lipid interactions
12:10- 12:30	**Qui Van** Quantifying the translational and rotational diffusion of membrane proteins and peptides
12:30 – 12:50	**Christoph Schmidt** Mechanosensitive membrane channels in primary cilia of kidney epithelial cells
12:50 – 14:00	**Lunch**
14:00 – 14:40	**Motomu Tanaka** (Heidelberg) Specific and generic roles of carbohydrates in regulating membrane mechanics and cell behaviors
14:40 – 15:00	**Daniel B. Werz** Towards the chemical synthesis of fluorescent glycosphingolipids
15:00 – 15:20	**Christian Ducho** Structural insights into protein-ligand-membrane interactions for the bacterial membrane protein Mray: synthesis and application of chemical probes
15:20 – 15:40	**Kai Tittmann** Structural basis for membrane bindings and concomitant catalytic activation of the peripheral membrane enzyme pyruvate oxidase
15:40 – 17:00	**Coffee Break / Poster Session**
17:00 – 17:40	**Samuel Kou** (Boston) Sequential Monte Carlo method for protein folding
17:40 – 18:00	**Axel Munk** Statistical jump analysis for single ion channel recordings
18:00 – 18:20	**Bert de Groot** Molecular dynamics simulations of lipid-protein interactions
18:20 – 18:40	**Adam Lange** The native conformation of the human VDAC 1 N-terminus
19:00	**Conference Dinner**

Tuesday, September 7th 2010

8:30 – 09:10	**Ulrich Koert (Marburg)** Structural and functional studies on synthetically modified
09:10 – 09:30	**Tim Salditt** Membrane fusion by x-rays: from model membranes to organelles
09:30 – 09:50	**Helmut Grubmüller** SNARE mediated liposome fusion in molecular detail
09:50 – 11:10	**Coffee Break / Poster Session**
11:10 – 11:50	**Fredrik Höök** (Gothenburg) SPR and LSPR combined with cell-membrane mimics for drug screening studies
11:50 – 12:10	**Marcus Müller** Free energy calculations of self-assembled structures: liquid-gel transition and fusion stalks
12:10 – 12:30	**Claudia Steinem** Fusion assay based on pore-spanning membranes
12:30 – 13:30	**Lunch**
13:30 – 14:10	**Reinhard Lipowsky (Golm)**) Multi-scale reorganization of bio-membranes
14:30 – 14:50	**Peter Jomo Walla** Discrimination between docking and fusion of liposomes using FCS and Förster energy transfer
14:50 – 15:30	**Axel Brunger (Stanford)** Single particle fluorescence microscopy of calcium triggered vesicle fusion
15:30 – 15:40	**Claudia Steinem** Concluding remarks
15:40	**Departure**

Speakers Abstracts

The interaction of antimicrobial peptides with lipid monolayers and bilayers

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Antimicrobial peptides attract a lot of interest as potential candidates to overcome bacterial resistance. So far, nearly all the proposed scenarios for their mechanism of action are associated with perforating and breaking down bacterial membranes after a binding process. We used DSC and FT-IR to study the influence of a linear and cyclic arginine- and tryptophan-rich antimicrobial hexapeptide having the same sequence (RRWWRF) on the thermotropic phase transitions of lipid membranes. In contrast to DPPG/DPPC bilayers, they induced a demixing in DPPG/DPPE bilayers and led to the appearance of two peaks in the DSC curves indicating a DPPG-peptide-enriched domain and a DPPE-enriched domain. These results could be confirmed by FT-IR-spectroscopic measurements using deuterated components. We therefore propose that the observed peptide-induced lipid demixing in PG/PE-membranes could be a further specific effect of the antimicrobial peptides operating only on bacterial membranes which contain PG and PE but not PC (1).
In supported lipid bilayers of pure and PG containing membranes the addition of antimicrobial peptides lead to drastic morphological changes as observed by fluorescence microscopy. Soon after addition of peptide lipid tubules seemed to emerge from the supported bilayer and budding of vesicles or formation of flat bilayer sheets could be observed in some cases. Local membrane heterogeneities induced by the peptide with the consequence of the induction of spontaneous curvature could be the reason for the morphological changes that again depend on membrane composition (2).
Infrared-reflection-absorption-spectroscopy (IRRAS) at the air-water interface is ideally suited to study the interaction of these peptides with the water-air surface and with lipid monolayers as models for bilayer membranes. Essentially only the peptides bound to or incorporated into the lipid monolayers are detected by IRRAS due to the limited penetration depth of the IR radiation. This facilitates the spectroscopic analysis of the amide bands of peptides and the determination of secondary structure elements. From angular dependent measurements it is possible to analyze the conformation and the orientation of molecules in the lipid/peptide films. We could show that model antimicrobial peptides without secondary structure in solution form α-helices when bound to negatively charged lipids but β-sheets when bound to the air-water surface. The power of the method will be shown by presenting different examples (3-6).

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M. Dyck, A. Kerth, A. Blume, M. Lösche, (2006) J. Phys. Chem. B 110, 22152-22159.
A. Erbe, A. Kerth, M. Dathe, A. Blume (2009) ChemBioChem 10, 2884-2892

Interaction of Peptide/PNA Hybrids in and between Membranes

Philipp E. Schneggenburger,[a] Antonina Lygina,[a] Karsten Meyenberg,[a] Annika Groschner,[a] Stefan Müllar,[a] Claudia Steinem,[a] Reinhard Jahn,[b] and Ulf Diederichsen[a]

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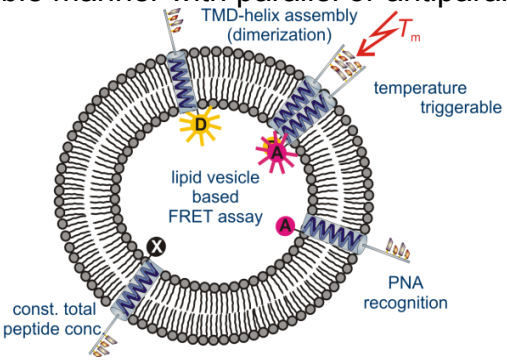
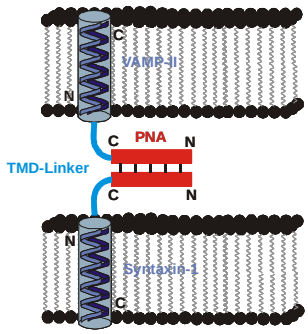
Specific recognition and aggregation of transmembrane domains (TMDs) mediated by functionalities outside the lipid bilayer is a well-known motif in membrane fusion as e.g. known from SNARE protein-mediated processes[1] and for the organization of membrane-spanning protein segments.[2] Based on general interest in the fusion mechanisms and protein/lipid interactions, the synthesis and analysis of protein TMDs and artificial membrane active peptide helix topologies is a valuable tool to study membrane incorporation, aggregation, fusogenicity, and dynamic processes within the lipid bilayer. Furthermore, synthesis allows modification of the TMDs like tagging with reporter groups and attachment of artificial recognition units. The design of the recognition unit can be derived from the biological lead to evaluate the mechanistical needs on the molecular level. Further, there is interest in organizing TMDs with respect to aggregate size, stability and orientation determined by the recognition units.

The first peptide/PNA conjugate discussed is based on a β-helical membrane spanning domain that is structurally derived from the d,l-alternating gramicidin A pore motif;[3] the second one is given by the transmembrane sequences of the native SNARE proteins. Peptide nucleic acid (PNA) recognition moieties were covalently attached to the respective TMDs. In analogy to cholesterol/DNA hybrids,[4] the recognition of TMD/PNA constructs can be obtained in a temperature dependent and reversible manner with parallel or antiparallel orientation of PNA strands.





The TMD/PNA constructs were used to estimate the impact of the membrane adjacent recognition on the TMD assembly by means of FRET analysis using covalently attached fluorescence probes.[5] Dimerization of the TMD/PNA conjugates within unilamellar lipid vesicles was observed at lower temperatures. Additionally, the SNARE derived TMDs covalently linked to PNA units were applied for in vitro vesicle fusion experiments revealing fusion activities comparable to neuronal SNARE proteins.

[1] R. Jahn, R. H. Scheller, *Nat. Rev. Mol. Cell Biol.* **2006**, 7, 631.
[2] Z. W. Hall, *Trends Curr. Biol.* **1992**, *2*, 8020.
[3] A. Küsel, Z. Khattari, P. E. Schneggenburger, A. Banerjee, T. Salditt, U. Diederichsen, *ChemPhysChem* **2007**, *8*, 2336.
[4] G. Stengel, R. Zahn, F. Höök, *J. Am. Chem. Soc.* **2007**, *129*, 9584. [5] P. E. Schneggenburger, S. Müllar, B. Worbs, C. Steinem, U. Diederichsen, *J. Am. Chem. Soc.* **2010**, *132*, 8020.

Towards determination of orientations in transmembrane peptides by pulse EPR spectroscopy

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Electron Paramagnetic Resonance (EPR) has emerged as a valuable tool to obtain structural parameters in membrane proteins, such as distances between amino acid residues or mobility of peptide chains, when combined with site-directed spin labelling techniques. Our efforts are directed towards expanding the current repertoire of spectroscopic tools to determine an additional parameter, i.e. the orientation of peptide chains within biological membranes. Such experiments require pulse EPR methods at high magnetic fields, where the anisotropy of the local magnetic interactions is spectrally resolved, as well as the site specific incorporation of rigid spin labels into the desired peptide position. To implement the methodology at high magnetic fields we have developed a new EPR probe head for distance measurements that operates at a frequency of 94 GHz. The new dual mode resonator allows pump and probe detection of spin label resonances within a spectral width of up to about 400 MHz, covering the whole spin label spectrum at this frequency. This development permits to expand the method of orientation selection, demonstrated by us in the past, to a general application with nitroxide spin labels. Secondly, in collaboration with the group of U. Diederichsen, new rigid nitroxide spin labels have been synthesized and incorporated in model peptides. First experimental data using our hardware and the spin label peptides are presented.

The power of in vitro systems in characterizing protein-lipid interactions

Petra Schwille

Biophysics – Schwille Lab, Biotechnologisches Zentrum, TU Dresden

In vitro systems like supported lipid bilayers and giant unilamellar vesicles (GUVs) have become very prominent in membrane protein research. GUVs in particular have been studied by membrane and lipid researchers for more than two decades. With their comfortable dimensions between single and hundreds of micrometers that are easily accessible to optical imaging and manipulation techniques, they have been proven ideal model systems to study membrane morphology and mechanical parameters, such as surface tension, elasticity, and local curvature, relevant for membrane structure and transformations. Moreover, since the advent of the raft hypothesis in cell biology, there has also been rising interest from the biological community to better understand the relevance of local lipid order for the lateral sorting and induction of functionality of membrane proteins. It is quite evident that the quantitative representation and local order of specific lipids in membranes of various organelles, in tight concert with the respective proteins inserted or attached to them, accounts to a large extent for biological functionality. However, since the exact relationships and also the structural features in live cells are often too complex or too small to be resolved quantitatively, minimal systems with reduced complexity, such as GUVs, pave the way to a more fundamental understanding of lipid-lipid and lipid-protein interactions of physiological importance. Moreover, inspired by the success of these minimal systems approaches to cell biological phenomena, many researchers nurture strong hopes that such a bottom-up approach does not stop at the membrane or at membrane-related processes, but that the GUV model system can be worked into more elaborate models of biomolecular self-organization.

Quantifying the translational and rotational diffusion of membrane proteins and peptides

Kerstin Weiß, Jörg Enderlein, and Qui Van

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Diffusion of proteins and peptides within lipid membranes is described by the classical Saffmann-Delbrück model, predicting a roughly logarithmic dependence of the diffusion coefficient on lateral size of the protein, or more precisely:

$$D_{Saffman} = \frac{k_B T}{4\pi\mu h}\left(\log\frac{\mu h}{\mu' R} - 0.5772\right)$$

where kB is Boltzmann's constant, T is the absolute temperature, □ is the viscosity of the lipid membrane, h is the thickness of the membrane, μ' is the viscosity of the surrounding medium, and R is the cylindrical radius of the diffusing protein. This model describes the diffusing protein as a cylindrical structure of given radius embedded in an infinite planar lipid bilayer. It does, in its simplest version, not take into account effects such as curvature, protein-protein or protein-lipid interactions. Recently, this simple model was challenged by both experimental [1, 2] as well as newer theoretical work [3-5] even for conditions close to infinite protein dilution and flatness (zero curvature) of the bilayer. In contrast, a very recent publication presents experimental results that support the validity of the Saffmann-Delbrück model [6]. The current project aims at measuring precise values of translational and rotational diffusion for a wide range of different protein sizes and environmental conditions (in particular nature of lipid membrane), which will allow for a critical re-evaluation and potential extension of the Saffmann-Delbrück model. For that purpose, we will use advanced techniques for preparing free-standing lipid bilayers, and our recently developed dual-focus fluorescence correlation spectroscopy (2fFCS) technique, which allows for precise and absolute measurements of diffusion of fluorescently labelled proteins in solution and in membranes. With these techniques, we will determine the size-diffusion relationship over a large range of molecular sizes, starting with small trans-membrane peptides up to large membrane proteins.

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2. Kriegsmann, J., et al., *Translational Diffusion and Interaction of a Photoreceptor and Its Cognate Transducer Observed in Giant Unilamellar Vesicles by Using Dual-Focus FCS.* ChemBioChem, 2009. **10**(11): p. 1823-1829.
3. Naji, A., A.J. Levine, and P.A. Pincus, *Corrections to the Saffman-Delbruck mobility for membrane bound proteins.* Biophys. J., 2007. **93**(11): p. L49-L51.
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5. Petrov, E.P. and P. Schwille, *Translational diffusion in lipid membranes beyond the Saffman-Delbruck approximation.* Biophysical Journal, 2008. **94**(5): p. L41-L43.
6. Ramadurai, S., et al., *Lateral Diffusion of Membrane Proteins.* J. Am. Chem. Soc., 2009. **131**: p. 12650-12656.

Mechanosensitive Membrane Channels in Primary Cilia of Kidney Epithelial Cells

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Mechanosensitive ion channels play an important role in cell function. They are involved in cell communication and act as emergency release valves to regulate osmotic pressure in cells. In order to understand their function and gating behaviour they can be reconstituted in artificial lipid bilayers and examined with electrophysiological and optical techniques such as single-channel recording and light microscopy.

Recent studies have shown that the primary cilium, long thought to be a vestigial cellular appendage with no function, has remarkable sensory abilities. Of particular interest, from both a biophysical and medical standpoint, are the primary cilia in kidney epithelial cells, which have been demonstrated to act as tiny flow sensors. The cilia are lined with mechanosensitive TRP channels (PC2), which allow the influx of cations into the cell in response to mechanical stimuli. We explore the mechanical response of this system using fluorescence microscopy and optical trapping techniques.

We have also designed a device for simultaneous electrical recording, fluorescence microscopy and optical trapping experiments to stimulate and characterize the opening of mechanosensitive channels. We form phospholipid bilayers on microfabricated porous silicon substrates because they combine the stability of solid supported membranes and the accessibility to both sides of the bilayer, which is necessary for electrical recordings.

We have produced a microchip for electrical recording using standard clean room techniques.
Apertures of micrometer size were etched into a silicon substrate forming porous microarrays. To electrically isolate the substrate, an oxide layer was grown by thermal oxidation. Integrated Ag/AgCl electrodes surrounding each microarray were fabricated by vapour deposition to make them individually addressable for electrical recordings and to be able to switch between the microarrays during the measurement.

Specific and Generic Roles of Carbohydrates in Regulating Membrane Mechanics and Cell Behaviors

Motomu Tanaka

Physical Chemistry of Biosystems, Institute of Physical Chemistry, University of Heidelberg
Cell Biophysics Group, Institute of Toxicology and Genetics, Karlsruhe Institute of Technology

Functional modification of solid surfaces with plasma membrane models draws an increasing attention as a straightforward strategy to bridge soft biological materials and hard inorganic materials. By the use of ultrathin polymer supports that mimic the generic role of extracellular matrix and glycocalyx, both artificial model membranes and native membranes (polymer-supported membranes) can be immobilized on various solid surfaces.
In the main part of my talk, I will introduce some of our recent studies where we physically model the roles of "soft" biopolymers (carbohydrates) in fine-adjustment of contacts at biological interfaces using various techniques, such as specular and off-specular X-ray and neutron scattering. In the second part, I will introduce you some examples how carbohydrate molecules coupled to soft surfaces can be used as external cues to regulate adhesion and morphology of cells.

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Towards the Chemical Synthesis of Fluorescent Glycosphingolipids

Daniel B. Werz

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Glycosphingolipids are a major part of the plasma membrane. They are found in the outer leaflet of the membrane with their hydrophilic carbohydrate portion directing into the extracellular space. It is hypothesized that clusters enriched in sphingolipids, cholesterol and certain proteins (membrane domains) are formed in the plane of the membrane ("raft hypothesis").[1] These membrane domain formations are driven by distinct lipid-lipid interactions and they are highly interesting in terms of lateral sorting, membrane signaling and trafficking; however, their cause of formation, their stability and dynamics are only partly understood. Consensus data on the size, function and lifetime of lipid rafts are elusive.

Synthesis within this project is directed to elucidate parameters which are important for lipid raft formation. Different parameters are the size and polarity of the carbohydrate head groups and the type of the fatty acid chain (saturated, unsaturated or hydroxylated) attached to the sphingosine. Special attention is focused to incorporate fluorescent tags into the lipid without changing its structural properties to a major extent. A solution to this problem might be the incorporation of polyene fatty acids that exhibit striking structural similarity to natural lipids which results in minimal effects on the lipid properties.[2]

The chemical synthesis of carbohydrate head groups, the sphingosine moiety as well as first results towards the creation of fluorescent fatty acids will be discussed in the talk.

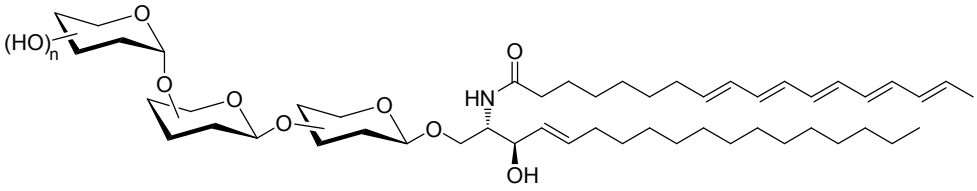

Figure 1. Glycosphingolipid with fluorescent fatty acid side chain.

Literature:

[1] K. Simons, E. Ikonen, *Nature* **1997**, *387*, 569-572.

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Structural insights into protein-ligand-membrane interactions for the bacterial membrane protein MraY: synthesis and application of chemical probes

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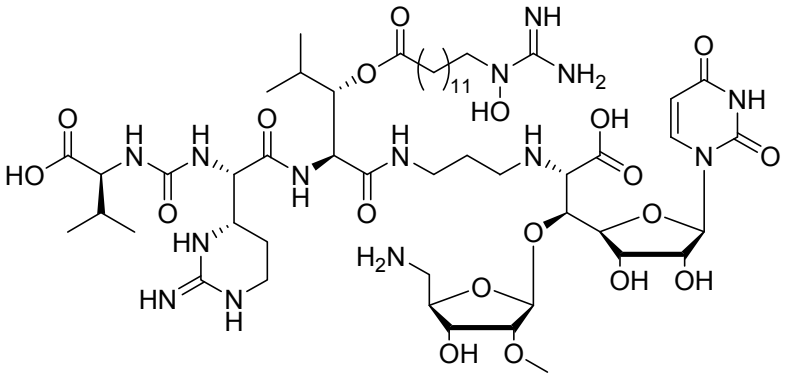
The emerging resistances of bacterial strains, e.g. methicillin-resistant Staphylococcus aureus (MRSA), towards established antibiotics urgently requires the development of novel antibacterial agents. One approach to achieve this goal is the systematic investigation of naturally occurring antibiotics with new or previously unexploited modes of action.
Muraymycins (e.g. muraymycin A1, see Figure) belong to the class of so-called nucleoside antibiotics and were isolated from a Streptomyces sp. as a collection of 19 compounds.[1] They inhibit the bacterial membrane protein translocase I (MraY), a key enzyme in the intracellular part of peptidoglycan biosynthesis and therefore an attractive target for new potential antibacterial drugs.[2] Some synthetic analogues of these natural products have already been prepared and investigated.[3]



Muraymycin A1

The overall goal of our project within the SFB 803 is to obtain molecular insights into inhibition of the bacterial membrane protein MraY. The synthesis of muraymycins as naturally occurring MraY inhibitors and of related analogues is investigated. These chemical probes will be tested for their MraY inhibitor potency under different conditions. Potential conformational changes of MraY upon inhibitor and/or cosubstrate binding will then be studied in collaborations using NMR and X-ray techniques. First results from synthetic studies on muraymycins and muraymycin analogues will be presented, particularly with respect to their unusual nucleoside core structure.[4-6]

Literature:
[1] L. A. Donald *et al.*, *J. Am. Chem. Soc.* **2002**, *124*, 10260-10261. [2] K.-I. Kimura, T. D. H. Bugg, *Nat. Prod. Rep.* **2003**, *20*, 252-273. [3] A. Yamashita *et al.*, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3345-3350. [4] A. P. Spork, S. Koppermann, C. Ducho, *Synlett* **2009**, 2503-2507. [5] A. P. Spork, C. Ducho, *Org. Biomol. Chem.* **2010**, *8*, 2323-2326. [6] A. P. Spork, S. Koppermann, B. Dittrich, R. Herbst-Irmer, C. Ducho, *Tetrahedron: Asymmetry* **2010**, *21*, 763-766.

Structural basis for membrane binding and concomitant catalytic activation of the peripheral membrane enzyme pyruvate oxidase

Kai Tittmann

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The thiamin- and flavin-dependent peripheral membrane enzyme pyruvate oxidase from E. coli catalyzes the oxidative decarboxylation of the central metabolite pyruvate to CO2 and acetate. Concomitant reduction of the enzyme-bound flavin cofactor triggers membrane binding of the outermost C-terminal domain, and shuttling of two electrons to ubiquinone 8, a membrane-bound mobile carrier of the electron transport chain. Binding to the membrane stimulates the catalytic efficiency by several orders of magnitude. The molecular mechanisms by which membrane binding and activation are governed were elucidated by X-ray structural analysis of the full-length enzyme and a proteolytically activated truncation variant lacking the last 23 C-terminal residues inferred in membrane binding. In conjunction with spectroscopic results, the structural data pinpoint a conformational rearrangement upon membrane binding and activation that exposes the auto-inhibitory C-terminus, thereby freeing the active site. In the activated enzyme, the side chain of Phe465 swings into the active site and wires both cofactors for efficient electron transfer. The isolated C-terminus, which has no intrinsic helix propensity, folds into a helical structure in the presence of micelles and lipids as revealed by circular dichroism and infrared reflection absorption spectroscopy. The structure of the isolated membrane-binding domain in the micelle-bound state was determined by liquid-state NMR spectroscopy demonstrating the domain to form an amphipathic helix.

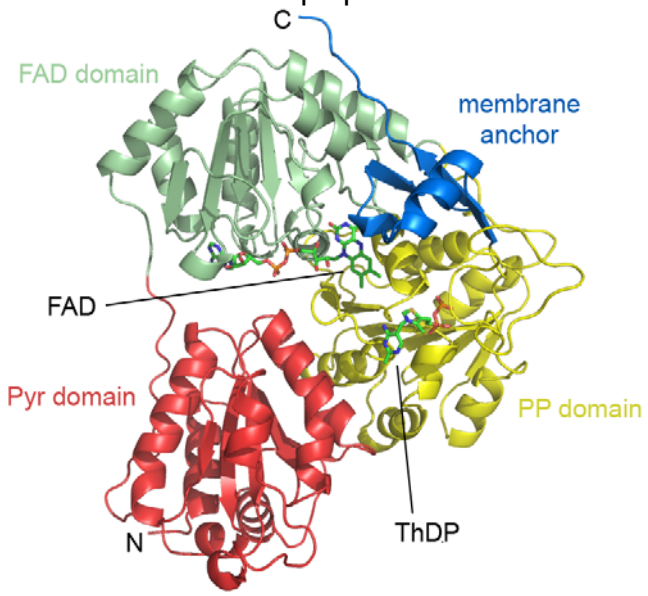

Fig. 1. Structure of a pyruvate oxidase monomer showing the individual domains and the bound cofactors.

Sequential Monte Carlo method in protein folding

Samuel Kou

Department of Statistics
Harvard University

Predicting the native structure of a protein from its amino acid sequence is a long standing problem. A significant bottleneck of computational prediction is the lack of efficient sampling algorithms to explore of the configuration space of a protein. In this talk we will introduce a sequential Monte Carlo method to address this challenge: fragment regrowth via energy-guided sequential sampling (FRESS). The FRESS algorithm combines statistical learning (namely, learning from the protein data bank) with sequential sampling to guide the computation, resulting in a fast and effective exploration of the configurations. We will illustrate the FRESS algorithm with both lattice protein model and real proteins.

Statistical Jump Analysis for Single Ion Channel Recordings

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The opening and closing states of single ion channel recordings maybe modelled as piecewise constant functions plus noise, thereby allowing for sub-states of the ion channel to be detected.
Our aim is to develop a fully automatic and computationally fast method to detect the signal's jumps, and to reconstruct the signal. To achieve this, we split the problem into finding the optimal solution given the number of jumps, and selecting the number of jumps in a data-driven way by a multi-resolution criterion. The validity of this approach has been proved for additive white noise [1,2]. We discuss the extension to the realistic situation where the data have been filtered in advance.

References:

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Scale Space Consistency of Piecewise Constant Least Squares Estimators - Another Look at the Regressogram. *IMS Lecture Notes Series* **55**, 65-84.
[2] **Boysen, L., Kempe, A., Munk, A., Liebscher, V., Wittich, O. (2009).**
Consistencies and rates of convergence of jump penalized least squares estimators. *Ann. Statist.* **37**, 157-183.

Molecular dynamics simulations of lipid-protein interactions

Rodolfo Briones, Camilo Aponte and Bert L. de Groot

Computational biomolecular dynamics group, Max Planck Institute for biophysical chemistry, Göttingen

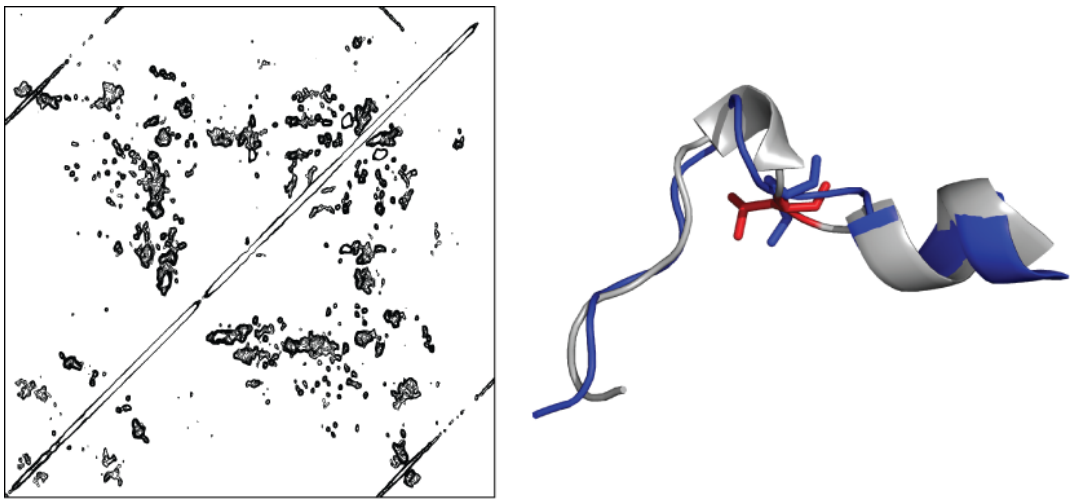
Lipid-protein interactions play an essential role for the function of integral membrane proteins. Likewise, integral and membrane-associated peptides and proteins drastically affect membrane behaviour. We present molecular dynamics simulations on the anion channel VDAC and the water channel AQP0 that highlight the interplay between membrane protein and the surrounding membrane. For VDAC, we find lipid headgroups interacting with E73, a glutamate buried in the hydrophobic part of the membrane. These local membrane-protein interactions are found to affect the global conformational dynamics of the protein. For AQP0, we compare simulation data to two-dimensional electron crystallography data that for the first time have allowed an experimental view at atomistic resolution of protein-surrounding lipids. In simulations of the 2D crystal, the simulations confirm the crystallographic lipid positions. For a channel tetramer embedded in a bilayer patch we address in how far the crystallographic positions are representative for annular lipids. In addition, preliminary data are presented for a model peptide composed of alternating D- and L amino acids of which EPR measurements identified two conformations with different lengths in a DMPC membrane. We present a suggested structural model for the shorter conformation, and speculate on the nature of the longer conformation.

The native conformation of the human VDAC1 N-terminus

Adam Lange

Max Planck Institute for Biophysical Chemistry

We have determined the conformation of the N-terminal domain of a functional human voltage-dependent anion channel (hVDAC1) in lipid bilayers [1]. Solid-state NMR spectroscopy reveals that the N-terminus assumes a well-defined structure and contacts a hydrophobic patch in β-strand 9 of the hVDAC1 β-barrel. Our data indicate that a rigid arrangement inside the aqueous pore is the native conformation of the N-terminus and that its removal induces a conformational change in the barrel.



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Structural and Functional Studies on Synthetically Modified Ion Channels

Simon Reitz, Menekse Cebi, Philipp Reiß, Gregor Studnik, Uwe Linne, Lars-Oliver Essen* and Ulrich Koert*

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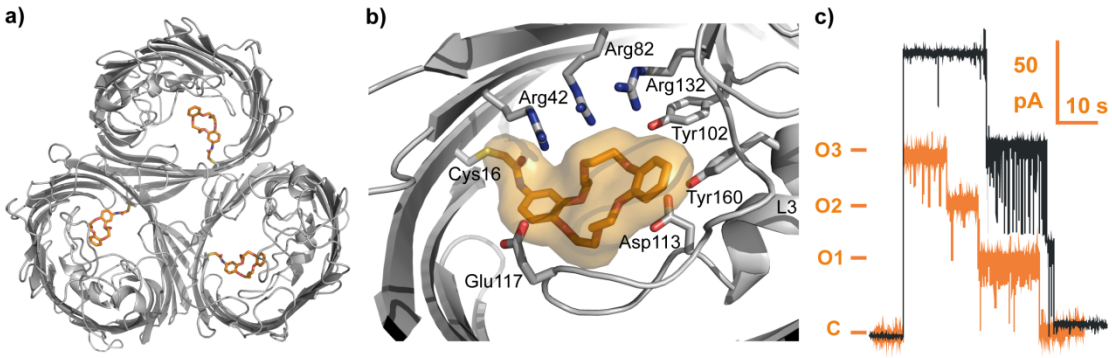
The attachment of synthetic modulators to biological ion channels is one of the challenges for chemical biology with potential applications in neurobiology and sensing.[1] While progress has been made on the modification of channels with a narrow ion conductance pathway,[2] the use of wider pores for ion-channel engineering has been mainly limited to hemolysin.[1a,3] The porins offer a broad variety of β-barrel architectures with pores of variable diameters which makes them promising candidates for attaching synthetic modulators.[4] Unlike the oligomeric hemolysins, porins have a conductance pathway built up by a single polypeptide chain, which eases synthetic modifications in the pore interior. Here we present functional and structural data for the implementation of synthetic modulators into the trimeric porin OmpF.[5]



Structural and functional data for an OmpF-crown ether hybrid: a) top view on OmpF trimer highlighting the dibenzo-18-crown-6 in orange; b) Surface representation for the dibenzo-18-crown-6 compound spanning between the L3 loop and the basic patch; c) Trimer-current trace of OmpF (black) and crown-hybrid (orange); 150 mM KCl at 140 mV.

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Membrane fusion by x-rays: from model membranes to organelles

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Understanding the physical mechanisms underlying membrane fusion requires a multi winged approach, involving model systems as well as biological membranes. We study fusion intermediates occurring in form of ordered passages or stalks connecting neighbouring bilayers in multilamellar model membrane stacks. The stalks exhibit long range crystalline order with rhombohedral symmetry in a fluid 'host' membrane stack, which is studied by high resolution x-ray diffraction under grazing incidence angles. Information on membrane curvature, and hydration interaction can be revealed by analyzing the quantitative electron density maps, collected for controlled environmental parameters and membrane composition. Phase diagrams can be analyzed in view of stabilizing or destabilizing agents for stalk formation [1].

While in these equilibrium phase, dehydration forces bring bilayers together favoring at some point the formation of stalks, it is specific membrane proteins and their interaction which set the local boundary conditions for membrane apposition in biological membrane fusion. In view of studying fusion in the presence of SNARE proteins, we have started a x-ray structural characterization of synaptic vesicles (SV) by small-angle x-ray scattering [2], and currently extent this work towards studies of SV dockled to and interaction with model bilayers.

Finally we present a novel high resolution x-ray imaging scheme capable of yielding a magnified hologram of a freely suspended lipid membrane illuminated by highly divergent and coherent x-ray beams. We propose this setup to image fusion trajectories at high resolution in future experiments [2].

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Hard x-ray phase contrast imaging of black lipid membranes
Applied Physics Letters 95, 203703 (2009); and unpublished extensions of the work

SNARE mediated liposome fusion in molecular detail

Herre Jelger Risselada, & Helmut Grubmüller

Neurotransmitter release at the synapse requires fusion of synaptic vesicles with the presynaptic plasma membrane. SNAREs are the core constituents of the protein machinery responsible for this membrane fusion, but the actual fusion mechanism remains unclear. We have simulated neuronal SNARE mediated membrane fusion in molecular detail. In our simulations, membrane fusion progresses through an inverted micelle fusion intermediate before reaching the hemifused state. The trans-membrane regions of the SNAREs were found to play a vital role in the initiation of fusion by causing distortions of the lipid packing of the outer membrane leaflets, and the C-termini of the trans-membrane regions are associated with the formation of the fusion pores. Our simulations also revealed that the presence of homodimerizations between the trans-membrane regions leads to the formation of unstable fusion intermediates that are under high curvature stress. The inherent mechanical stress in the linker region of the SNARE complex was found to drive both the subsequent formation and expansion of fusion pores. We show that multiple SNARE complexes mediate membrane fusion in a cooperative and synchronized process. Finally, we show that after fusion, the zipping of the SNAREs extends into the membrane region, in agreement with the recently resolved X-ray structure of the fully assembled state.

SPR and LSPR combined with cell-membrane mimics for drug screening studies

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During the past decade, conventional surface plasmon resonance (SPR) has started to play a central role in the drug screening process. Recently, the through-put capacity of these systems has increased by integrating array-based SPR systems with imaging readout and advanced microfluidic liquid handling. However, what still remains a holy grail in this line of industrial R&D is the limited success of SPR to probe the interaction of low-molecular-weight drug candidates with lipid-membrane-residing proteins. While water-soluble proteins can be immobilized at sufficiently high densities to probe the binding of low-molecular-weight drug candidates, the fact that lipids must surround membrane proteins puts sever constrains on the surface densities that can be reached while still keeping the proteins in a native state. The solution to this problem will most likely require both new interfacial lipid-based self assembly approaches and surface-based transducer concepts with even higher sensitivity.

While working on contributing to potential solutions to these problems, we observed that a local change in refractive index upon molecular transport across the membrane of tethered lipid vesicles could be easily resolved using conventional SPR.[1] With 30% of all membrane proteins acting as molecular channels, and 70% of all current drugs being targeted towards membrane proteins, this concept might indeed provide a means to use SPR to study the influence of drug candidates on membrane protein function – despite the fact that the interfacial refractive index contrast induced by the drug itself cannot be resolved. However, while minute changes in the refractive index inside tethered vesicles can be measured with high signal-to-noise ratio, the high transport efficiency of membrane channels leads to millisecond uptake and release rates of the sub attoliter volumes enclosed by lipid vesicles. This thus calls for rapid exchange of the liquid outside the tethered liposomes, which in turn requires both advanced fluidic control and readout from as small regions as possible. While the lateral propagation of conventional SPR limits the smallest regions that can be probed to around 50 μm2, changes in the localized SPR response associated with discrete nanoscale metal particles is in principle limited by the dimension of the particles themselves.

In this presentation, I will discuss our attempts to use evanescent wave sensing to probe membrane-protein controlled molecular transport[2], as well as our recent efforts to merge small-scale LSPR sensing[3] with liquid handling providing millisecond solution-exchange rates over micron sized areas. I will also present how LSPR-active nanoholes can be utilized as substrate-spanning pores, thus enabling flow-through sensing offering significantly improved rates of uptake as well as capture efficiencies[4], especially if combined with material-specific-surface chemistries that direct the molecular recognition reactions to the most sensitive regions of the LSPR sensors[5].

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Free-energy calculations of self-assembled structures: liquid-gel transition and fusion stalks

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Using a solvent-free, coarse-grained model for amphiphilic membranes [1], we develop methods for accurately measuring free energies by computer simulation. Two examples will be discussed: a) Using reweighting techniques we accurately determined the phase coexistence of the liquid and the gel phase of our model membranes and compute the line tension and interface stiffness between the two coexisting phases [2]. b) We determine the excess free energy of hourglass-shaped connections (i.e., stalks) between two apposed bilayer membranes in the liquid phase. In order to calculate the free energy by simulation in the canonical ensemble, we reversibly transfer two apposed bilayers into a configuration with a stalk in three steps (see figure) [3]. First, we gradually replace the intermolecular interactions by an external, ordering field. Second, the external, ordering field is changed as to transform the non-interacting system from the apposed bilayers to two-bilayers connected by a stalk. On the third branch of the transformation path, we reversibly replace the external, ordering field by non-bonded interactions. Using expanded-ensemble techniques, the free energy change along this reversible path can be obtained with an accuracy of 10-3kBT per molecule in the nVT-ensemble. Moreover, calculating the chemical potential, we obtain the free energy of a stalk in the grandcanonical ensemble (i.e. at constant membrane tension). Employing semi-grandcanonical techniques, we compute the change of the excess free energy upon altering the molecular architecture. This computational strategy can also be applied to compute the free energy of self-assembled phases in copolymer systems [4], and the excess free energy of defects or interfaces [5].

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Fusion assay based on pore-spanning membranes

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Fusion of biological membranes is a central requirement for many cellular processes. It involves at least two distinct steps, binding or apposition of two membranes and their subsequent merger followed by the mixing of aqueous compartments encapsulated by these membranes. Experiments based on artificial membrane systems have significantly contributed to our current knowledge on membrane fusion processes. However, there are still a number of drawbacks associated with these assays. Thus, we aim to establish a new vesicle-planar membrane fusion assay to be able to gain insight into protein-mediated fusion processes starting from docking, via hemifusion to full fusion. To achieve this goal, membranes suspending the pores of a highly ordered porous material were established, which have the advantage that they are very robust, and mechanically stable. Moreover, both membrane sides can be addressed individually allowing the application of a transmembrane potential, fusion modulating compounds or an electrochemical gradient. Our results show that the fusion of unilamellar vesicles with these pore-suspending membranes can be readily followed by time-lapsed fluorescence microscopy.

In a first approach, pore-spanning membranes were achieved by painting a lipid dissolved in n-decane on a functionalized porous silicon substrate. The membrane was doped with the fluorescence dye Oregon Green DHPE, which allows following the membrane formation process by means of fluorescence microscopy. Single fusion events, initiated by the presence of Ca2+, were observed upon the addition of large unilamellar vesicles doped with Texas Red DHPE in a time resolved manner. Lipid mixing during the fusion process was followed by the occurring Förster resonance energy transfer (FRET), from which the diffusion constant of the lipids in the plane of the bilayer can be obtained. Simultaneously, the release of a water soluble dye entrapped in the vesicle lumen was observed.

In a second approach, solvent-free pore-spanning membranes were prepared by spreading and fusion of giant unilamellar vesicles onto functionalized porous silicon substrates. In this case, fusion of large unilamellar vesicles with these pore-spanning membranes was initiated by a molecular recognition process. The pore-spanning membranes were doped with a maleimido-functionalized lipid, to which the peptide H6WGC was coupled via the cysteine side chain. Large unilamellar vesicles were added containing the lipid DOGS-Ni-NTA. Via complex formation of the histidine residues to Ni-NTA, the two membranes come in close proximity, which is the prerequisite for fusion. A detailed statistical analysis of single vesicle events revealed the dependence of the fusion efficiency on the DOGS-Ni-NTA content in the vesicles.

Multi-Scale Reorganization of Bio-Membranes

Reinhard Lipowsky

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Because of their fluidity, biomimetic and biological membranes can easily reorganize themselves and can, thus, quickly adapt to changes in their environment. Important examples for such reorganization processes are provided by cluster and domain formation within membranes [1,2], molecular recognition during membrane adhesion [3], membrane fusion [4,5], and wetting of membranes in contact with several aqueous phases [6,7]. These different processes, which involve a wide range of time and length scales, will be discussed from a theoretical point of view emphasizing the fruitful interplay between theory and experiment.

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Discrimination between docking and fusion of liposomes using FCS and Förster Energy Transfer

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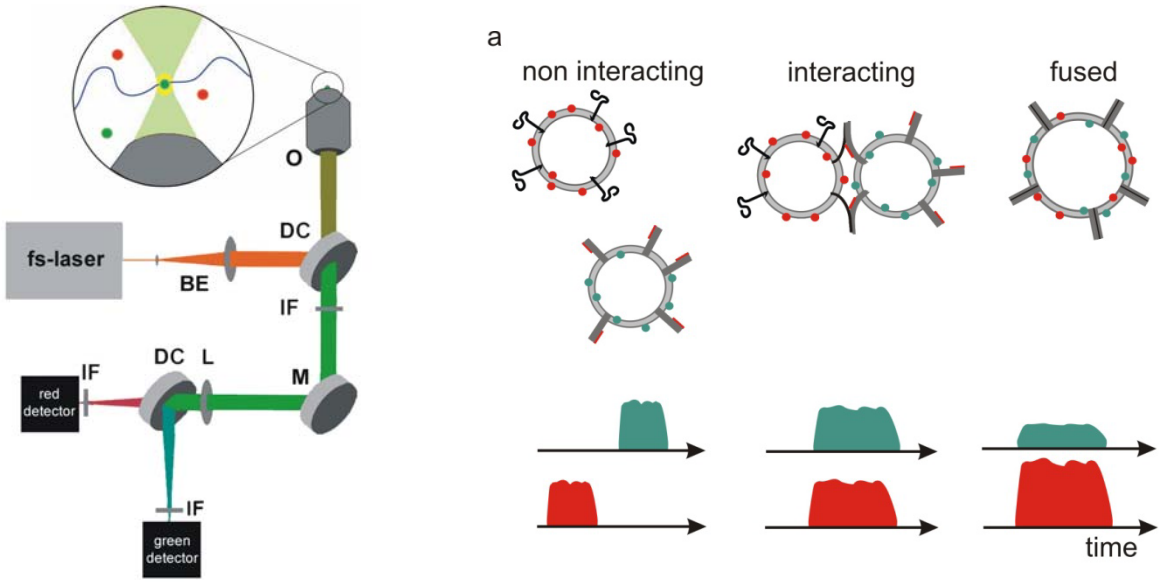
Membrane fusion must be highly regulated in order to guarantee the functioning of processes like endosomal transport or neural signal transmission. So far, however, important molecular details of how the membrane fusion processes are regulated and triggered are still unknown. One reason is that standard assays can not resolve a decisive intermediate step in which vesicles are docked to the other membrane awaiting a signal for fusion. In our contribution we will present a method that is based on simultaneous two-photon fluorescence cross-correlation and Förster energy transfer analysis that enables resolving this decisive intermediate step. With this assay principle it is now possible to elucidate which proteins are key players in the regulation of membrane fusion and how, for example, such fusions are triggered. We present first results that resolve decisive intermediate steps in the regulation of neural signal transmission.



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Single particle fluorescence microscopy studies of calcium triggered vesicle fusion

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Ca^{2+} triggers neurotransmitter release to initiate signal transmission in all synapses. Synaptotagmin 1 is the Ca^{2+}-sensor for fast synchronous release, complexin is a modulator of release, and neuronal SNAREs are essential for synaptic vesicle fusion. We reconstituted this entire machinery and implemented simultaneous observation of lipid and content dye fluorescence in single vesicles in order to differentiate between instances of docking, hemifusion, and complete fusion (relevant for neurotransmitter release), respectively. Without Ca^{2+} fusion events are observed rarely, so the experiment begins in a defined state of docked vesicles. Upon Ca^{2+}-injection, half of the docked donor (synaptobrevin/synaptotagmin I) vesicles completely fuse with acceptor (syntaxin/SNAP-25/complexin) vesicles within 0.27 sec at ambient temperature. The Ca^{2+} response is cooperative. In contrast, neuronal SNAREs alone produce a much slower response. Our experiments suggest a mechanism where synaptotagmin 1 is a Ca^{2+}-dependent kinetic regulator of SNARE-dependent fusion, and complexin further accelerates this process.

Posters

Name	Title
Aeffner, S.	Stalk structures in model membrane systems studies by X-ray scattering
Beerlink, A.	Black lipid membranes studies by X-ray phase contrast imaging
Briones, R.	Lipid-protein interactions and dynamical properties of VDAC-1 channel
Castorph, S.	Structure parameters of synaptic vesicles quantified by small-angle X-ray scattering
Daoulas, K.	DFT-based models for amphiphilic membranes: bridging particle – based and Field-theoretical descriptions
Fuhrmanns, M.	Effects of the influenza fusion peptides on the phase behaviour of lipid mixtures
Gattin, Z.	Investigation of VDAC2 with solid-state NMR
Ghosh, S.	Interaction of synaptic vesicles with model lipid membranes
Groschner, A. **Lygina, A.**	Synthesis of SNARE analogues to analyze the mechanism of membrane docking and fusion
Grunwald, M.	Fluorescence correlation spectroscopy to resolve the docked intermediate of membrane fusion
Höfer, I.	Investigation of the influence of molecular recognition mechanism on the fusion of vesicles with pore-spanning membranes
Holkenbrink, A.	Synthesis of different glycosphingolipids
Kaufeld, T.	Mechanosensing across the cell membrane: Mechanically activated TRP channels in model lipid bilayers and MDCK cells
Marrelli, G.	The role of membrane proteins in pore formation and membrane fusion: Computer simulation of coarse-grained models
Polupanow, T.	Synthesis of modified gramicidin A for analysis of membrane-peptide interactions and its water permeability
Ries, Oliver	Synthesis of O-Acylated 3-Hydroxyleucine derivates for the preparation of Muraymycins or MraY inhibitors
Risselada, H.J.	Fast roads and dead ends in SNARE-dependent membrane fusion
Saßen, C.	Insertion of MscL into pore-suspending membranes
Schild, L.	Endpoint optimization for uniform sampling schemes in NMR experiments
Sicoli, G.	Rotation of a few degrees, relative translation by a few Angstroms: organization in membrane studies by EPR spectroscopy
Sitte, A.	Analysis of the membrane binding mechanism of the peripheral membrane protein pyruvate oxidase from Escherichia coli
Spork, A.P.	Synthesis of the muraymycin core structure and analogues for the investigation of the bacterial membrane protein MraY
Villinger, S.	Functional dynamics in the voltage dependent anion channel in micellar and bicellar environment
Weichbrodt, C.	Electrophysiological investigation of the VDAC ion-channel
Weiß, K.	Quantifying the translational and rotational diffusion of membrane proteins and peptides

Stalk structures in model membrane systems studied by X-ray scattering

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The fusion of two biological membranes is a key step in processes like exocytosis, synaptic transmission n, fertilization or viral infection. It has been proposed to proceed via an intermediate lipidic structure termed a “stalk” [1]. Due to its localized (few nm) and highly transient nature, a major hurdle for structural studies has been the absence of suitable model systems.
Properties of the lipid bilayer matrix are frequently correlated to lipid polymorphism. It has been discovered that certain phospholipids arrange into a liquid crystalline equilibrium phase of rhombohedral symmetry. Reconstruction of the electron density distribution in the unit cell from X-ray diffraction data revealed that this phase is made up of stalk structures arranged on a regular lattice [2].
We use this method to study the effect of lipid composition and cholesterol content on stalk formation and stalk structure by laboratory-scale [3] and synchrotron experiments. The aim is to provide experimental data on how lipid composition affects the structure and free energy of stalks.

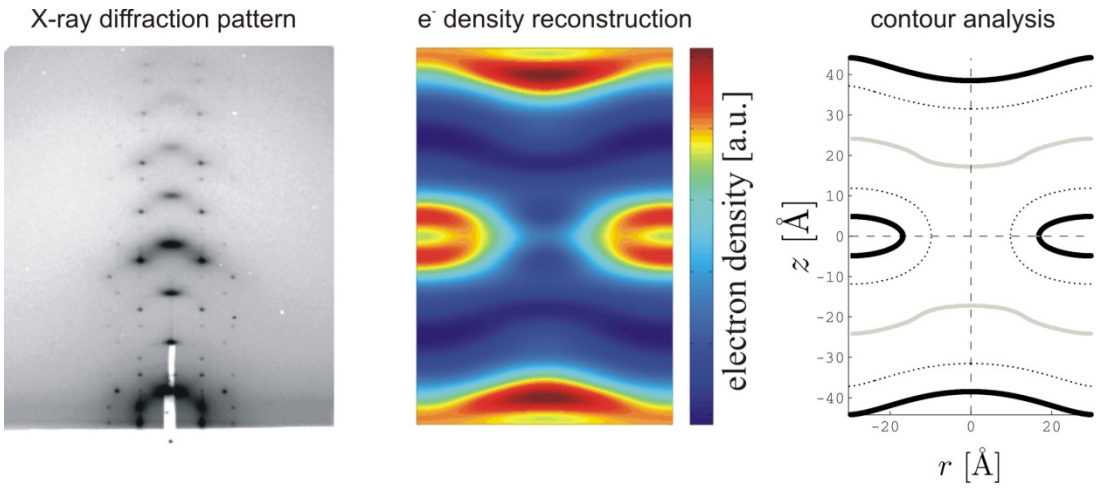

Fig. 1: left) GISAXS pattern of the rhombohedral phospholipid phase recorded at beamline ID01 at the ESRF (Grenoble, France). center) Resulting electron density distribution in a stalk. right) Contours of the regions of highest (black) and lowest (gray) electron density can be obtained from the experiment-tal data and allow to apply analytic bending energy models [4,5].

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Black Lipid Membranes studied by X-Ray Phase Contrast Imaging

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Membranes are considered as the most important interfaces in biology, and can be visualized under physiological conditions by optical techniques such as phase contrast and fluorescence light microscopy. While the contour lines and large lateral domains of biological membranes can be imaged, the density profile of the membrane and associated changes cannot be resolved by visible light. We report on hard x-ray phase contrast imaging of black lipid membranes (BLMs) [1], which are freely suspended over a micro machined aperture in an aqueous solution [2]. This new way of membrane structure analysis allows investigating bio molecular and organic substances in aqueous environments by parallel and divergent beam propagation imaging, using partially coherent multi-keV x-ray radiation [3-4]. The width of the thinning film is significantly smaller than the detector pixel size, but can be resolved from quantitative analysis of the intensity fringes in the Fresnel diffraction regime down to its native thickness of about 5nm. We have put forward a simplified but extendable model, which enables the theoretical description of image formation and characterization of membrane thickness and its decrease during the thinning process from a bulk to a bimolecular film [5]. The structural changes can be obtained from both the loss of contrast and the asymmetry of the detected Fresnel fringes. On the basis of the recent experiments, future investigations will be performed to study the interactions of membranes, as they are for example known from synaptic fusion, with high spatial resolution.

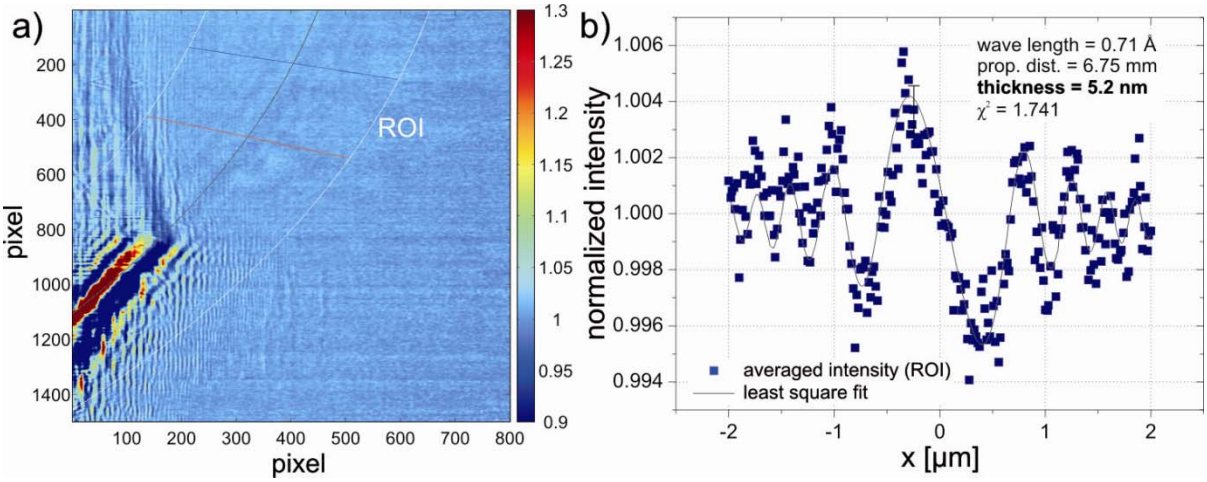

Fig. 1: a) Hard X-ray phase contrast image (performed at 17.5 keV) of a thinned, bulged BLM made of DPhyPC in aqueous buffer. b) Averaged Fresnel fringe intensity of the depicted ROI from (a) with least-square fit to data and fit parameters.

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Lipid-Protein Interactions and Dynamical Properties of VDAC-1 Channel

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VDAC-1 channels are one of the main components of the outer mitochondrial membrane. It is responsible for the transport of ATP and other anions and it is involved in apoptosis and cancer [1]. The X-ray and NMR structures [2-4] showed VDAC as a 19 β-barrel structure with an N-terminal α-helix bound to its interior. The type of amino acid in E73X facing the membrane and the N-terminal helix could to be associated with local perturbations on the lipids and changes of the intrinsic VDAC dynamics [5]. To address these points we measured the local average thickness and several structural properties of several mutants and N-terminal deletions (replacements) of VDAC-1, modelled by MD, inserted in DPMC phospholipid patches. The results show that the main distortions of the membrane are located around E73 in its charged state. The major fluctuations of VDAC barrel were also correlated with the charged E73 but also with the absence or alternative configurations of the N-terminal helix. The motion amplitude described as PCA eigenvectors suggest that the helix stabilize or increase barrel rigidity. The structural deformations of VDAC correspond with changes of an ideal ellipse fitted to the β-barrel structure. These results help to understand the intrinsic dynamic of VDAC and its possible interaction mechanism with lipid membranes.

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Structure Parameters of Synaptic Vesicles Quantified by Small-Angle X-Ray Scattering

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The release of neurotransmitters from neurons, in response to stimulation, forms the basis of communication in the nervous system. Neurotransmitters are stored in small membraneous organelles, synaptic vesicles (SVs), within the presynaptic terminal. These vesicles undergo an elaborate cycle of fusion with the plasma membrane (releasing neurotransmitter), followed by retrieval and reformation and transport back to the plasma membrane for further rounds of fusion [1].

In recent years there has been enormous progress in our knowledge of the molecular composition and structure of synaptic vesicles [2]. However, we still lack a detailed view of the physical properties of this trafficking organelle as it proceeds through its life-cycle.

Here we use small-angle x-ray scattering (SAXS) to determine the average radial density profile ρ(r) and the size polydispersity of SVs [3, 4]. We show that SAXS can be used to study the supra-molecular structure of an entire functional organelle under quasi-physiological conditions. The profile ρ(r) of SVs including structural parameters of the protein layers, as well as the polydispersity function p(R), are derived with no free prefactors on an absolute scale. The measured SV structure on length scales between the constituent biomolecules and the SV size confirms the main aspects of recent numerical modeling [2], which was based on the crystal structures of the constituent proteins and stoichiometric knowledge from biochemical studies. In addition, we present first evidence of a laterally anisotropic structure, indicative of larger protein clusters.

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DFT-BASED MODELS FOR AMPHIPHILIC MEMBRANES: BRIDGING PARTICLE-BASED AND FIELD-THEORETICAL DESCRIPTIONS

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The multi-functionality of biological membranes is achieved through the high complexity and diversity of their molecular components. However, similarities are observed between membranes with very different chemical composition regarding collective phenomena, i.e., processes involving large numbers of molecules. Fusion and fission events occurring in lipid as well as in polymeric membranes are a typical example [1]. This universality suggests that the physical mechanisms of such processes can be understood within minimal models, incorporating those features/interactions that are necessary to reproduce the phenomena of interest. We develop a minimal model for amphiphilic membranes invoking a generic, bead-spring representation of the molecular architecture. The non-bonded interactions are defined via a functional, having the form of a third-order expansion with respect to the local densities of the hydrophobic and hydrophilic beads [2]. The presence of the water is taken implicitly into account via the "virial" coefficients of the hydrophilic segments. The density-functional representation of the interactions allows for: a) A straightforward connection of the model parameters with thermodynamic properties of the membranes, e.g., the density of the hydrophobic core, b) a very efficient study of the model within particle-based Monte Carlo [2] and multi-body DPD simulations [3] and c) obtaining a fast but approximate solution of the same model within Self Consistent Field (SCF) theory.

At the first part of our presentation we discuss representative results obtained from the particle-based simulations of planar, amphiphilic bilayers and demonstrate how thermodynamic and mechanical properties of the membrane are affected by the parameters of the model. At the second stage, we develop a three-dimensional SCF theory approach to the same model and compare its predictions regarding representative membrane properties with the results of the particle based-simulations. The SCF theory allows for a fast estimation of the free energies of different morphologies observed in membranes, helping to understand the pathways of various collective phenomena. We consider the membrane fusion event, focusing on one, specific, intermediate morphology: the "stalk" structure. For this case preliminary results concerning the effect of the amphiphile asymmetry on the stability of the stalk will be presented and compared to the predictions obtained from the particle based simulations via a thermodynamic integration scheme [4].

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Effects of the Influenza HA Fusion Peptides on the Phase Behavior of Lipid Mixtures

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We present molecular dynamics studies focusing on the influenza HA fusion peptide as an agent of fusion. Using a coarse-grained model, we started the simulations from a random mixture of lipids, solvent and peptides to determine the phases that spontaneously assemble. By comparing the adopted phases to the ones adopted in the absence of peptides, conclusions in terms of changes in preferred curvature could be drawn.

The phases spontaneously formed indicate a systematic shift of the phase diagram towards more positive mean curvature in the presence of the peptides. In particular, the lamellar phase was favored over the stalk phase, and the stalk phase over the inverted hexagonal phase. For the latter phase boundary, the wildtype peptides were found to have a larger effect than the non-fusogenic G1V and W14A mutants, which appears to be connected to the ability of the wildtype peptides to align with the positive curvature component of the stalks with their boomerang shape. As a separate phenomenon, we observed the formation of bicontinuous cubic phases, both for the wildtype and mutant peptides, indicating a stabilization of negative Gaussian curvature.

A possible rationalization of these effects is that the peptides induce a positive curvature component, both via their additional contribution to the surface and, in the case of the wildtype peptides, via their kinked structure. This positive curvature can manifest as an overall increased mean curvature. However, the induction of positive curvature can also be limited to only one of the two principal curvatures and be compensated by a drop in the other and thereby manifest as negative Gaussian curvature. While this formation of saddle-splay curvature is normally associated with a significant energetic penalty, the peptides' preferred location in regions of high Gaussian curvature might act to lower the energetic costs by replacing the lipids in those areas. The peptides might therefore possess a dual role in the induction of bicontinuous cubic phases, forcing a positive curvature component on the system and lowering the energetic costs of Gaussian curvature.

Which bicontinuous phase is adopted is found to depend on the peptide present, with the wildtype inducing the single diamond phase and the G1V and W14A mutants inducing the double diamond phase in consistence with the wildtype's higher ability to stabilize a positive curvature component due to its kinked shape. While the stabilization of double bicontinuous cubic phases is normally associated with peptides that facilitate fusion, this relation is also not strict in experimental results, suggesting an involvement of the fusion peptides in a different stage of the fusion process. The formation of the single diamond phase in the presence of the wildtype peptides might indicate an importance of stalk-pore complexes, which have been observed as intermediate states of vesicle fusion in simulations.

Investigation of VDAC2 with solid-state NMR

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Voltage-dependent anion channels (VDACs), also known as mitochondrial porins, are 30-35 kDa pore-forming proteins found in the mitochondrial outer membrane (MOM) of eucariots. The first mitochondrial VDAC-porin was discovered by Schein, Colombini, and Finkelstein in 19761. To date, multiple VDAC isoforms (VDAC1, VDAC2, and VDAC3) have been identified in a variety of organisms, including yeast, plants, mouse, and humans. Although all VDACs are highly preserved in all eucariotic kingdoms some studies revealed important differences in the regulatory functions within the different cell types. For example, genetic studies have shown that VDAC2 knockout mice are embryonic lethal, whereas both VDAC1 and VDAC3 knockout mice are viable2, which might suggest that VDAC2 has some different functions from VDAC1 and VDAC3. The structural information about VDAC1 (mouse and human) is known only from 20083-5. Recently, we have determined the native conformation of the N-terminal part of human VDAC1 in liposomes by solid state NMR6 which was difficult to be observed by solution-state NMR.

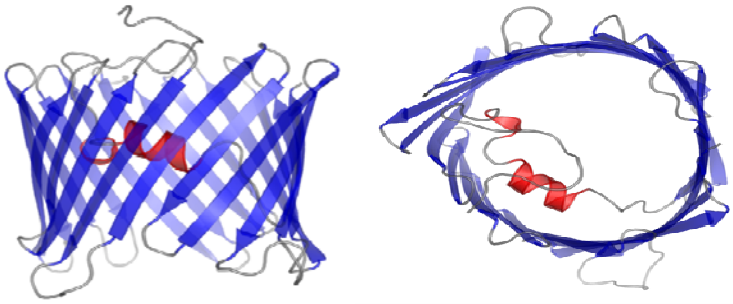

Figure 1. Homology model of human VDAC2 performed with MODELLER. The structure of mouse VDAC1[3] was used as the template.

The main interest of this study is the determination of the structure of the N-terminal part of VDAC2 and the study of its dynamics in a lipid bilayer. Although the N-teminal part of VDAC2 is 11 amino acids longer than of VDAC1, the part where the α-helix is expected in VDAC2 has more than 90% similarity with VDAC1 so we expect that the N-terminal part of VDAC2 will be similar to the N-terminal part of VDAC16 due to this high sequence similarity. The preliminary results obtained by solid state NMR experiments and molecular dynamics simulations on homology model of VDAC2 will be presented.

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Interaction of Synaptic Vesicles with Model Lipid Membranes

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Synaptic vesicles (SVs) are trafficking organelles in the presynaptic nerve terminals responsible for transport and release of neurotransmitters into the synaptic cleft. The adhesion and fusion of these vesicles with the plasma membranes are essential steps of the SNARE mediated and Ca^{2+} dependent exocytosis process. For a detailed and high resolution structural analysis of this process, we have performed an advanced interface sensitive x-ray scattering experiment on a system where the inner leaflet of the synaptic membrane has been modelled by a controlled lipid monolayer at the air-water interface. The x-ray reflectivity and grazing incidence x-ray diffraction (GIXD) measurements show the interaction of SVs with this model system. This interaction is manifested by the structural changes of the monolayer induced by the adhering SVs. Such an interaction was further investigated on the solid supported single bilayer. Both these monolayer and bilayer results suggest a significant role of the anionic lipid phosphatidylinositol-4,5-biphosphate (PIP_2) in this interaction. The SV-associated protein synaptotagmin is assigned to bind the PIP_2-rich microdomains to explain the observed results.

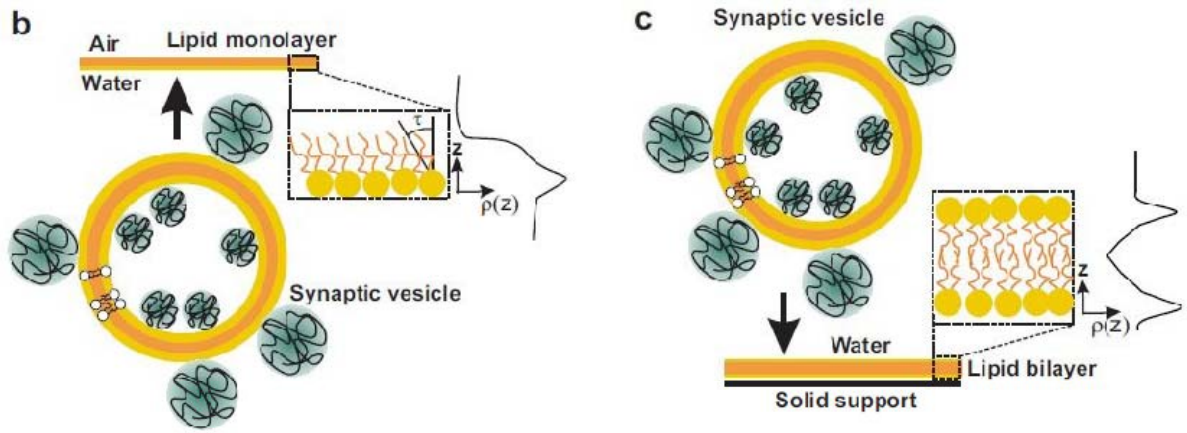

Fig.: Schematic illustration of model systems: (left) SVs were injected into the subphase of a lipid monolayer formed at the air-water interface, (right) SVs were introduced on top of a lipid bilayer formed on a solid support. The respective electron density profiles (ρ(z)) from the lipid monolayer and bilayer are also shown. τ is the tilt angle of the alkyl chains of the lipid molecules in the gel phase.

Synthesis of SNARE analogues to analyze the mechanism of membrane docking and fusion

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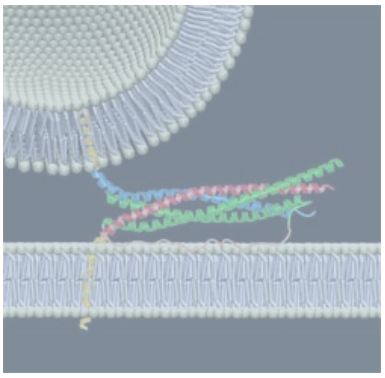

All intracellular transport processes, ranging from secretion in yeast to neurotransmitter release in the brain, depend on the ability of membranes to fuse with each other. This usually requires a set of three membrane proteins known as SNAREs (soluble N-ethylmaleimide-sensitive attachment protein receptors). Upon membrane contact, the SNARE motifs spontaneously assemble into a stable four-helix coiled-coil recognition complex combining three helices from the t-SNARE (target-membrane SNARE) with one from the v-SNARE (vesicle-membrane SNARE).[1,2]

However, it is not clear yet whether fusion is caused by force exerted on the membranes (transmitted to the transmembrane domain by a stiff linker) or by other mechanisms. In our studies, we replaced the SNARE motifs of neuronal SNARE proteins by artificial recognition units. These model systems have been synthesized using solid phase peptide synthesis. Well-known coiled-coil forming peptides or PNA double strands were chosen as recognition units. Fusion of vesicles mediated by the model systems containing the TMD and the respective recognition unit was indicated.[3,4] The first fusion experiments with vesicles containing these artificial SNARE biooligomers showed in vitro fusion activities comparable to that of neuronal SNARE proteins. Further experiments are to be carried out to investigate the mechanism of the fusion process.

Subsequently, the SNARE complex is disassembled by the concerted action of α-SNAP and ATPases associated with the N-ethylmaleimide-sensitive factor (NSF).

The inhibition of the disassembly process was addressed by targeting the α-SNAP/NSF machinery. Synaptobrevin (v-SNARE) was mutated at defined amino acid positions, thereby preventing α-SNAP recognition. Mutated synaptobrevin sequences contained residues with inversed surface charges or sterically demanding side chains to inhibit the α-SNAP/NSF mediated SNARE complex disassembly.[5] Experiments with these mutants by SDS-PAGE indicate successful inhibition.

Another approach to inhibit the SNARE disassembly process is to modify the well-defined recognition unit between the v-SNARE, one of the t-SNAREs and the complexin helix.[4] The sequence of complexin was synthesized as α-peptide to take benefit from a conformationally rigid and structurally well defined helix.

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Fluorescence correlation spectroscopy to resolve the docked intermediate of membrane fusion

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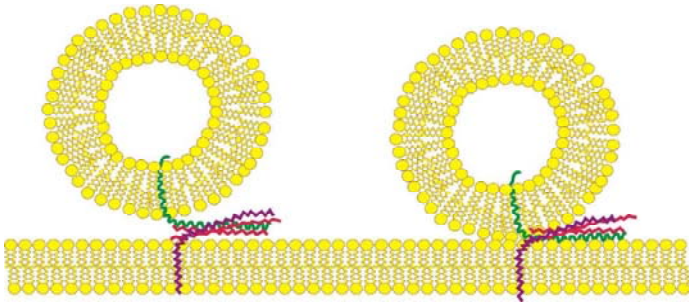
Membrane fusion in neuronal exocytosis is mediated by SNARE-proteins. In this process, synaptic vesicles carry the neurotransmitters to the presynaptic end of the axon and release them from the cell during fusion with the presynaptic membrane. SNARE-mediated membrane fusion of artificial liposomes is a well established way to investigate the exocytosis in neurons. Our focus is on the docked intermediate of membrane fusion. To visualize this process in vitro, we label two liposome populations with red and green lipid-anchored fluorophores which can undergo FRET (fluorescence resonance energy transfer)[1]. Each population is reconstituted with complementary sets of SNARE-proteins. Combining fluorescence cross-correlation spectroscopy with fluorescence lifetime analysis we can simultaneously detect the populations of the docked and the fused intermediates[2].



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Investigation of the influence of molecular recognition mechanisms on the fusion of vesicles with pore-spanning membranes

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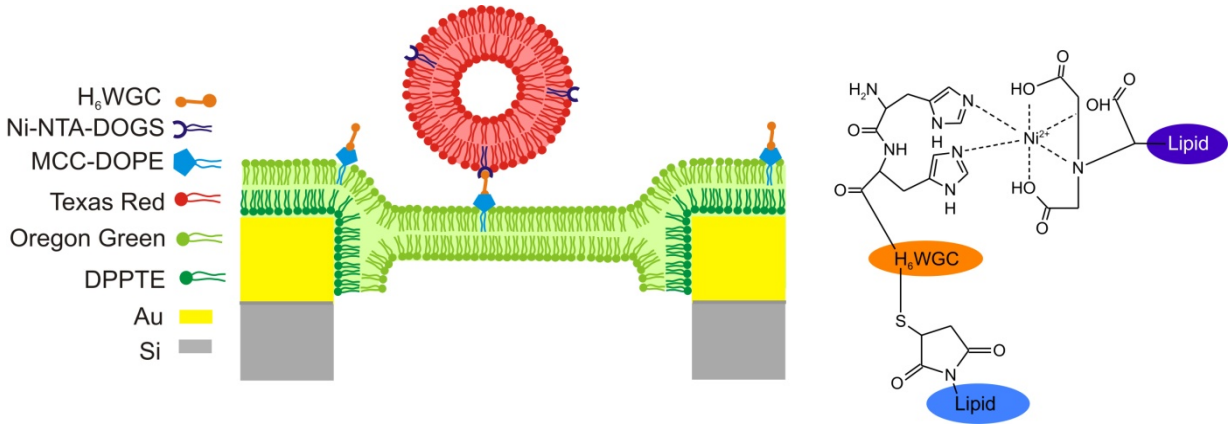
In an effort to understand the molecular mechanism of membrane fusion, a great variety of in vitro fusion assays has been developed to monitor the different steps that occur during this process like hemifusion and fusion pore formation.
We present a new and versatile fusion assay based on pore-spanning membranes that can overcome the drawbacks of other model membrane systems like black lipid membranes or solid supported membranes.
The fusion of large unilamellar vesicles (600 nm in diameter) with pore-spanning membranes (micro-BLMs) allows for the investigation of single fusion events by means of fluorescence microscopy. Vesicles are doped with Texas Red DHPE, while micro-BLMs prepared by the painting-technique are doped with Oregon Green DHPE. Lipid mixing can be followed by the occurrence of Förster Resonance Energy Transfer (FRET). Content release during single fusion events can be verified by inclusion of the water-soluble dye pyranine into the vesicles.
In a second approach we investigated fusion processes that were mediated by molecular recognition. The peptide H6WGC was bound to pore-spanning membranes via reaction with maleimide-functionalized lipids. Membrane merger is driven by the interaction between the His-tag of the peptide and DOGS-Ni-NTA located in the vesicle membrane. We were able to show that those fusion processes are specific and strongly depend on the receptor densities in the membrane.



Synthesis of Different Glycosphingolipids

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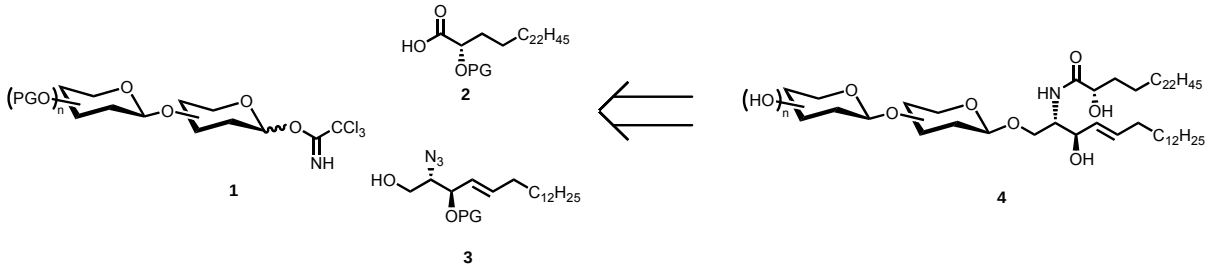
Glycosphingolipids can be found in all eucaryotic cell membranes, but mainly in the plasma membrane. They are known to form clusters, so called membrane domains, which can move within the fluid bilayer. Such lipid rafts seem to play an important role in the regulation of different cellular processes like signal transduction. From a retrosynthetic point of view, glycosphingolipids consist of three major building blocks; a carbohydrate component, a fatty acid and a sphingoid base.[1]
Due to great structural variety of glycosphingolipids it would be interesting to investigate the influence of different structural motifs on domain formation. Therefore glycosphingolipids with a variable carbohydrate and fatty acid part have been built up.

Sugar building blocks such as 1 can be synthesized according to literature-known strategies[2] with some modifications in the protecting group pattern. d-Erythro-(2S,3R)-sphingosine (3) is the main sphingoid base in mammalian tissue and therefore used for all prepared glycosphingolipids. For the synthesis of 3 several routes are known.[3] A variation in the fatty acid part is based on the compounds found in eucaryotic cells. Different chain lengths, the grade of saturation and α-hydroxylation (2) of the fatty acids are considered.
Synthetically the first step is the glycosylation reaction of 1 and 3. Azide reduction of the sphingosine, peptide coupling with the fatty acid and further global deprotection lead to glycosphingolipids such as 4.



Scheme 1. Retrosynthesis of a glycosphingolipid 4.

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Mechanosensing across the cell membrane: Mechanically activated TRP channels in model lipid bilayers and MDCK cells

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It has been shown that the primary cilium has remarkable sensory abilities. Of particular interest, from both a biophysical and medical standpoint, are the primary cilia in kidney epithelial cells, which have been demonstrated to act as tiny flow sensors. The cilia are lined with mechanosensitive TRP ion channels (PC2), proteins that allow the influx of cations into the cell in response to mechanical stimuli.
The aim of this project is to study the mechanical and electrical response of the PC2 proteins in two different systems: Directly in primary cilia of renal epithelial (MDCK) cells and reconstituted in planar model lipid bilayers.

To observe and stimulate polycystin-2 channels and to examine the electrical response a setup has been built which combines an epi-fluorescence microscope with an optical trap and also offers the possibility to perform electrophysiological experiments.

To perform low noise electrical recordings with ion channels reconstituted in planar lipid bilayers on porous substrates we fabricated a microchip suitable for electrical recording using standard cleanroom techniques.
Apertures of micrometer size are etched into a silicon nitride membrane forming several small porous microarrays and visualized by Scanning Electron Microscopy.

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The role of membrane proteins in pore formation and membrane fusion: computer simulation of coarse-grained models

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Using a coarse-grained model we study collective phenomena in membranes in presence of an inclusion (peptide). The lipids are described as amphiphilic chains composed of a hydrophobic and a hydrophilic block of monomers. The molecular architecture is represented through a bead-spring model where additional bending potential between two consecutive segments is included. The non-bonded interactions are captured via a density functional which is a third-order expansion in the density of the two component types. The model is solvent-free, i.e. the presence of the water is taken implicitly into account through the coefficients of the expansion. This model can be studied with efficient multi-body DPD simulations. The parameters of the model are tuned to promote the assembly of the lipids to a bilayer in the liquid phase and to reproduce characteristic macroscopic properties of the membrane such as: bending rigidity, area compressibility, and line tension of the pore.

Initially we consider two opposed membranes under tension where the rupture of a single bilayer competes with the fusion event. We perform a final tuning of our parameters so that fusion prevails and we identify the critical tension for this process to start. We study the fusion pathway, which we observe to follow the sequence: stalk, hemifusion, pore formation and fusion.

In order to study the influence of a hydrophobic inclusion on the fusion process we analyse the local and long range effects on the structure of the membrane. We describe a peptide either as a large particle interacting with a Lennard-Jones potential with the lipids or as a cluster of hydrophobic monomers, connected via strong harmonic forces. We observe that the effect of the inclusion on membrane properties exceeds the range of the direct particle-lipid interactions. Just after the region in contact with the peptide the radial density profile shows a depletion zone, which is prone to pore formation. In the same region the thickness is smaller than its equilibrium value. We conclude comparing the simulation results regarding the variation of the membrane thickness with the predictions of an elastic model.

Synthesis of modified gramicidin A for analysis of membrane-peptide interactions and its water permeability

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[2] Institut für Biophysik, Johannes Kepler Universität, Linz, Austria
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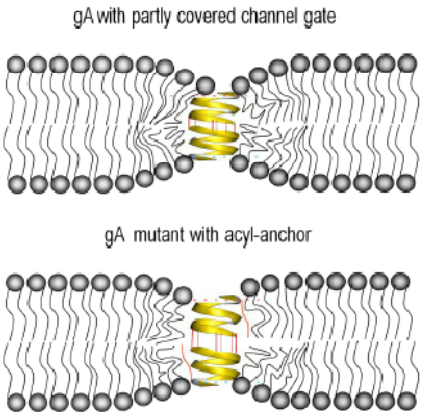
[4] Max-Planck-Institut für Biophysikalische Chemie



The linear pentadecapeptide antibiotic gramicidin A (gA) is a naturally occurring product of Bacillus brevis known to form ion channels in synthetic and natural membranes.[1] Two principal structural models are known, a head-to-head β6.3 - helical dimer and a double-helical β5.6 - structure.[2] These structures demonstrate single-file transfer through the channels. However, recent atomistic molecular dynamics simulations clearly identified lipid head groups as deterrents of the water flux due to their ability to block the channel entrance.[3] Based on this observation, we suggest modifications of the peptide's sequence to test the hypothesis that a reduction of the lipid head group interference increases the water permeability. In order to counteract and to regulate the stability and function of the gramicidin within membranes gA-derivatives with covalently attached fatty acids and other modifications such as a phospholipid anchor were synthesized. Further modifications as the attachment of charged residues and modifying the length of gramicidin A occur to be crucial for augmentation of water permeability and regulation of the hydrophobic mismatch. Circular dichroism experiments of acylgramicidin show the β6.3-helical conformation, the preferred conformation upon incorporation in membrane bilayers of gramicidin A. Furthermore, water flux, single channel and FRET measurements will be done in order to provide additional structural and functional information.

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Synthesis of O-Acylated 3-Hydroxyleucine Derivatives for the Preparation of Muraymycins as MraY Inhibitors

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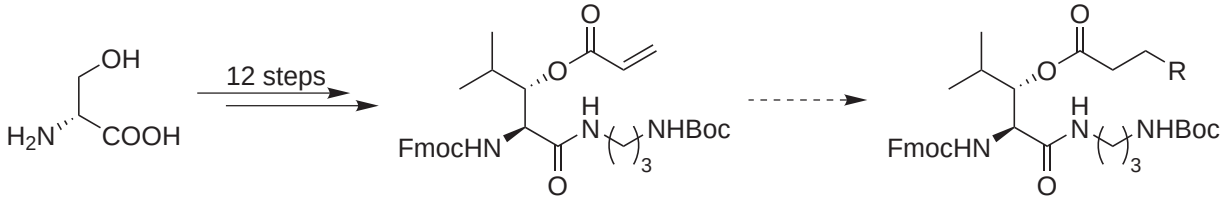
In 2002, McDonald et al. discovered nineteen structurally related naturally occurring nucleoside antibiotics called muraymycins, which block peptidoglycan biosynthesis by inhibition of the bacterial transmembrane protein MraY. In general, muraymycins consist of a uracil-uronic acid substructure linked to a short peptide chain. Structural diversity is achieved through the absence or presence of an aminoribose unit attached to the uronic acid moiety and a differentially O-lipidated (2S,3S)-3-hydroxyleucine moiety, respectively.[1]

In context of our work regarding the total synthesis of muraymycins and analogues thereof, it is of particular interest to establish a convenient synthesis of O-lipidated 3-hydroxyleucine derivative building blocks. Our synthetic route employs d-serine 1 as starting material, and conversion of 1 into 2 should enable the introduction of different lipid moieties by olefin metathesis, finally leading to lipidated analogues 3. The lipidation pattern plays a crutial role in MraY inhibition, making the rapid access to muraymycin analogues with different lipid structures highly desirable.



1 **2** **3**

Figure 1. Envisaged synthesis of *O*-lipidated 3-hydroxyleucine derivatives **3** (R = functionalised, unfunctionalised or branched lipid chain)

Furthermore, the synthesis of N-Alkyl-N-hydroxyguanides for the preparation of the lipid side chain of the biologically most potent muraymycin A1 is currently investigated. Previously reported syntheses were carried out in aqueous media and are therefore not applicable for the derivatisation of lipophilic compounds.[2-4] Thus it is important to establish an efficient synthesis which can be performed in organic solvents.

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Fast roads and dead ends in SNARE-dependent membrane fusion

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Neurotransmitter release at the synapse requires Ca^{2+} induced fusion of synaptic vesicles with the presynaptic plasma membrane. SNAREs are the core constituents of the proteins machinery responsible for fusion. The mechanism of Ca^{2+} triggered synaptic fusion, however, is still not well understood. Using near-atomic molecular dynamics simulations, we shed some light on the key determinants that are essential for both inducing and mediating fast and efficient SNARE-dependent fusion between a synaptic vesicle and the presynaptic plasma membrane. We show that the presence of four oligomerized neuronal SNARE complexes can, depending on their relative arrangement and secondary structure of both the Syntaxin and Synaptobrevin linker region, solely induce strong positive curvatures in the target membrane. These findings suggest an interplay between the SNARE complexes and other known curvature mediators/sensors such as Synaptotagmin, the calcium sensor in synaptic fusion, to lower the initial fusion barrier i.e. the formation of a stalk. After stalk formation, our simulations revealed that fusion, dependent on both the number and relative arrangement of SNARE complexes present, can progress through two topological different lipidic hemifusion pathways which considerably differ in fusion rate. We demonstrate that the steric hinderance of fusion proteins that comprise the stalk can force fusion through the rapid pathway by preventing an elongated expansion of the stalk that would otherwise lead to the formation of highly stable fusion intermediates. We further demonstrate that, when fusion is forced through the rapid pathway, the presence of cholesterol in the plasma membrane enhances fusion pore opening and reduces transient leakage.
Controversially, the same presence of cholesterol also increases the preference for an elongated stalk expansion i.e. the slow fusion pathway. Our simulations therefore underscore that rapid SNARE-dependent fusion necessarily requires a feedback mechanism which prevents that fusion, under enhanced fusiogenic conditions, progresses through the more preferable but slower pathway and which is in-vivo likely facilitated by the presence of additional fusion mediators.

Insertion of MscL into pore-suspending membranes

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Research on lipid membranes has long been confined to black lipid membranes (BLMs) and solid supported membranes. Whereas the former principally allow access to both sides of the membrane but suffer from low stability, the latter exhibit high stability at the expense of only unilateral access and reduced mobility of the lipids. Pore-suspending membranes, in contrast, provide higher stability than BLMs through support of the membrane via the pore rims as well as two aqueous compartments and a lipid mobility comparable to that observed in BLMs which is why they are the model of choice for channel protein investigations (Fig. 1).[1,2]

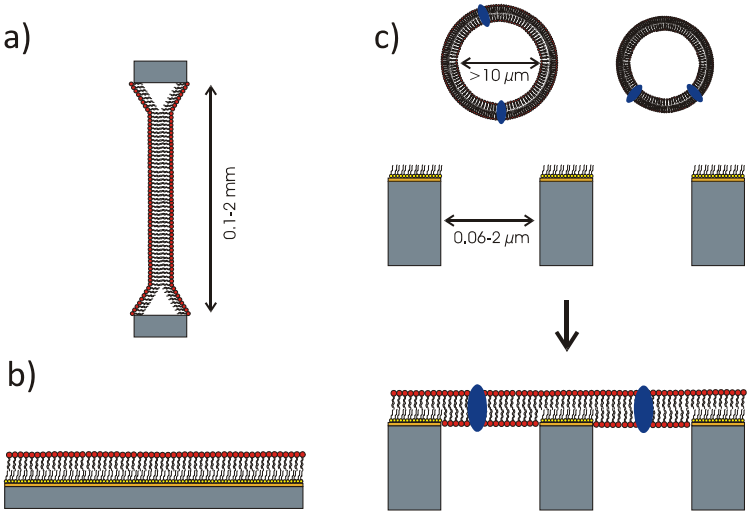

Figure 1 Schematic representation of a) a classical BLM, b) a solid supported membrane, c) a pore-suspending membrane with inserted proteins obtained *via* spreading of proteo-GUVs.

Mechanosensitive channels are thought to be the molecular basis for such fundamental yet diverse phenomena as pain, touch, hearing, balance, osmo- or cardiovascular regulation. This is due to their ability to detect mechanical force and transduce it into a chemical or electrical signal. The *E. coli* mechanosensitive channel of large conductance (MscL) is a homopentameric, integral membrane protein, each subunit comprising 136 amino acids with two transmembrane helices. It responds to changes in membrane tension, acting as a release valve, e.g. in case of an osmotic downshock. With an open pore diameter of >2.5 nm it exhibits a conductance of 2.5 nS (200 mM KCl).[3]

The objective of this project is to make our new membrane model system accessible to more delicate and demanding proteins. Therefore, a solvent free approach is employed to incorporate MscL into pore-suspending membranes. To date, expression and purification of MscL as well as reconstitution into GUVs have been established. Highly covered porous substrates have been obtained as is proven by means of confocal laser scanning microscopy (CLSM) and scanning ion conductance microscopy (SICM).

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Endpoint Optimization for Uniform Sampling Schemes in NMR Experiments

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Our aim is to find an optimal sampling scheme for multidimensional NMR experiments. The optimal sampling may depend on the reconstruction methods used, and we distinguish between parametric and non-parametric reconstruction methods. For simplicity we first concentrate on NMR experiments with one indirect time. We develop a mathematical framework and start by optimizing the endpoint for a uniform sampling scheme via numerical and analytical methods.

Rotation of a few degrees, relative translation by a few Angstroms: organization in membrane studied by EPR spectroscopy

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The specific functionalities of several bio-macromolecules are often associated to conformational changes. To monitor such conformational changes may provide new insights related to specific interactions which occur in long- and short-distances ranges. EPR spectroscopy combined with site directed spin labelling represents a valuable tool to get both structural and dynamic features. A multi-frequency approach can guarantee high accuracy in long-range distance determination and the increasing spectroscopic resolution may provide insights even for local short range changes and/or relative orientation of the spin probes.
Double resonance EPR techniques are currently applied on spin labelled trans-membrane peptides and peptide which exhibit a temperature-dependence into the formation of α-helix.
The contribution of the separation between the backbone and the spin label to the observed distance distribution can be determined by analyzing different peptides where the commercially available MTSL spin probes and novel synthetic spin probes are applied.
The simultaneous detection of different species which are stable intermediate represents the major achievement by our techniques with respect to more traditional spectroscopic methods. Low and high field EPR studies on those systems pointed out dynamic aspects related to the nature of the labelled system.
Furthermore, limits of the High-Field (HF)-PELDOR set up related to commercially available resonators have been overcome by the construction of a new dual mode resonator. Preliminary results on the new resonator are also presented; those results allow us to widen the range of applications into orientation selection studies.

Analysis of the membrane binding mechanism of the peripheral membrane protein pyruvate oxidase from Escherichia coli

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Pyruvate oxidase from E. coli (EcPOX) is a homotetrameric enzyme that is involved in cellular energy metabolism. EcPOX requires the cofactors thiamin diphosphate and FAD for activity and catalyzes the oxidative decarboxylation of pyruvate into CO_2 and acetate. This redox reaction generates two reducing equivalents which are initially transferred to the flavin cofactor. Reduction of FAD triggers a conformational change of the enzyme, which leads to solvent exposure of the membrane anchor and eventual membrane association of EcPOX. At the membrane, the two reducing equivalents are finally transferred to membrane-dissolved ubiquinone 8 (Q8), a mobile carrier of the respiratory chain. Membrane binding of EcPOX is conferred by the C-terminal 23 amino acids (alpha-peptide). In the resting state, the C-terminal membrane anchor is clamped to the protein surface and exhibits no defined secondary structure. Previous work in our group revealed that the isolated peptide forms an amphipathic helix in the presence of membranes and micelles, as predicted by others before. A central aim of the project is to identify principles that underpin conformational stabilization of the alpha-peptide in the unstructured enzyme-bound versus the helical membrane-bound state. Additionally, the mechanism of directed electron transfer from the enzyme active site to membrane-bound Q8 shall be analyzed.

Pure alpha-peptide was afforded either by recombinant expression as SUMO-fusion protein or by solid-phase peptide synthesis. Using circular dichroism and infrared reflection absorption spectroscopy the transition of the alpha peptide from an unstructured conformation in solution to an α-helical conformation in the presence of lipids and micelles was characterized. A preliminary liquid-state NMR-structure of the alpha-peptide in the presence of SDS-micelles indicates the formation of an amphipathic helix.
Electron transfer between reduced EcPOX and quinone cosubstrates was analyzed by steady-state and transient (stopped-flow) kinetics. Thermodynamics of quinone binding was characterized by circular dichroism spectroscopy. The structural basis for redox coupling shall be provided by X-ray structure analysis of an EcPOX:quinone complex.

Synthesis of the muraymycin core structure and analogues for the investigation of the bacterial membrane protein MraY

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Due to the emerging resistances of bacteria towards established antibiotics, there is an urgent need for the development and exploration of novel antibacterial agents, which ideally should display new or yet unexploited modes of action. In this regard the bacterial membrane protein translocase I (MraY), a key enzyme in the early stages of peptidoglycan biosynthesis, represents an attractive target. So far, there is no clinically approved antibiotic addressing MraY as a target, but there are natural products, the so-called 'nucleoside antibiotics', which efficiently inhibit this membrane protein. Within this group one of the most promising classes for further investigations are the muraymycins (e.g., muraymycin C4 1, Fig.).[1] This collection of 19 compounds isolated from a Streptomyces sp. displays a high potential for detailed structure-activity relationship (SAR) studies as there are known MraY-inhibiting truncated analogues with reported remarkable activities against for example Staphylococcus aureus (e.g., 2 and 3, Fig.).[2]

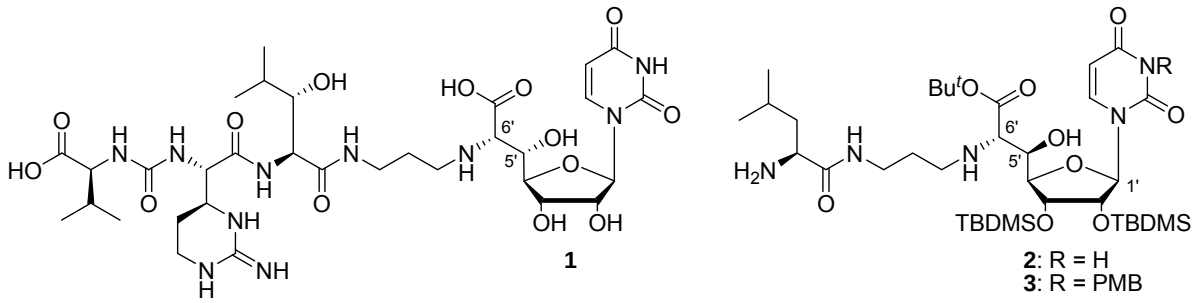

Fig. Muraymycin C4 **1** as a representative of the naturally occurring muraymycins. Truncated 5'-*epi*-muraymycin analogues **2** and **3** with reported biological activity.

Thus, a valuable building block of the nucleosidic core structure was synthesized for the preparation of muraymycins and analogues (without figure).[3] Furthermore, compounds 2 and 3 were chosen as lead structures for the development of novel truncated analogues. Therefore the corresponding 5'-deoxy analogues of 2 and 3 have been prepared both with the (6'S)-configuration of the naturally occurring muraymycins and with the (6'R)-configuration in a diastereoselective fashion (without figure).[4] The synthesis of 5'-hydroxy derivatives with different stereochemical configurations is also further investigated.[3,5] The evaluation of the antibacterial potency of the prepared analogues is expected to yield not only further SAR insights but also an improved understanding of the general mode of action and mode of inhibition of the bacterial membrane protein MraY.
The results from this synthetic work towards MraY inhibitors will be presented.

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Functional dynamics in the voltage dependent anion channel in micellar and bicellar environment

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The voltage-dependent anion channel (VDAC), located in the outer mitochondrial membrane, controls cell life and death by acting as a gatekeeper for the entry and exit of mitochondrial metabolites (1-3). Here we reveal functional dynamics of isoform one of VDAC (VDAC1) by a combination of solution NMR spectroscopy, MD simulation and Gaussian Network Model (GNM) analysis. Slow (μs-ms), but not fast (ps-ns) dynamics are significantly increased for the N-terminal six β-strands of VDAC1 in micellar solution, in agreement with increased B-factors observed in the same region in the bicellar crystal structure of VDAC1. The increased B-factors and the corresponding slow dynamics are well predicted by low-frequency modes derived from GNM analysis and by low-pass filtered MD simulation. The latter reveals that a charge on the membrane-facing glutamate 73 (E73) accounts for the elevation of N-terminal protein dynamics as well as a thinning of the nearby membrane. Mutation or chemical modification of E73 strongly reduces the long time scale dynamics in solution, implying a partial charge on E73 as the main cause of the dynamics. Since E73 is necessary for hexokinase-I-induced VDAC channel closure and inhibition of apoptosis (4), our results imply that slow dynamics in the N-terminal part of the barrel are essential for VDAC interaction and gating.

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Electrophysiological Investigation of the VDAC Ion-Channel

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The Voltage-Dependent Anion Channel (VDAC) plays an important role in cell life and apoptosis since it is the main porin of the outer mitochondrial membrane (OMM).[1] An important step in apoptosis is the release of pro-apoptopic factors, e.g. cytochrome-c, from the mitochondrial matrix to the cytosol due to changes in the OMM permeability. A deeper understanding of this yet not fully understood process is of great value for the treatment of different related diseases such as stroke, Alzheimer, Parkinson and cancer. As VDAC is believed to play a pivotal role in this process, the alterations of its electrophysiological properties under different conditions are investigated.

For the determination of its electrophysiological properties, human VDAC1, expressed in E. coli, purified and finally refolded in the detergent LDAO, is reconstituted in artificial membranes. These membranes are prepared via the Müller-Rudin-technique on a porous alumina substrate containing pores with a diameter of 60 nm.[2] The quality of these so-called nano-Black-Lipid-Membranes (nano-BLMs) is verified via electrochemical impedance spectroscopy (EIS), VDAC is reconstituted and single channel recordings are made.

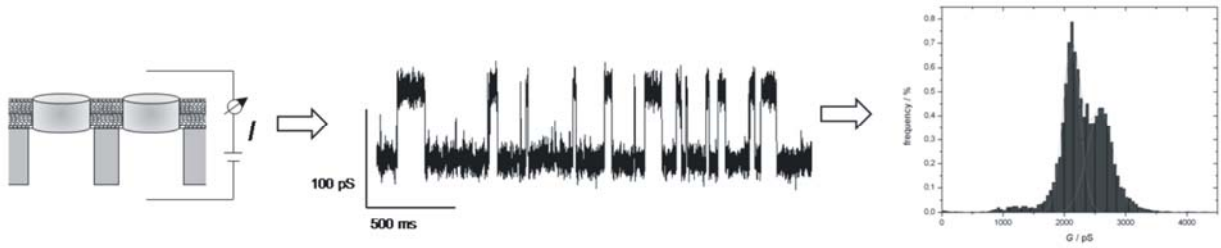

Figure 1. Schematic diagram of the setup of a single channel recording with subsequent evaluation.

In order to obtain information about the conductance states, open lifetimes and open probabilities of VDAC, holding potentials in the range of about ±100 mV are applied and the resulting current steps are recorded.

Statistical evaluation of the data shows that VDAC inserted in artificial bilayers tends to be in a fully open state with a conductance of about 4 nS in 1 M KCl without transmembrane potential. With increasing potential the channel partially closes, wich results in a decrease in conductance to about 40 % at ±80 mV.

Acknowledgment: The hVDAC1 was a kind gift of Prof. C. Griesinger, MPIBPC, Göttingen

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Quantifying the translational and rotational diffusion of membrane proteins and peptides

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Diffusion of proteins and peptides within lipid membranes is described by the classical Saffmann-Delbrück model. Recent publications have both challenged and supported this model, and cur-rently it is not clear how valid the assumptions and conclusions of this model are. The current project aims at measuring precise values of translational and rotational diffusion for a wide range of different protein sizes and environmental conditions (in particular nature of lipid membrane), which will allow for a critical re-evaluation and potential extension of the Saffmann-Delbrück model.

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